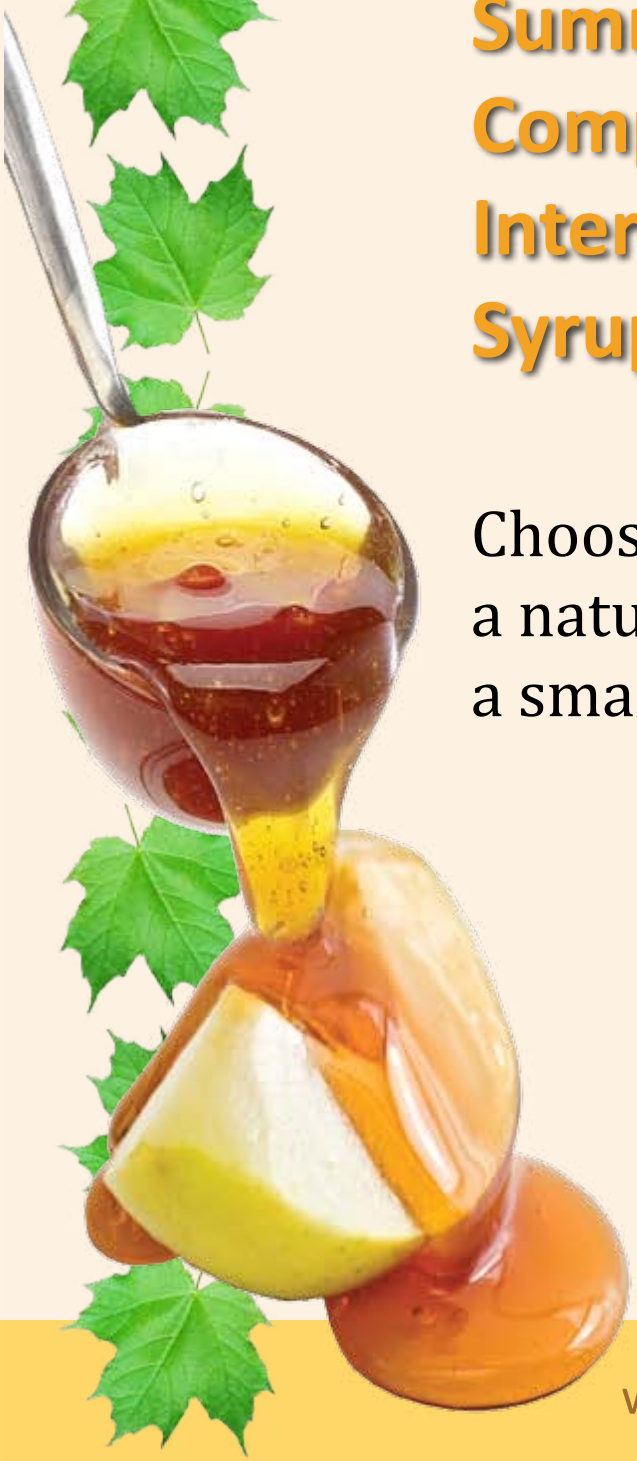
NUTRITION AND HEALTH BENEFITS OF PURE MAPLE SYRUP

Summary of Information Compiled by the International Maple Syrup Institute



Choose ***Pure Maple Syrup,***
a natural sweetener and
a smart food choice.

ENTER

March 2012

www.internationalmaplesyrupinstitute.com

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Maple Syrup Marketing Strategy and Positioning Statement

The Opportunity:

The effective presentation of nutritional and health benefits information for pure maple syrup has the potential to dramatically improve consumer perceptions of pure maple syrup. This, in turn, could significantly increase maple syrup's sales volume and resulting market share. This may occur wherever sweeteners are used and in the consumer driven pourable sweet condiments market (table syrups), which is currently dominated in North America by corn syrups, particularly high fructose corn syrup (HFCS).

Background:

Pure maple syrup presently represents a very small share of the market in the United States, Canada and overseas. In the U.S., for example, maple syrup, along with honey, accounts for about 1% of all sweeteners delivered for food and beverage use.

2005 U.S. Per-capita Sweeteners – Food and Beverage Use

(Annual pounds, dry basis, per USDA)

Total	Refined Sugar	Corn Sweeteners	Honey, Maple, etc.
142.6 lbs.	44%	55%	1%

Refined sugar consumption is 63 pounds per person per year in the United States, while it is about 18% lower in Canada at 52 pounds per person per year. In the United States in 2005, per capita use of maple and honey combined was 1.4 pounds per person per year while it was 1.8 pounds per person per year in Canada.

In the direct to consumer market, maple syrup competes mostly within the pourable sweet condiments (table syrups) market where corn syrups, particularly HFCS, have a commanding market share. In the table syrup market, which is used largely as a topping on foods such as pancakes, waffles and ice cream, it is estimated that maple syrup has about a 5% share of servings in the U.S, with the rest going mostly to corn syrups bearing labels like Log Cabin and Aunt Jemima. The dominate share position of the corn based brand name syrups is driven by their significantly lower retail price and heavy advertising and promotion. This is helped by the fact that the corn industry is heavily subsidized in the U.S., estimated at about $4 billion per year.

All sweeteners now have a less than wholesome image, due in large part to highly publicized studies of the potential harmful health effects of processed foods in North America's high calorie, low nutrition diet, coupled with an obesity epidemic. This is particularly true for corn syrups, especially HFCS, where 58% of U.S. consumers now believe it poses a health risk right behind mad cow disease and mercury contamination in seafood. It is believed that Canadians have a similar outlook.

"it's natural, tastes great and does cost more, but it is a better contributor to a healthy diet than other commonly consumed sweeteners"

A leader in raising public concerns about the corn industry has been Michael Pollen. In his best selling book entitled "The Omnivore's Dilemma" and other books and articles he has authored, he targets the corn industry as a root cause of U.S. health problems. In various forms, it is in about 80% of the processed foods we consume from soft drinks, cake mixes and mayonnaise to tooth paste and meat. Meat is included in this list because "corn is what feeds the steer that is converted into the beef we eat". Corn sweeteners make up 10% of all calories consumed by the average adult in the U.S. and 20% among children. The average person in the U.S. consumes about 60 pounds per year of HFCS alone. Four of the top 10 leading causes of premature death in North America, including cancer and diabetes, have well established links to our "corn-laced" diet.

Despite the very specific concerns about corn syrups, consumers are cautioned about all sugars and urged through various means to cut back on sweets and sweeteners of all kinds for a healthier life and to help curtail the obesity epidemic. Thus, maple syrup, as a caloric sweetener, is caught up in this negative imagery.

Until now, maple syrup has not been able to set itself strongly apart from this growing concern about caloric sweeteners. Maple syrup is clearly viewed as better than corn syrup, but the basic message from government, medical and academia health experts is that "North Americans consume too much of all types of sugar". The nutritional and potential health benefits research findings clearly demonstrate that pure maple syrup has considerable nutritional value, contains antioxidants which may be beneficial to human health and may have additional health advantages.

Currently, a typical consumer might position maple syrup in their mind as "it's natural and tastes great, but it's much more expensive than brand name (corn based) syrups". Effective communication of the nutritional and other potential benefits of maple syrup, contributing to good health, can shift this mindset to "it's natural, tastes great and does cost more, but it is a better contributor to a healthy diet than other commonly consumed sweeteners". This new realization of the nutritional and potential health benefits of pure maple syrup may not narrow the real price disparity when compared to other sweeteners, but it will decrease the impact price has on purchase decisions.

Business Goal:

Initial Goal: Increase the consumption of maple syrup by current users, and thereby increase purchase volume of maple syrup.

Eventual Goal: Increase the acceptance and consumption of maple syrup among all consumers worldwide.

Marketing Strategy:

Create and increase an awareness of the superior nutritional and other potential health benefits of maple syrup compared to other commonly used sweeteners.

POSITIONING STATEMENT

Target Audience:

Initial Focus: Current maple syrup users, particularly light and moderate users.

Eventual Focus: All people who like the maple flavor and use sweeteners.

Frame of Reference:

Common sweeteners used as toppings and ingredients with a particular focus on corn syrup as it is used in maple flavored "table syrups", but also including honey*, cane sugar and brown sugar.

*Honey may have other health related benefits because of its uniqueness (i.e. treatment of allergies).

Unique Benefits of Pure Maple Syrup:

- Pure maple syrup delivers more overall nutritional value than many common sweeteners and has one of the lowest calorie levels.
- Pure maple syrup provides enhanced antioxidant levels compared to other common and popular foods, such as apples and broccoli.
- Pure maple syrup may have other health benefits that are currently being studied.

Nutritional Value for Various Sweeteners

% of Recommended Daily Value (DV) Per ¼ cup (60 ml)

	Maple Syrup		High Fructose Corn Syrup		Honey		Brown Sugar		White Sugar	
	(1/4 cup / 80 g)		(1/4 cup / 78 g)		(1/4 cup / 85 g)		(1/4 cup / 55 g)		(1/4 cup / 51 g)	
	% DV	**mg**	% DV	mg	% DV	mg	% DV	mg	% DV	mg
Riboflavin	**37**	**0.59**	1	0.01	2	0.03	0	0.0	1	0.01
Thiamin	**1**	**0.01**	0	0.0	0	0.0	0	0.0	0	0.0
Manganese	**95**	**1.89**	4	0.07	4	0.07	2	0.04	0	0.0
Zinc	**6**	**0.58**	0	0.02	2	0.19	0	0.02	0	0.0
Magnesium	**7**	**16.5**	0	0.0	1	1.75	2	5.0	0	0.0
Calcium	**5**	**58.0**	0	0.0	0	5.0	4	45.8	0	0.48
Iron	**1**	**0.09**	0	0.02	3	0.36	3	0.39	0	0.03
Selenium	**1**	**0.4 µg**	1	0.55 µg	1	0.66 µg	1	0.65 µg	1	0.3
Potassium	**5**	**167**	0	0.0	1	44.0	2	73.3	0	0.96
Calories	**216**		220		261		216		196	

Source: USDA Nutrient Database and Canadian Nutrient File

Notes: The values shown are the overall minimum values for the minerals and nutrients and the overall maximum values for the calories reported by the USDA Nutrient Database and the Canadian Nutrient File. The percent daily values (% DV) were calculated using the Health Canada recommended daily intake values for an average 2,000 calorie diet.

Antioxidant value for common foods

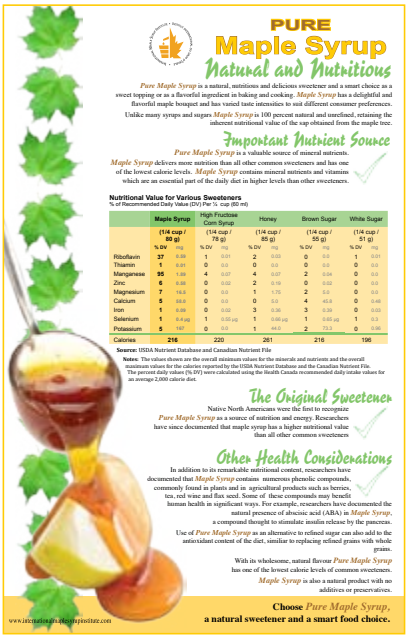
ORAC Value 100g of fresh product	**µmol TE²/100g**	**ORAC Value per serving**		**µmol TE²/ serving**
Brocoli, raw	1,362	Banana, raw	1 medium (118g)	1,037
Banana, raw	879	Broccoli, raw	½ cup (46g)	627
Carrot, raw	666	Carrot, raw	1 (72 g)	480
Maple Syrup	600	Maple Syrup	¼ cup (60 ml/80 g)	480
Cabbage, raw	508	Tomato, raw	1 medium (123 g)	415
Tomato, raw	337	Cantaloupe	½ cup (85 g)	268
Cantaloupe	315	Cabbage	½ cup (37 g)	188

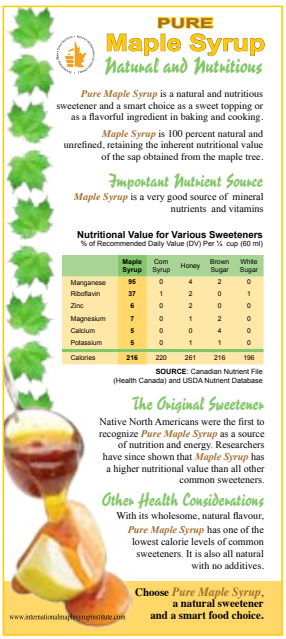
USDA Database for the Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods. Results showing the antioxidant power of maple syrup were obtained from Brunswick Laboratories, a USDA-certified facility.

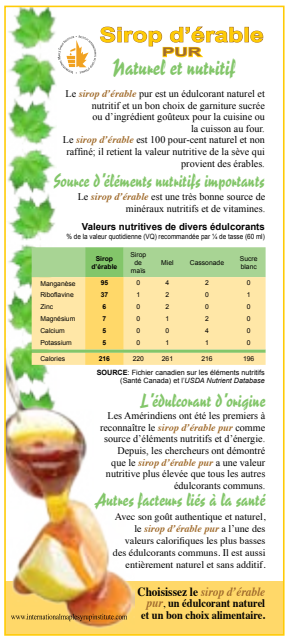
IMSI Poster and Rackcard

Provided for you on this CD are print ready pdfs of posters and rackcards, available in English and French. GIve your customers the facts on Maple Syrup: Natural and Nutritous.

Click on the thumbnails below to see a larger view. For print, be sure to use the print ready files provided on the cd.







Your local print supplier can output these files for you. The poster should be printed on 12x18, then be trimmed back to 11x17. Print on 28lb gloss stock or light card.

The rack cards trim back to 3.5x8.5 inches. Print on a light card stock, and consider having French on one side, English on the other.

Summary Statements of Maple Syrup Nutritional and Health Benefits Information

The following statements are grouped by type of nutritional information, representing what is being said about maple syrup based on this literature review, as well as examples of marketing statements being made by members of the maple industry in the public forum. As maple syrup is a natural product with ranges of values, some variations do exist in published values of nutrition information.

Purity

- Pure Maple Syrup is a unique and natural product produced exclusively by the concentration of sap from the maple tree. It is a natural sweetener that contains no added sugar, colouring agents, artificial flavourings, preservatives or other additives.
- The range of colour (grades) of pure maple syrup is due to natural changes in the sap as the production season progresses, not by the addition of any caramel colouring agents.

Minerals, Vitamins and General Nutrition

- Maple Syrup is a significant source of several nutrients. Pure maple syrup has natural variations, but on average, a 4 Tbsp serving of Maple Syrup supplies us with more than 100% of our daily intake of manganese, 37% of riboflavin, 18% of zinc, 7% of magnesium, 5% of calcium and 5% of potassium. www.domorewithmaple.com and Canadian Nutrient File
- In addition to carbohydrates, vitamins and minerals, maple syrup and its products also contain phenolic compounds. These compounds are found in sap and are concentrated in maple syrup. www.domorewithmaple.com
- The following chart from the Federation of Quebec Maple Producers shows the percentage of the recommended Daily Value of 6 nutrients as well as the calories and grams of sugar for maple syrup. The values are also given for honey, sugar and brown sugar for comparison.

Per 60 ml portion in %DV*	Maple syrup of Canada	Honey	Sugar	Brown sugar
Manganese	100	3	0	9
Riboflavin (B_2)	37	2	1	0
Zinc	18	2	0	1
Magnesium	7	1	0	7
Calcium	5	0	0	5
Potassium	5	1	0	6
Calories	217	261	196	211
Sugars (in G)	54	71	51	54

Legend : Excellent source of | Good source of | Source of

*DV: The *Daily Value* is the amount deemed sufficient to meet the daily needs of the majority of healthy individuals. Source: Canadian Nutrient File (Health Canada)

- Based on the minimum values obtained from the analysis of over 600 samples of maple syrup from different regions across Quebec, the following claims can currently be made on maple syrup labels in Canada:

  ✓ Excellent source of manganese and Vitamin B2
  ✓ Good source of energy
  ✓ 4 mg of polyphenols per 60 mL serving

FPAQ Nutrition Information Fact Sheet

- A serving of ¼ cup of maple syrup contains:

  ✓ 100% of the recommended Daily Value for manganese – important factor in energy production, healthy bone formation and antioxidant defences necessary for normal brain and nerve function
  ✓ 34% of the recommended Daily Value of riboflavin – aids in metabolic processes
  ✓ 11% of the Daily Value of zinc – essential for normal reproduction and growth as well as a healthy immune system.

www.canadamaple.com

- The trace mineral manganese is an essential cofactor in a number of enzymes important in energy production and antioxidant defenses. One role of manganese is as a component of the key oxidative enzyme superoxide dismutase, which disarms free radicals produced within the mitochondria (the energy producing factories of cells). One ounce of maple syrup supplies 22.0% of the daily value of manganese. *www.whfoods.com*

Minerals, Vitamins and General Nutrition: Examples of Marketing Statements

- A sweetener that's better for you – yes, it exists! *www.purecanadamaple.com*
- Maple syrup is sweet - and we're not just talking flavor. Maple syrup, as an excellent source of manganese and a good source of zinc, can also be sweet for your health. www.*whfoods.com/genpage.php?tname=foodspice&dbid=115*
- Pure maple syrup is one of Natures perfect creations. A completely unrefined organic sweeter that contains vitamins and minerals (calcium, potassium, iron, B2, B5, B6, and niacin). Guess what...it tastes great! *www.oldstatefarms.com*
- Savour the flavour of liquid gold. Delicately sweet straight from the maple tree. *www.mapleorchardfarms.com/*
- Sugar, corn syrup and maple syrup… Are they all the same? Not really! Although all three are sweetening agents, maple syrup contains more vitamins and minerals than its two distant cousins. A 60 millilitre (1/4 cup) portion of maple syrup provides 100% of the recommended daily allowance of manganese, 37% of riboflavin, 18% of zinc, 7% of magnesium and 5% of calcium and potassium. *www.siropderable.ca*

- Maple's not just for breakfast anymore. Whether it's for baking, cooking, marinating, toppings, spices, basting for barbecuing season or for a treat, nothing says Canada like Mmm...maple! *www.whitemeadowsfarms.com*

- The natural goodness of maple syrup, with over 40 antioxidants, low glycemic index, and natural nutrition always make maple syrup a smart choice. *www.themaplestore.com/ (Jakeman's)*

- Health benefits of real maple syrup are far more comprehensive than you might expect. The only product in our diet coming directly from a plant's sap, this natural sweetener features over 54 antioxidants that can help delay or prevent diseases caused by free radicals, such as cancer or diabetes. In addition, maple syrup features high levels of zinc and manganese, keeping the heart healthy and boosting the immune system. *www.purecanadamaple.com/benefits-of-maple-syrup/*

- "These new scientific findings underscore the nutritional message whereby food that undergoes little to no processing provides greater health benefits," said very enthused dietitian Hélène Laurendeau. "100% pure Maple syrup is a natural, non-refined product, which gives it an edge over other sweetening agents. We have reason to be proud of our maple syrup, whose unique flavour makes it a versatile addition to countless culinary creations." (In reference to antioxidant research conducted by Seeram and ABA research conducted by Desjardins) *www.ishs.org/news/?p=1588*
www.ishs.org/news/?p=1588

Antioxidants

- Phenolic compounds are widely distributed in plants. Over two dozen phenolic compounds have been isolated in maple syrup and evidence suggests that many more are present. In view of the well-established antioxidant activity these substances possess, it is suggested that it is the complexity of the mixture that makes maple syrup of particular interest rather than any one compound. These beneficial phenolic compounds make maple syrup a healthy choice as a sweetener.
Reference: 1.Abou-Zaid, M. M.; Nozzolillo, C.; Tonon, A.; Coppens, M.; Lombardo, A. D. A. High performance liquid chromatography characterization and identification of antioxidant polyphenols in maple syrup. Pharm. Biol. 2008, 46, 117-125.
Additional information: This research isolated 24 phenolic compounds but evidence indicated the presence of many more. The total phenolic content of the maple syrup, however, is very small in proportion to the sugar content (about 20 ± 5 mg/10 mL of syrup, 0.2% by dry weight). However, it is suggested that the biological activity may aid in overcoming any negative effects of the high sugar content of the syrup on humans, thus, indulging a sweet tooth, and is most likely, as suggested by Theriault et al. (2006), not related so much to any particular compound as to the overall complexity of the mixture.

- Over 20 antioxidant compounds have been discovered in pure maple syrup that have been linked to human health. Several of these antioxidant compounds are reported to have anti-cancer, anti-bacterial, and anti-diabetic properties. The amount of the effect is yet to be determined but the presence of these beneficial compounds in maple syrup is very interesting from a human health perspective.
  (Note: The number of antioxidant compounds discovered increased from 20 to 54 in 2011 from further research by these scientists.)
  Reference: 9.Li, L.; Seeram N. Maple syrup phytochemicals include lignans, coumarins, a stilbene, and other previously unreported antioxidant phenolic compounds. J. Agric. Food Chem. 2010, 58, 11673-11679.
  Reference: News release, with video, from University of Rhode Island concerning research conducted by N. Seeram can be found at *www.uri.edu/news/releases/?id=5256*

- The results from research conducted by Legault, et al. in 2010 indicated that pure maple syrup possesses an interesting in vitro inhibition of cancer cell growth, with the strongest effect being found against prostate and lung cancer cells. Maple syrup extracts have also exhibited in vitro antioxidant and nitric oxide inhibition activities. The value of antioxidants in a protective role against cancer is well known. The ability of maple syrup to inhibit nitric oxide is also important as several works have shown that the overproduction of nitric oxide is a result of inflammation, a factor in the formation and development of cancer. Inhibiting nitric oxide results in inhibiting inflammation and thus may help to prevent diseases such as cancer.
  Reference: 8.Legault, J.; Girard-Lalancette, K.; Grenon, C.; Dussault, C.; Pichette, A. Antioxidant activity, inhibition of nitric oxide overproduction, and in vitro antiproliferative effect of maple sap and syrup from Acer saccharum. J. Med. Food 2010, 13, 460-468.

- Phenolic compounds, widely distributed in plants, contribute to the colour and aroma of the food and may also have potential health benefits, including preventing or delaying the formation of cancer. The phenolic compounds present in maple syrup have antioxidant and antiradical activities as well as potential antimutagenic activities.
  Reference: 10.Theirault, M.; Caillet, S.; Kermasha, S.; Lacroix, M. Antioxidant, antiradical and antimutagenic activities of phenolic compounds present in maple products. Food Chem. 2006, 98, 490-501.

- Use of alternatives to refined sugar, such as pure maple syrup, can add to the cumulative antioxidant content of the diet by replacing refined sugar. This would increase antioxidant consumption similar to replacement of refined grains with whole grains.
  Reference: 12.Phillips, K.; Carlsen, M.; Blomhoff, R. Total antioxidant content of alternatives to refined sugar. J. Amer. Diet. Assoc. 2009, Jan. 109(1):64-71.

- Maple syrup has been found to have antioxidant, antimutagenic, and human cancer cell antiproliferative properties (Ref: 8, 10).

- Phenolic content was found to be higher at the beginning and at the end of the harvest season, for all three producers in the study conducted by Kermasha et. al. in 1995, the highest levels being at the end of the season. This differs from the study by Legault et. al. in 2010 where it was found that phenolic compounds were 29% lower and antioxidant activity 24% lower at the end of the season compared to the beginning. Phenolic compound concentrations were also found to differ among producers. These differences may be due to variations in harvest and processing as well as soil and climatic conditions.
  Reference: 6.Kermasha, S.; Goetghebeur, M.; Dumont, J. Determination of phenolic compound profiles in maple products by high performance liquid chromatography. J. Agric. Food Chem. 1995, 43, 708-716.

Antioxidants: Examples of Marketing Statements

- "Not all sweeteners are created equal. When choosing a sweetener, pure maple syrup may be a better choice because of the range of antioxidant compounds not found in other sweeteners." N. Seeram, *www.purecanadamaple.com/next-champion-food/*
- Researchers from the University of Rhode Island (URI) have identified 54 compounds in maple syrup, many with antioxidant properties and potential health benefits. In laboratory studies, they acted as anti-cancer and anti-inflammatory agents. Initial studies also suggest that compounds exist in maple syrup that may inhibit enzymes relevant in Type 2 diabetes management. *www.ishs.org/news/?p=1588www.ishs.org/news/?p=1588*
- Over four dozen phenolic compounds, many with antioxidant properties and potential health benefits, have been identified in maple syrup. This does not mean that you should eat enormous quantities of maple syrup to increase your intake of antioxidants and other beneficial compounds. It does mean, however, that if you are going to use a sweetener, maple syrup is a good choice because it has these phenolics.
- The antioxidant activity of pure maple syrup is comparable to that of Gala red apples, broccoli, and bananas. (FPAQ Antioxidant Fact Sheet)
- A 60 ml serving of maple syrup (1/4 cup) provides 10 to 38% of the recommended daily allowance of antioxidants according to several nutritionists in the United States. (FPAQ Antioxidant Fact Sheet)
- Variations exist in the antioxidant capacity among samples of maple syrup. Despite this, the antioxidant capacity of maple syrup puts it in good position as an antioxidant among fruits and vegetables. (FPAQ Antioxidant Fact Sheet)
- Maple syrup contains antioxidants, which can delay or prevent free radical induced diseases, such as diabetes and cancer. A ¼ cup portion of maple syrup contains as much antioxidant activity as one serving of raw tomato or broccoli. www.canadamaple.com

Abscisic Acid And Diabetes

- Abscisic acid in maple syrup occurs in concentrations that are of significance to have an effect on human health, according to the effective thresholds of abscisic acid reported by Guri et al. in 2007 (Clinical Nutrition 26:107-116). The physiological properties of abscisic acid have been known within the plant world for some time, but its health benefits for humans has only recently been realized. Along with other effects, it is known to stimulate insulin release through pancreatic cells and to increase sensitivity of fat cells to insulin, which makes it a powerful weapon against metabolic syndrome and diabetes. (Federation of Quebec Maple Producers, Press Release, March 4, 2010)

- Research conducted by Yves Desjardin and his colleagues of Laval University has found that both maple syrup and sap contain equally important quantities of terpenes, and in particular, abscisic acid (ABA), a phytohormone whose health benefits have only recently been discovered. Along with other effects, ABA is known to stimulate insulin release through pancreatic cells and to increase the sensitivity of fat cells to insulin, making it a useful weapon against metabolic syndrome and diabetes. News-Medical.Net

- Research by Dr. Yves Desjardins at Laval University has found maple syrup to contain high levels of abscisic acid, a promising phytohormone that could provide health benefits. Abscisic acid is known to stimulate the release of insulin by pancreatic cells, increase fat cell sensitivity and promote muscle sugar absorption. Because of this, it acts as a potential therapeutic agent against metabolic syndrome and diabetes. *www.ishs.org/news/?p=1588www.ishs.org/news/?p=1588*

- Geneviève Béland, Director of Promotion and Market Development for the Federation of Quebec Maple Syrup Producers, on Yves Desjardins research on ABA:

  "These findings show that maple products contain a whole host of complementary active elements. The sugar molecules which provide the energy and sweetness in maple products are inherently complemented by abscisic acid molecules because they encourage insulin homeostasis. Further studies are obviously needed before we can more accurately understand how eating maple products effects insulin behaviour. Studying maple products is of particular interest to the food science sector when we consider that all the bioactive molecules of the sugar maple are carried in its sap and that these molecules are forty times more concentrated in maple syrup." News-Medical.Net

- Maple syrup may prove to be relevant in Type 2 diabetes management, although the findings must be verified in clinical trials. "We discovered that the polyphenols in maple syrup inhibit enzymes that are involved in the conversion of carbohydrate to sugar," said Seeram. "In fact, in preliminary studies maple syrup had a greater enzyme-inhibiting effect compared to several other healthy plant foods such as berries, when tested on a dry-weight basis. By 2050, one in three people will be afflicted with Type 2 diabetes and more and more people are looking for healthier diets, so finding a potential anti-diabetic compound in maple syrup is interesting for the scientific community and the consumer," said Seeram. *www.purecanadamaple.com/next-champion-food/*

Glycemic Index

- The Glycemic Index (GI) is a scale that ranks foods on how they affect blood glucose levels. By consuming foods with a low GI rating (55 or lower), we can potentially help prevent or control heart disease, diabetes and obesity. Maple Syrup (GI: 54) compares favourably with other sweetening products such as sugar (GI: 58) and honey (GI: 87). www.domorewithmaple.com

Heart Health

- The zinc supplied by maple syrup, in addition to acting as an antioxidant, has other functions that can decrease the progression of atherosclerosis. Zinc is needed for the proper function of endothelial cells and helps to prevent the endothelial (inner lining of blood vessels) damage caused by oxidized LDL cholesterol and other oxidized fats. Endothelial membranes that are low in zinc are much more prone to injury. The manganese supplied by maple syrup is also good for the heart. Studies have found that adults deficient in manganese have decreased levels of HDL ("good" cholesterol). *www.whfoods.com*

Immune System

- Zinc and manganese, both supplied by maple syrup, are important to a healthy immune system. Researchers have studied, particularly in children, the effects of zinc deficiency and zinc supplementation on the immune response and the number of white blood cells. In these studies, zinc deficiency has been shown to compromise numbers of white blood cell and immune response, while zinc supplementation has been shown to restore conditions to normal. The manganese in maple syrup is also important to the immune system as an antioxidant component, helping to lessen inflammation and support healing. In addition, manganese may also act as an immunostimulant. *www.whfoods.com*

Men's Health

- Maple syrup may help to support reproductive health, particularly in men. Zinc is concentrated more highly in the prostate than in any other human tissue, and low levels of zinc in this gland relate to a higher risk for prostate cancer. Zinc is even used therapeutically by healthcare practitioners to help reduce prostate size. Manganese may also play a role in supporting men's health since, as a catalyst in the synthesis of fatty acids and cholesterol, it also participates in the production of sex hormones, thus helping to maintain reproductive health. *www.whfoods.com*

Probiotics

- Research has been conducted to evaluate the capacity of maple sap or its concentrate to be used in the development of new probiotic products. Probiotics are beneficial microorganisms that function internally to promote healthy digestion, boost the immune system, and contribute to general health. Maple sap is a good candidate as the basis for a new probiotic product due to its nutritional value and the fact that most commercially available probiotics to date are based on dairy products. A maple based probiotic would be an ideal means of delivering probiotics to humans, particularly for those with lactose intolerance or allergies to dairy. Trial probiotic products were developed from maple sap and appear to have good potential for supporting and delivering probiotics.

Reference: 7.Khalf, M.; Dabour, N.; Kheadr, E; Fliss, I. Viability of probiotic bacteria in maple sap products under storage and gastrointestinal conditions. Bioresource Technology. 2010, 101, 7966–7972.

Maple Nutrition and Health Journal Articles and Other Research

Below are abstracts from a variety of scientific journals on maple nutrition and health. Click on the pdf link below each abstract to open the full article.

1. High performance liquid chromatography characterization and identification of antioxidant polyphenols in maple syrup

Reference: Abou-Zaid, M. M.; Nozzolillo, C.; Tonon, A.; Coppens, M.; Lombardo, A. D. A. High performance liquid chromatography characterization and identification of antioxidant polyphenols in maple syrup. Pharm. Biol. 2008, 46, 117-125.

Abstract: Maple syrup of four grades (extra-light, light, medium and dark) of the 2007 crop was provided by three maple producers from St. Joseph's Island, Ontario. Twenty-four phenolic compounds were isolated from a medium grade syrup and identified on the basis of spectral and chemical evidence. They were a) benzoic acid and several hydroxylated and methoxylated derivatives (gallic acid; 1-O-galloyl-β-D-glucose; γ-resorcylic acid); b) cinnamic acid derivatives (p-coumaric acid; 4-methoxycinnamic acid; caffeic acid; ferulic acid; sinapic acid; and the ester chlorogenic acid); c) flavonoids, the flavanols catechin and epicatechin, and the flavonols kaempferol and its 3-O-β-D-glucoside, 3-O-β-D-galactoside, quercetin and its 3-O-β-D-glucoside; 3-O-β-L-rhamnoside and 3-O-rhamnoglucoside (rutin). Traces obtained at 280 and 350 nm in HPLC runs of the ethyl acetate soluble fractions of eight samples indicated the presence of many more phenolic substances, most at very low concentration with some variabilities in peak heights, but not in retention times, among the syrups. In view of the well-established antioxidant activity these substances possess, it is suggested that it is the complexity of the mixture rather than any one compound that may serve to counter the presence of the high concentration of sugars in the syrup.

1. Full article

2. Characterization of the pyrazines formed during the processing of maple syrup

Reference: Akochi-K.; Alli, I.; Kermasha, S. Characterization of the pyrazines formed during the processing of maple syrup. J. Agric. Food Chem. 1997, 45, 3368-3373.

Abstract: Pyrazine formation in maple syrup was investigated during the boiling of maple sap at 105 °C for 220 min. In general terms, there was an induction period, characteristic of the type of pyrazine, associated with the formation of all identified pyrazines. No pyrazine was detected before 60 min of heating at 105 °C; 2,5-dimethyl-and trimethylpyrazine were formed after 60 min of heating, whereas methyl-, 2,6-dimethyl-, ethyl-, 2,3-dimethyl-, and 2-ethyl-3-methylpyrazine were detected after 120 min of heating. The total level of

pyrazines increased from 3.42 ng/g after 60 min of heating to 72.32 ng/g in the final syrup. The formation rate constants (0.04-0.13 ng of pyrazines/min) were determined from the slopes of plots of concentrations versus time of heating. These plots were consistent with pseudo-zero-order reactions. The formation of these pyrazines was influenced by the heating time and by the pH of the boiling sap. The pH values of the sap increased from 7.2 to 9.2 during the first 40 min of boiling, then decreased to 7.3; the decrease in pH values was associated with an increase in the total soluble solids, mainly sugars, from 3% in the sap to 65% in the syrup. Consequently, the levels of sucrose, glucose, and fructose increased from 23.21, 0.09, and 0.09 mg/g, respectively, in the sap to 416.97, 3.25, and 1.82 mg/g in the syrup.

2. Full article

3. The chemical composition of maple syrup

Reference: Ball, D. W. The chemical composition of maple syrup. J. Chem. Educ. 2007, 84, 1647-1650.

Abstract: This article is an introduction to the chemistry of maple sap and syrup: in particular, what makes this sweet liquid maple syrup instead of just a concentrated sugar solution. The types of sugars, the trace ingredients, and the mineral content make maple syrup more than just simple sugar water.

3. Full article

4. Determination of the glycemic index of selected foods (white bread and cereal bars) in healthy persons

Reference: Chlup, R. et al. Determination of the glycemic index of selected foods (white bread and cereal bars) in healthy persons. Biomed. Papers. 2004, 148, 17-25. (not maple syrup)

Abstract: The glycaemic index (GI) is a measure of the food power to raise blood glucose (B-glucose) concentration after a meal. For healthy eating, foods with low GI are recommended.However, for many foods in the European Union the GI has not been defined yet. The aims of this prospective open-label study were: (1) to determine the GI of white bread and juicy cereal bars FIT (Úsovsko, Czech Republic) by means of the glucometer Optium (Abbott/Medisense); (2) to compare the GI of tested foods determined in the morning and in the evening hours; (3) to compare the GI of tested foods in men and women and (4) to assess the variability of the GI. Methods: To determine the GI, measured portions of food containing 50 g of carbohydrates were eaten by 11 healthy volunteers. B-glucose curves were constructed from B-glucose values at time 0, 15, 30, 45, 60, 60, 120 min after the meal. The GI was calculated by dividing the incremental area under the curve (IAUC) for the tested food by that for the standard food (IAUCS). In each volunteer each food was tested 5 times so that 5 GI's was obtained and the average was calculated. The GI for each tested food was calculated as the mean from the respective average GI's of the 11

volunteers. MS Excel and the statistical program SPSS v. 10.1 were used to analyze the data. Results: (1) The mean values of the GI for white bread was 70.3 % and for juicy cereal bars was 101.0 %, as determined in a total of 139 tests in the whole group of 11 volunteers. There was a difference when comparing white bread vs. glucose ($p = 0.012$) and white bread vs. cereal bars ($p = 0.026$) but no difference between glucose and cereal bars. (2) There was no significant difference between the GI determined in the morning and in the evening hours either for the total of 139 tests or for the individual tested foods. (3) No significant difference could be seen between the GI in men and women when comparing glucose, cereal bars and white bread. (4) There was a wide variability of GI in all tested foods: the standard deviation of GI for white bread was 30.7 %, for juicy cereal bars 38.0 %. Conclusions: The GI's for white bread and juicy cereal bars were determined. There was no difference either between the GI values determined in the morning vs. the evening hours or between the values in men vs. women. The results show wide variability. An accurate standard method for the determination of GI needs to be defined, carefully used and re-evaluated to enable a comparison of the results with various methods of other working groups.

4. Full article

5. Molecular models of compounds in maple syrup

Reference: Coleman, W. F. Molecular models of compounds in maple syrup. J. Chem. Educ. 2007, 84, 1650.

Abstract: The same issue of J. Chem. Educ. includes an article by David Ball dealing with the chemical composition of maple syrup. This Featured Molecule for maple syrup is drawn from that paper. The molecules modeled here (2,3-dimethylpyrazine and syringaldehyde) are identified in Table 4 as probable contributors to the taste of maple syrup.

5. Full article

6. Determination of phenolic compound profiles in maple products by high performance liquid chromatography

Reference: Kermasha, S.; Goetghebeur, M.; Dumont, J. Determination of phenolic compound profiles in maple products by high performance liquid chromatography. J. Agric. Food Chem. 1995, 43, 708-716.

Abstract: A high-performance liquid chromatography method, using ultraviolet and electrochemical detectors, was developed for the analyses of phenolic and furfural compounds in maple products. The concentrations of compounds were calculated using external standards that conformed to linear behavior. Most of compounds identified in saps, concentrates, and syrups were related to lignin derivatives. Statistical analyses of data showed that 5-(hydroxymethyl)-2-furaldehyde (HMF) concentrations and phenolic profiles were significantly different as related to harvest time and maple products. Although HMF concentrations were not significantly different as related to the producers,

a highly significant difference was observed for phenolic profiles. An increase in the relative proportion of phenolic acids and a decrease in that of aldehydes and alcohols were observed during the reverse osmosis of maple sap. The thermal evaporation resulted in an increase in the amount of HMF, ferulic acid, vanillin, and syringyl aldehyde with a concomitant drastic decrease in sinapic acid.

6. Full article

7. Viability of probiotic bacteria in maple sap products under storage and gastrointestinal conditions

Reference: Khalf, M.; Dabour, N.; Kheadr, E; Fliss, I. Viability of probiotic bacteria in maple sap products under storage and gastrointestinal conditions. Bioresource Technology. 2010, 101, 7966–7972.

Abstract: This study was undertaken to develop new probiotic products based on liquid maple sap or its concentrate. Sap and concentrate, with or without inulin (2%) were inoculated with Bifidobacterium lactis Bb12 and Lactobacillus rhamnosus GG valio at initial counts of 107-108 CFU/ml. Viability was assessed over four weeks of storage at 4°C and under in vitro simulated gastrointestinal conditions using dynamic gastrointestinal model known as TIM-1. Viability was maintained throughout the storage period at the same order of 107 to 108 CFU/ml. Inulin significantly enhanced the survivability during passage through the gastrointestinal tract simulator. The developed products could be an excellent alternative for delivering probiotics, especially for individuals suffering lactose intolerance to dairy products.

7. Full article

8. Antioxidant activity, inhibition of nitric oxide overproduction, and in vitro antiproliferative effect of maple sap and syrup from Acer saccharum

Reference: Le gault, J.; Girard-Lalancette, K.; Grenon, C.; Dussault, C.; Pichette, A. Antioxidant activity, inhibition of nitric oxide overproduction, and in vitro antiproliferative effect of maple sap and syrup from Acer saccharum. J. Med. Food 2010, 13, 460-468.

Abstract: Antioxidant activity, inhibition of nitric oxide (NO) overproduction, and antiproliferative effect of ethyl acetate extracts of maple sap and syrup from 30 producers were evaluated in regard to the period of harvest in three different regions of Québec, Canada. Oxygen radical absorbance capacity (ORAC) values of maple sap and syrup extracts are, respectively, I2±6 and 15±5 µmol of Trolox equivalents (TE)/mg. The antioxidant activity was also confirmed by a cell-based assay. The period of harvest has no statistically significant incidence on the antioxidant activity of both extracts. The antioxidant activity of pure maple syrup was also determined using the ORAC assay. Results indicate that the ORAC value of pure maple syrup (8±2 µmol of TE/mL) is lower than the ORAC value of blueberry juice (24±1 µmol of TE/mL) but comparable to the ORAC

values of strawberry (10.7±0.4 µmol of TE/mL) and orange (10.8±0.5 µmol of TE/mL) juices. Maple sap and syrup extracts showed to significantly inhibit lipopolysaccharide-induced N0 overproduction in RAW264.7 murine macrophages. Maple syrup extract was significantly more active than maple sap extract, suggesting that the transformation of maple sap into syrup increases NO inhibition activity. The highest NO inhibition induced by the maple syrup extracts was observed at the end of the season. Moreover, darker maple syrup was found to be more active than clear maple syrup, suggesting that some colored oxidized compounds could be responsible in part for the activity. Finally, maple syrup extracts (50% inhibitory concentration=42±6 µg/mL) and pure maple syrup possess a selective in vitro antiproliferative activity against cancer cells.

8. Full article

9. Maple syrup phytochemicals include lignans, coumarins, a stilbene, and other previously unreported antioxidant phenolic compounds

Reference: Li, L.; Seeram N. Maple syrup phytochemicals include lignans, coumarins, a stilbene, and other previously unreported antioxidant phenolic compounds. J. Agric. Food Chem. 2010, 58, 11673-11679.

Abstract: Twenty-three phenolic compounds were isolated from a butanol extract of Canadian maple syrup (MS-BuOH) using chromatographic methods. The compounds were identified from their nuclear magnetic resonance and mass spectral data as 7 lignans [lyoniresinol (1), secoisolariciresinol (2), dehydroconiferyl alcohol (3), 50-methoxy-dehydroconiferyl alcohol (4), erythro-guaiacylglycerol-ß-O-40-coniferyl alcohol (5), erythro-guaiacylglycerol-ß-O-40-dihydroconiferyl alcohol (6), and [3-[4-[(6-deoxy-R-L-mannopyranosyl)oxy] 3-methoxyphenyl]methyl]-5-(3,4-dimethoxyphenyl)dihydro-3-hydroxy-4-(hydroxymethyl)-2(3H)-furanone (7)], 2 coumarins [scopoletin (8) and fraxetin (9)], a stilbene [(E)-3,30-dimethoxy-4,40-dihydroxystilbene (10)], and 13 phenolic derivatives [2-hydroxy-30,40-dihydroxyacetophenone (11),1-(2,3,4-trihydroxy-5-methylphenyl)ethanone (12), 2,4,5-trihydroxyacetophenone (13), catechaldehyde (14), vanillin (15), syringaldehyde (16), gallic acid (17), trimethyl gallic acid methyl ester (18), syringic acid (19), syringenin (20), (E)-coniferol (21), C-veratroylglycol (22), and catechol (23)]. The antioxidant activities of MS-BuOH (IC50 > 1000 µg/mL), pure compounds, vitamin C (IC50 =58 µM), and a synthetic commercial antioxidant, butylated hydroxytoluene (IC50 = 2651 µM), were evaluated in the diphenylpicrylhydrazyl (DPPH) radical scavenging assay. Among the isolates, the phenolic derivatives and coumarins showed superior antioxidant activity (IC50 < 100 µM) compared to the lignans and stilbene (IC50 >100 µM). Also, this is the first report of 16 of these 23 phenolics, that is, compounds 1, 2, 4-14, 18, 20,and 22, in maple syrup.

9. Full article

10. Quebecol, a novel phenolic compound isolated from Canadian maple syrup

Reference: Li, L. and N. Seeram.Quebecol, a novel phenolic compound isolated from Canadian maple syrup Quebecol, a novel phenolic compound isolated from Canadian maple syrup. Journal of Functional Foods. March 2011.

Abstract: The province of Quebec in Canada leads the world's production of maple syrup, a natural sweetener obtained by thermal evaporation of sap collected from maple (Acer) species. As part of our laboratory's detailed chemical investigation of Canadian maple syrup, a novel phenolic compound, 2,3,3-tri-(3-methoxy-4-hydroxyphenyl)-1-propanol, assigned the common name of quebecol, was obtained. Quebecol was isolated using a combination of chromatographic methods and identified by detailed 1D and 2D nuclear magnetic resonance (NMR) and mass spectral (MS) analyses. Liquid chromatography mass spectral (LC-MS) analyses revealed that quebecol is not originally present in maple sap. This observation, as well as the lack of a feasible biosynthetic pathway to explain its origin, suggests that quebecol is formed during the processing and/or extraction of maple syrup. Thus, the identification and biological evaluation of non-natural, process-derived compounds in maple syrup are warranted since such molecules may contribute towards the biological activities reported for this natural sweetener.

10. Full article

11. Antioxidant, antiradical and antimutagenic activities of phenolic compounds present in maple products

Reference: Theirault, M.; Caillet, S.; Kermasha, S.; Lacroix, M. Antioxidant, antiradical and antimutagenic activities of phenolic compounds present in maple products. Food Chem. 2006, 98, 490-501.

Abstract: The phenolic compounds in maple sap and syrup were extracted at different periods of the season and were separated to collect the glycosylated compounds and the aglycone compounds. The antioxidant and antiradical activities of each phenolic compound were studied using the thiobarbituric acid reactive substances (TBARS) assay and the N,N-diethyl-p-phenylenediamine (DPD) decoloration test to measure the free radical scavenging. The results showed that in general the phenolic compounds had a good antioxidant and antiradical properties. The glycosylated compounds from maple sap and maple syrup showed a better activity than the aglycones. The antimutagenic effects of each phenolic compounds from maple sap and syrup were also investigated as the inhibition of SOS induction by chemical agents in Salmonella typhimurium TA1535/pSK1002 containing the fusion gene umuC-lacZ.

Induction of the SOS gene (umuC) expression was assayed by measuring accumulated b-galactosidase activity using a modified Umu test. The antimutagenic properties were studied per se and after metabolisation by S9 fraction. The results showed that an optimum of antimutagenic properties of the glycosylated metabolites phenolic compounds

from sap and syrup was observed at 75% of the season for the sap and at 25% of the season for the syrup. A higher antimutagenic activity was observed at 25% and 100% of the season for aglycones present in syrup and at 75% of the season for aglycones present in sap.

11. Full article

12. Aromatic compounds and their antioxidant activity from Acer saccaharum

Reference: Yoshikawa, K.; Kawahara, Y.; Arihara, S.; Hashimoto, T. Aromatic compounds and their antioxidant activity from Acer saccaharum. J. Nat. Med. 2010, in press (published online Aug 5, 2010, DOI 10.1007/s11418-010-0450-5).

Abstract: A new lignan glycoside, 5-(300,400-dimethoxyphenyl)-3-hydroxy-3-(40-hydroxy-30-methoxybenzyl)-4-hydroxymethyl-dihydrofuran-2-one 40-O-a-L-rhamnopyranoside (1), with seven known compounds, compound 2, koaburside, icariside E4, cleomiscosin C, cleomiscosin D, scopoletin, and 50-demethylaquillochin, were isolated from the EtOH extract of the wood of Acer saccharum (Aceraceae). Their structures were determined by 1D and 2D nuclear magnetic resonance (NMR) and mass spectroscopy analysis. All of the isolated compounds, 1–8, were tested for their antioxidant activity in superoxide dismutase (SOD)-like assay.

12. Full article

13. Total antioxidant content of alternatives to refined sugar

Reference: Phillips, K.; Carlsen, M.; Blomhoff, R. Total antioxidant content of alternatives to refined sugar. J. Amer. Diet. Assoc. 2009, Jan. 109(1):64-71.

Abstract: Background - Oxidative damage is implicated in the etiology of cancer, cardiovascular disease, and other degenerative disorders. Recent nutritional research has focused on the antioxidant potential of foods, while current dietary recommendations are to increase the intake of antioxidant rich foods rather than supplement specific nutrients.

Many alternatives to refined sugar are available, including raw cane sugar, plant saps/syrups (eg, maple syrup, agave nectar), molasses, honey, and fruit sugars (eg, date sugar). Unrefined sweeteners were hypothesized to contain higher levels of antioxidants, similar to the contrast between whole and refined grain products.

Objective - To compare the total antioxidant content of natural sweeteners as alternatives to refined sugar.

Design - The ferric-reducing ability of plasma (FRAP) assay was used to estimate total antioxidant capacity. Major brands of 12 types of sweeteners as well as refined white sugar and corn syrup were sampled from retail outlets in the United States.

Results - Substantial differences in total antioxidant content of different sweeteners were found. Refined sugar, corn syrup, and agave nectar contained minimal antioxidant

activity (0.01 mmol FRAP/100 g); raw cane sugar had a higher FRAP (0.1 mmol/100 g). Dark and blackstrap molasses had the highest FRAP (4.6 to 4.9 mmol/100 g), while maple syrup, brown sugar, and honey showed intermediate antioxidant capacity (0.2 to 0.7 mmol FRAP/100 g). Based on an average intake of 130 g/day refined sugars and the antioxidant activity measured in typical diets, substituting alternative sweeteners could increase antioxidant intake an average of 2.6 mmol/day, similar to the amount found in a serving of berries or nuts.

Conclusion - Many readily available alternatives to refined sugar offer the potential benefit of antioxidant activity.

13. Full article

14. The Chemical Composition of 80 Pure Maple Syrup Samples Produced in North America

Reference: Stuckel, J. G., and N. Low. The Chemical Composition of 80 Pure Maple Syrup Samples Produced in North America. Food Research International. 1996, 29(3-4), 373-379.

Abstract: A total of 80 pure maple syrup samples received from primary producers in Canada and the United States were analyzed for their chemical composition, pH and °Brix. The major carbohydrates found in maple syrup (sucrose, glucose and fructose) were determined employing anion exchange high performance liquid chromatography (HPLC) with pulsed amperometric detection. The sucrose content was found to range from 51.7 to 75.6%; glucose and fructose contents ranged from 0.00 to 9.59% and 0.00 to 3.95%, respectively. The major organic acid present in maple syrup was malic acid. Trace amounts of citric, succinic and fumaric acid were also present. All organic acids were determined by ion exchange HPLC analysis with UV detection at 210 nm. Malic acid levels ranged from 0.1 to 0.7%. Citric, succinic and fumaric acids were found to be present at levels less than 0.06 ppm. Inductively coupled plasma atomic emission spectroscopy was employed for the analysis of potassium, magnesium and calcium, the main minerals found in maple syrup. Potassium was found to be present in the greatest concentration ranging from 1005 to 2990 mg/L. Magnesium and calcium ranged from 10 to 380 mg/L and 266 to 1702 mg/L, respectively. The Karl Fischer titration method was employed to determine maple syrup moisture content. The moisture content of maple syrup ranged from 26.5 to 39.4%. The pH and °Brix values for maple syrup ranged from 5.6 to 7.9, and 62.2 to 74.0°, respectively.

14. Full article

15. Maple Sap and Syrup are a Rich Source of Abscisic Acid and Polyphenols with Potential Benefits To Health – Presentation by Yves Desjardins, Laval University, Québec, Canada given at the International Horticulture Conference, Lisbon, Portugal, August 2010

Abstract: For centuries maple sap and syrup have been a staple of North-American native people and are consumed now-a-days throughout the world as edulcoration produce and natural sweeteners, appreciated for their quality and delicate taste. Maple sap is collected in the Spring when freeze/thaw cycles cause the sweet sap to rise in the tree and flow from especially made taps in the trunk for collection. The sap is boiled to concentrate the sugar and forms a rich 66°Brix syrup. Apart from sugar, the natural sap contains minerals, oligosaccharides, some proteins, polyphenols and phytohormones. We hereby present original results on the content of maple sap and syrup in phytohormones and especially in abscisic acid (ABA), in ABA-conjugates and its metabolites. We show that this sesquiterpene can be traced in large concentration in both the sap and the syrup. The metabolites thus resist heat and technological process leading to the consumable produce. Moreover, the largest form of sesquiterpene in the sap and syrup were phaseic acid and dihydrophaseic acid, accounting for almost 90% of this class of molecules while ABA and its 7'-OH form accounted for close to 10% of this terpenoid in the sap and syrup. Recently ABA and their metabolites have been suggested to act as autocrine cytokine molecules in human granulocytes and were shown to stimulate the release of insulin by pancreatic Langherans Islets (Guri et al. 2007, Clin. Nutr. 26:107-116). The high titer of ABA in maple products may explain why they are better tolerated by those suffering from diabetes and metabolic disorders than those consuming other sources of sugars.

15. Full article

Maple Syrup Nutritional and Health Benefits: Fact Sheets and Other Information

BOOKS

North American Maple Syrup Producers Manual

Koelling, M. R., R. B. Heiligmann, and T. D. Perkins. 2006. **North American Maple Syrup Producers Manual**, 2nd ed. Ohio State University Extension Bulletin 856. 329 pp.

FACT SHEETS AND INFORMATION

Click on the pdf symbol to open the fact sheet listed.

A. **Glycemic Index Fact Sheet** – Quebec Federation of Maple Syrup Producers

B. **Antioxidant Value Fact Sheet** – Quebec Federation of Maple Syrup Producers

C. **Nutritional Information of Maple Syrup Fact Sheet** – Quebec Federation of Maple Syrup Producers

D. **Press Release of Abscisic Acid Research** (Yves Desjardins) – Quebec Federation of Maple Syrup Producers

E. **Complete Press Kit of Maple Information** – Quebec Federation of Maple Syrup Producers

F. **Definition of maple syrup** – FDA (United States Food and Drug Administration)

G. **Charts comparing nutritional value and antioxidant value of maple syrup to other sweeteners and foods**

H. **Maple from Tree to Table Culinary Education Guide**
www.domorewithmaple.com/

I. **Quebec Maple, a Natural, Nutritional Ingredient – A Culinary Curriculum**
developed by Daniel LaGarde, CEC, Executive Chef, Do More With Maple!
www.domorewithmaple.com/

J. **Canadian Nutrient File – Maple Syrup, bulk**

Canadian Nutrient File – Maple Syrup, prepackaged
www.hc-sc.gc.ca/fn-an/nutrition/fiche-nutri-data/index-eng.php

K. **USDA National Nutrient Database – Maple Syrup**
www.nal.usda.gov/fnic/foodcomp/search/

Maple Syrup Nutrition and Health – Websites

1. The World's Healthiest Foods
www.whfoods.com/genpage.php?tname=foodspice&dbid=115
The George Mateljan Foundation for the World's Healthiest Foods was established by George Mateljan to discover, develop and share scientifically proven information about the benefits of healthy eating, and to provide the personalized support individuals need to make eating The World's Healthiest Foods enjoyable, easy, quick and affordable. Outlines, history, description, storage, etc. in addition to a thorough description of the health benefits of maple syrup. (incorrect sugar content stated at 60%)

2. Canadian Organic Maple Syrup – Health Benefits
www.canadianorganicmaple.com/health_benefits.cfm
Presents maple syrup health benefits material from "The World's Healthiest Foods" website.

3. Benefits of maple syrup – Nature Cure Methods
naturecure.ygoy.com/2010/07/19/benefits-of-maple-syrup/
Presents maple syrup health benefits material from "The World's Healthiest Foods" website.

4. Health Benefits of Maple Syrup – Suite 101
www.suite101.com/content/health-benefits-of-maple-syrup-a189781
Article on health benefits and nutrition of maple syrup, drawing information from a number of other websites, including whfoods.com.

5. URI pharmacy researcher finds beneficial compounds in pure maple syrup – University of Rhode Island
www.uri.edu/news/releases/?id=5256
March 22, 2010 - University of Rhode Island researcher Navindra Seeram, who specializes in medicinal plant research, has found more than 20 compounds in maple syrup from Canada that have been linked to human health, 13 of which are newly discovered in maple syrup. In addition, eight of the compounds have been found in the Acer (maple) family for the first time. (research published in J. Agric. Food Chem. 2010, 58, 11673-11679)

6. Health Check – Maple Syrup Benefits – Turn to 10 NBC
www2.turnto10.com/lifestyles/2010/apr/08/health_check_maple_syrup_benefits-ar-108979/
Video from a news report from a local NBC television station reporting on findings from research conducted by N. Seeram at the University of Rhode Island.

7. Health News – Maple syrup reduces cancer, diabetes risk
Published: March 26, 2010
www.upi.com/Health_News/2010/03/26/Maple-syrup-reduces-cancer-diabetes-risk/UPI-11371269647350/
Discusses antioxidant related research results from projects conducted by N. Seeram (University of Rhode Island, published in J. Agric. Food Chem. 2010, 58, 11673-11679) and J. Legault, et al. (Quebec, published in J. Med. Food 2010, 13, 460-468.)

8. Here's why maple syrup is very good for your health – Healthzone.ca
www.healthzone.ca/health/dietfitness/diet/article/783558--here-s-why-maple-syrup-is-very-good-for-your-health
Discusses research results from N. Seeram (University of Rhode Island, J. Agric. Food Chem. 2010, 58, 11673-11679) with some additional commentary from the Federation of Quebec Maple Syrup Producers.

9. The secret to prevent diabetes with maple syrup – Spotlight.vitals.com
spotlight.vitals.com/2010/04/the-secret-to-prevent-diabetes-with-maple-syrup/
Discusses antioxidant related research results from projects conducted by N. Seeram (University of Rhode Island, published in J. Agric. Food Chem. 2010, 58, 11673-11679) and J. Legault, et al. (Quebec, published in J. Med. Food 2010, 13, 460-468.)

10. Research reveals maple syrup and maple water contain abscisic acid – News Medical
www.news-medical.net/news/20100305/Research-reveals-maple-syrup-and-maple-water-contain-abscisic-acid.aspx
Article posted March 5, 2010. Discusses research that has found maple syrup to contain beneficial compounds, most notably abscisic acid, from a project conducted by Yves Desjardins (Quebec, presented at 28th International Horticultural Congress in Portugal) and also mentions the health benefits of maple syrup supported by research conducted by N. Seeram (University of Rhode Island, published in J. Agric. Food Chem. 2010, 58, 11673-11679). Reference: Federation of Quebec Maple Syrup Producers

11. Pure Canadian Maple Syrup – Quebec Federation of Maple Syrup Producers
www.purecanadamaple.com
www.siropderable.ca
Extensive information about maple syrup nutrition and health benefits. Why and how maple syrup should be used as a sugar alternative. The website also has good information on a media room page, recipes, and other maple information. Includes a page of links to recent coverage of maple syrup in the media.

12. Nutritional value comparison - Citadelle
www.citadelle-camp.coop/maple-syrup/All-about-Maple/Nutritional-Value.aspx
Nutritional value chart comparing energy, fat, sodium, carbohydrate, sugar, protein, potassium, calcium, magnesium, phenolic compounds, and glycemic absorption of various sweeteners.

13. Nutrition facts and analysis for syrups, maple – SELFNutritionData know what you eat
nutritiondata.self.com/facts/sweets/5602/2
Nutrition facts and information for maple syrup, in chart and graph format. Four choices of serving sizes, and corresponding mineral, vitamin, etc. content, including % DV. (i.e. the fatty acid chart shows 20 mg of omega-6 fatty acids per tablespoon of maple syrup)

14. Maple syrup – curezone.com
curezone.com/foods/maple_syrup.asp
Discusses pure maple syrup and how it is composed of balanced sugars, minerals, vitamins and amino acids which make it unique from other sweeteners. Warns consumers about the use of paraformaldehyde tablets and lead in maple syrup.

15. Maple Syrup Calories and Nutrition Facts – PEERtrainer
www.peertrainer.com/DFcaloriecounterB.aspx?id=6880
Information appears to be copied from curezone.com.

16. Nutritional facts – New Brunswick Maple Association
maple.infor.ca/nutritional_facts
Presents nutritional information, including vitamins, amino acids, antioxidants, sugars, and minerals. Charts comparing nutritional value of maple syrup to other sweeteners and nutrients and antioxidant value of maple syrup to other foods.

17. Nutritional facts pure maple syrup vs. white sugar – livestrong.com
www.livestrong.com/article/326813-nutritional-facts-pure-maple-syrup-vs-white-sugar/
Compares minerals and nutrition of maple syrup and white sugar.

18. Pure maple syrup nutrition – livestrong.com
www.livestrong.com/article/270564-pure-maple-syrup-nutrition/
Discusses the nutritional value of maple syrup, including macronutrients, vitamins, minerals, and other benefits.

19. Maple syrup nutritional facts – maple syrup source
www.maplesyrupsource.com/maple_syrup_nutritional_information.php
General nutritional facts for maple syrup, including detailed nutritional information chart.

20. Maple Syrup Education – Nutritional Value – Michigan Maple Syrup Association
www.mi-maplesyrup.com/education/syrupeducation.htm
Provides information for the chemical composition of maple syrup with a chart and short summary of the different components. Reference: M. F. Morselli 1975. Nutritional Value of Maple Syrup. National Maple Syrup Digest 14(2):12. Revised by Henry J. Marckres (2003)

21. Nutritional Information for Pure Maple Syrup – MapleSource.com – Bascom Family Farms
www.maplesource.com/Info_Center/syrup/nutritional.php
Brief discussion of nutritional information of pure maple syrup – sugars, minerals, nutrition facts.

22. The master cleanse diet
www.themastercleansediet.org/
themastercleanse.org/
The Master Cleanse diet (also known as the lemonade diet or the maple syrup diet) is a diet consisting of fresh lemon juice, pure maple syrup, cayenne pepper and water. The master cleanse is used for detoxification and weight loss. It was created in 1946 by Stanley Burroughs as a healthy and natural means for detoxification, to flush the body of toxins, pesticides and other impurities.

23. Are syrups a sulfite health issue? – Associated content
www.associatedcontent.com/article/1072607/are_syrups_a_sulfite_health_issue.html?cat=5
Discusses sulfites in syrups. In the maple syrup section, it states: "Maple syrup has its own sulfite health issue due to its naturally occurring sulfites. However, the good news here is that sulfite levels are so low that most sulfite sensitive people can handle maple syrup in moderate amounts."

24. Nutritional Information – Massachusetts Maple Producers Association
www.massmaple.org/nutrition.php
General nutritional information and chart of nutritional value.

25. Maple Syrup Health Benefits – Maple Syrup World
www.maplesyrupworld.com/pages/Maple-Syrup-Health-Benefits.html
Brief statements on nutritional value of maple syrup and information on anti-diabetic and anti-cancer properties of maple syrup from J. Med. Food 2010, 13, 460-468.

26. Omega-6 fatty acids – University of Maryland Medical Center
www.umm.edu/altmed/articles/omega-6-000317.htm
Good information on omega-6 fatty acids, what they are and why they are beneficial to human health. (maple syrup is not discussed in this article but does contain omega-6 fatty acids – 20 mg/tbsp listed on nutritiondata.com)

27. Antioxidant – Wikipedia
en.wikipedia.org/wiki/Antioxidant
Information on antioxidants in general.

28. Antioxidants Topic Overview – WebMD
www.webmd.com/food-recipes/tc/antioxidants-topic-overview
Information on antioxidants in general.

29. Maple Syrup Facts – Do More With Maple! (Quebec Delegation Chicago)
www.domorewithmaple.com/maplesyrupfacts.html
This website provides information about maple syrup, including an extensive culinary curriculum for using maple syrup with nutrition information and health benefits. The purpose of this site is to educate people and encourage more widespread use of maple syrup. (This culinary curriculum for maple products is being sponsored by the following organizations; the Federation of Québec Maple Syrup Producers, Decacer (Equinox Maple Flakes), Citadelle Maple Producers Cooperative, Heritage Yamaska (La Coulée d'Abbotsford) and Lapierre Maple Farm.)

30. Canadian scientist slams maple syrup study touting health benefits – POSTMEDIA NEWS

www.canada.com/health/Canadian+scientist+slams+maple+syrup+study+touting+health+benefits/4545863/story.html
Article discussing a McGill University scientist's viewpoint on the findings of the research conducted by N. Seeram from the University of Rhode Island. His viewpoint is that it is irresponsible to promote maple syrup as a health food because of its high sugar content and that the study amounts to an advertisement for maple syrup.

31. 54 Beneficial Compounds Discovered in Pure Maple Syrup – Science Daily March 30, 2011
www.sciencedaily.com/releases/2011/03/110330131316.htm
Updated information on research conducted by N. Seeram at the University of Rhode Island. An additional 34 beneficial compounds were found in maple syrup in addition to the 20 found previously. This includes the discovery of a new phenolic compound that has been named Quebecol.

32. Maple syrup could help fight cancer, diabetes industry-funded study – POSTMEDIA NEWS
www.canada.com/health/Maple+syrup+could+help+fight+cancer+diabetes+industry+funded+study/4542296/story.html
Discussing the research conducted by N. Seeram at the University of Rhode Island on the beneficial compounds of maple syrup, particularly the implications in relation to diabetes.

33. Maple Syrup Has 54 Beneficial Compounds – And a Whole Lot of Sugar – Treehugger.com
www.treehugger.com/files/2011/04/maple-syrup-54-beneficial-compounds.php?campaign=th_rss&utm_source=feedburner&utm_medium=feed&utm_campaign=Feed3A+treehuggersite+%28Treehugger%29
Article discussing the health of maple syrup based on the results of the research in Rhode Island conducted by N. Seeram, professing the benefits and Schwarcz from McGill, refuting the value of maple syrup as a health food because of the high sugar content, with excerpts from Seeram and Schwarcz.

34. Maple Syrup May be a Healthy Sugar Alternative – Personal Liberty Digest, April 5, 2011
www.personalliberty.com/news/maple-syrup-may-be-a-healthy-sugar-alternative-800476704/
Nice concise little article on the health benefits implicated in research conducted by Seeram at the University of Rhode Island.

35. Could Maple Syrup be the Next Superfood? – International Society for Horticultural Science
www.ishs.org/news/?p=1588
Very good article outlining the potential health benefits of maple syrup based on the research conducted by Seeram at the University of Rhode Island and Desjardins at Laval University.

The following pages contain the linked posters, rackcards, journals and info sheets. For easier navigation and explanantion, please click the appropriate link to the left: IMSI Poster and Rackcard, Journal Articles, and Fact Sheets and Other Information, and navigate to the articles provided from those source pages.

Please note that printready files for the poster and rackcards, English and French, are provided in a separate folder on this CD, as are the individual Journal Articles and Info Sheets.

PURE Maple Syrup

Natural and Nutritious

Pure Maple Syrup is a natural, nutritious and delicious sweetener and a smart choice as a sweet topping or as a flavorful ingredient in baking and cooking. ***Maple Syrup*** has a delightful and flavorful maple bouquet and has varied taste intensities to suit different consumer preferences.

Unlike many syrups and sugars ***Maple Syrup*** is 100 percent natural and unrefined, retaining the inherent nutritional value of the sap obtained from the maple tree.

Important Nutrient Source

Pure Maple Syrup is a valuable source of mineral nutrients. ***Maple Syrup*** delivers more nutrition than all other common sweeteners and has one of the lowest calorie levels. ***Maple Syrup*** contains mineral nutrients and vitamins which are an essential part of the daily diet in higher levels than other sweeteners.

Nutritional Value for Various Sweeteners

% of Recommended Daily Value (DV) Per ¼ cup (60 ml)

	Maple Syrup		High Fructose Corn Syrup		Honey		Brown Sugar		White Sugar	
	(1/4 cup / 80 g)		(1/4 cup / 78 g)		(1/4 cup / 85 g)		(1/4 cup / 55 g)		(1/4 cup / 51 g)	
	% DV	**mg**	% DV	mg	% DV	mg	% DV	mg	% DV	mg
Riboflavin	**37**	**0.59**	1	0.01	2	0.03	0	0.0	1	0.01
Thiamin	**1**	**0.01**	0	0.0	0	0.0	0	0.0	0	0.0
Manganese	**95**	**1.89**	4	0.07	4	0.07	2	0.04	0	0.0
Zinc	**6**	**0.58**	0	0.02	2	0.19	0	0.02	0	0.0
Magnesium	**7**	**16.5**	0	0.0	1	1.75	2	5.0	0	0.0
Calcium	**5**	**58.0**	0	0.0	0	5.0	4	45.8	0	0.48
Iron	**1**	**0.09**	0	0.02	3	0.36	3	0.39	0	0.03
Selenium	**1**	**0.4 µg**	1	0.55 µg	1	0.66 µg	1	0.65 µg	1	0.3
Potassium	**5**	**167**	0	0.0	1	44.0	2	73.3	0	0.96
Calories	**216**		220		261		216		196	

Source: USDA Nutrient Database and Canadian Nutrient File

Notes: The values shown are the overall minimum values for the minerals and nutrients and the overall maximum values for the calories reported by the USDA Nutrient Database and the Canadian Nutrient File. The percent daily values (% DV) were calculated using the Health Canada recommended daily intake values for an average 2,000 calorie diet.

The Original Sweetener

Native North Americans were the first to recognize ***Pure Maple Syrup*** as a source of nutrition and energy. Researchers have since documented that maple syrup has a higher nutritional value than all other common sweeteners

Other Health Considerations

In addition to its remarkable nutritional content, researchers have documented that ***Maple Syrup*** contains numerous phenolic compounds, commonly found in plants and in agricultural products such as berries, tea, red wine and flax seed. Some of these compounds may benefit human health in significant ways. For example, researchers have documented the natural presence of abscisic acid (ABA) in ***Maple Syrup***, a compound thought to stimulate insulin release by the pancreas.

Use of ***Pure Maple Syrup*** as an alternative to refined sugar can also add to the antioxidant content of the diet, similiar to replacing refined grains with whole grains.

With its wholesome, natural flavour ***Pure Maple Syrup*** has one of the lowest calorie levels of common sweeteners.

Maple Syrup is also a natural product with no additives or preservatives.

Choose *Pure Maple Syrup*, a natural sweetener and a smart food choice.

www.internationalmaplesyrupinstitute.com

Sirop d'érable PUR

Naturel et nutritif

Le ***sirop d'érable*** pur est un édulcorant naturel, nutritif et délicieux et un bon choix de garniture sucrée ou d'ingrédient goûteux pour la cuisine ou la cuisson au four. Le ***sirop d'érable*** a un goût d'érable enchanteur et goûteux et a diverses intensités de goût pour répondre aux différentes préférences des consommateurs. Par opposition à plusieurs sirops et sucres, le ***sirop d'érable*** est 100 pour-cent naturel et non raffiné; il retient la valeur nutritive de la sève qui provient des érables.

Source d'éléments nutritifs importants

Le ***sirop d'érable*** est une précieuse source de minéraux nutritifs. Le ***sirop d'érable*** offre plus de nutriments que tous les autres édulcorants communs et a l'une des valeurs calorifiques les plus basses parmi ceux-ci. Le ***sirop d'érable*** contient des minéraux nutritifs et des vitamines qui sont une partie essentielle de l'alimentation quotidienne dans des niveaux plus élevés que les autres édulcorants.

Valeurs nutritives de divers édulcorants

% de la valeur quotidienne (VQ) recommandée par ¼ de tasse (60 ml)

	Sirop d'érable		Sirop de maïs		Miel		Cassonade		Sucre blanc	
	(1/4 de tasse / 80 g)		(1/4 de tasse / 78 g)		(1/4 de tasse / 85 g)		(1/4 de tasse / 55 g)		(1/4 de tasse / 51 g)	
	% VQ	**mg**	% VQ	mg	% VQ	mg	% VQ	mg	% VQ	mg
Riboflavine	**37**	**0.59**	1	0.01	2	0.03	0	0.0	1	0.01
Thiamine	**1**	**0.01**	0	0.0	0	0.0	0	0.0	0	0.0
Manganèse	**95**	**1.89**	4	0.07	4	0.07	2	0.04	0	0.0
Zinc	**6**	**0.58**	0	0.02	2	0.19	0	0.02	0	0.0
Magnésium	**7**	**16.5**	0	0.0	1	1.75	2	5.0	0	0.0
Calcium	**5**	**58.0**	0	0.0	0	5.0	4	45.8	0	0.48
Fer	**1**	**0.09**	0	0.02	3	0.36	3	0.39	0	0.03
Sélénium	**1**	**0.4 µg**	1	0.55 µg	1	0.66 µg	1	0.65 µg	1	0.3
Potassium	**5**	**167**	0	0.0	1	44.0	2	73.3	0	0.96
Calories	**216**		220		261		216		196	

Source : Fichier canadien sur les éléments nutritifs et *USDA Nutrient Database*

Note : Les valeurs qui figurent ici sont les valeurs minimales globales des minéraux et éléments nutritifs et les valeurs maximales globales pour les calories présentées par l'*USDA Nutrient Database* et le Fichier canadien sur les éléments nutritifs. Les pourcentages de valeur quotidienne (% VQ) ont été calculés en se servant des valeurs d'apport quotidien recommandée de Santé Canada pour un régime d'environ 2 000 calories.

L'édulcorant d'origine

Les Amérindiens ont été les premiers à reconnaître le ***sirop d'érable pur*** comme source d'éléments nutritifs et d'énergie. Depuis, les chercheurs ont démontré que le ***sirop d'érable pur*** a une valeur nutritive plus élevée que tous les autres édulcorants communs.

Autres facteurs liés à la santé

En plus de son remarquable contenu nutritionnel, les chercheurs ont trouvé que le ***sirop d'érable*** contient de nombreux composés phénoliques, communément trouvés dans des plantes et des produits agricoles comme les baies, le thé, le vin rouge et les graines de lin. Certains de ces composés peuvent améliorer la santé humaine de façon significative. Par exemple, des chercheurs ont documenté la présence d'acide abscissique dans le ***sirop d'érable***, un composé qu'on croît pouvoir stimuler la libération d'insuline du pancréas.

L'utilisation du ***sirop d'érable pur*** au lieu du sucre raffiné peut aussi augmenter la teneur d'antioxydants dans le régime, dans le même ordre d'idées que consommer des grains entiers au lieu de grains raffinés.

Avec son goût authentique et naturel, ***sirop d'érable pur*** a l'une des valeurs calorifiques les plus basses des édulcorants communs.

Le ***sirop d'érable*** est aussi un produit naturel et sans additif ou agent de préservation.

Choisissez *le sirop d'érable pur*, un édulcorant naturel et un bon choix alimentaire.

www.internationalmaplesyrupinstitute.com

PURE Maple Syrup

Natural and Nutritious

Pure Maple Syrup is a natural and nutritious sweetener and a smart choice as a sweet topping or as a flavorful ingredient in baking and cooking.

Maple Syrup is 100 percent natural and unrefined, retaining the inherent nutritional value of the sap obtained from the maple tree.

Important Nutrient Source

Maple Syrup is a very good source of mineral nutrients and vitamins

Nutritional Value for Various Sweeteners

% of Recommended Daily Value (DV) Per ¼ cup (60 ml)

	Maple Syrup	Corn Syrup	Honey	Brown Sugar	White Sugar
Manganese	**95**	0	4	2	0
Riboflavin	**37**	1	2	0	1
Zinc	**6**	0	2	0	0
Magnesium	**7**	0	1	2	0
Calcium	**5**	0	0	4	0
Potassium	**5**	0	1	1	0
Calories	**216**	220	261	216	196

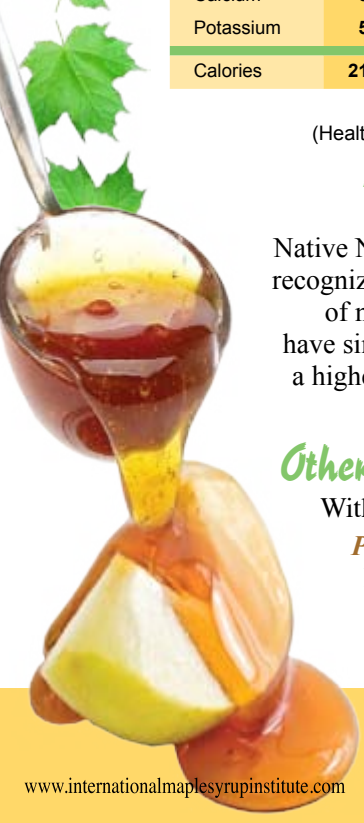
SOURCE: Canadian Nutrient File (Health Canada) and USDA Nutrient Database

The Original Sweetener

Native North Americans were the first to recognize ***Pure Maple Syrup*** as a source of nutrition and energy. Researchers have since shown that ***Maple Syrup*** has a higher nutritional value than all other common sweeteners.

Other Health Considerations

With its wholesome, natural flavour, ***Pure Maple Syrup*** has one of the lowest calorie levels of common sweeteners. It is also all natural with no additives.



Choose *Pure Maple Syrup*, a natural sweetener and a smart food choice.

www.internationalmaplesyrupinstitute.com

Sirop d'érable PUR

Naturel et nutritif

Le *sirop d'érable* pur est un édulcorant naturel et nutritif et un bon choix de garniture sucrée ou d'ingrédient goûteux pour la cuisine ou la cuisson au four.

Le *sirop d'érable* est 100 pour-cent naturel et non raffiné; il retient la valeur nutritive de la sève qui provient des érables.

Source d'éléments nutritifs importants

Le *sirop d'érable* est une très bonne source de minéraux nutritifs et de vitamines.

Valeurs nutritives de divers édulcorants

% de la valeur quotidienne (VQ) recommandée par ¼ de tasse (60 ml)

	Sirop d'érable	Sirop de maïs	Miel	Cassonade	Sucre blanc
Manganèse	**95**	0	4	2	0
Riboflavine	**37**	1	2	0	1
Zinc	**6**	0	2	0	0
Magnésium	**7**	0	1	2	0
Calcium	**5**	0	0	4	0
Potassium	**5**	0	1	1	0
Calories	**216**	220	261	216	196

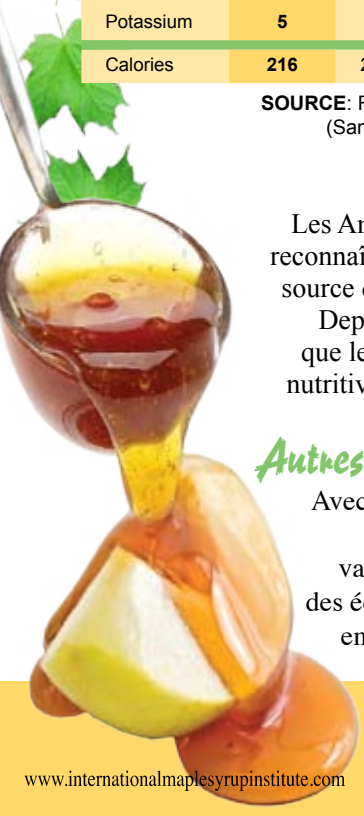
SOURCE: Fichier canadien sur les éléments nutritifs (Santé Canada) et l'*USDA Nutrient Database*

L'édulcorant d'origine

Les Amérindiens ont été les premiers à reconnaître le *sirop d'érable pur* comme source d'éléments nutritifs et d'énergie. Depuis, les chercheurs ont démontré que le *sirop d'érable pur* a une valeur nutritive plus élevée que tous les autres édulcorants communs.

Autres facteurs liés à la santé

Avec son goût authentique et naturel, le *sirop d'érable pur* a l'une des valeurs calorifiques les plus basses des édulcorants communs. Il est aussi entièrement naturel et sans additif.



Choisissez le *sirop d'érable pur*, un édulcorant naturel et un bon choix alimentaire.

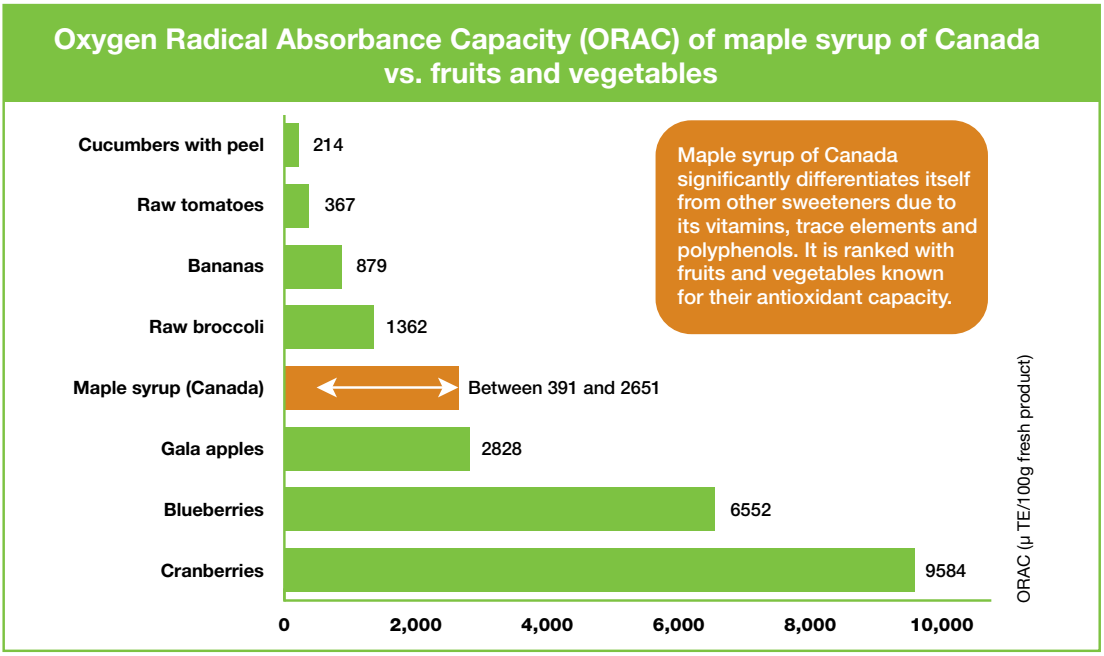
www.internationalmaplesyrupinstitute.com

Promotional Spec Sheet: Antioxidant capacity for maple syrup of Canada

Summary

- Maple syrup of Canada contains active antioxidant elements: polyphenols, trace elements, and vitamins.
- Its antioxidant activity is comparable to that of Gala red apples, broccoli, or bananas.
- A 60 ml serving of syrup (1/4 cup) provides 10 to 38% of the recommended daily allowance of antioxidants according to certain nutritionists in the United States.

Results



Source: USDA National Nutrient Database for Standard Reference *http://www.nal.usda.gov/fnic/foodcomp/search/*

The antioxidant capacity of maple syrup of Canada was determined with the ORAC method. Three laboratories were involved in this large-scale study (18 to 45 samples of various grades from three regions of Quebec; all laboratories received the same samples). The table above shows that the antioxidant capacity of maple syrup is quite variable. This variation should be studied thoroughly, since none of three laboratories involved in the analysis of the samples showed an equivalent ORAC value despite having equivalent samples. The maple syrup grade seems to have an impact on the antioxidant capacity, since the average antioxidant value of Dark syrup is three times greater than that of Extra-Light syrup.

Based on the minimum (391 µmolTE/100 g fresh product) and maximum (2,651 µmolTE/100 g fresh product) values observed, a serving of maple syrup of Canada provides 10 to 38% of the recommended daily allowance of antioxidants according to certain nutritionists in the United States, which amounts to 3,000 to 5,000 ORAC units per day.

Applications and outlooks

The antioxidant capacity of maple syrup of Canada gives it a prime position among fruits and vegetables. Used as a food ingredient, it enhances the quality of prepared dishes with an added health benefit. Additional studies are necessary to explain the variation in values.

Collaborators: Mr. Richard Béliveau, Ph.D., cancer researcher at Sainte Justine Hospital in Montreal and co-author of the bestseller *Foods to Fight Cancer*. Mr. Boxin Ou, Ph.D., principal research scientist at Brunswick Laboratories, affiliated with the United States Department of Agriculture (USDA)

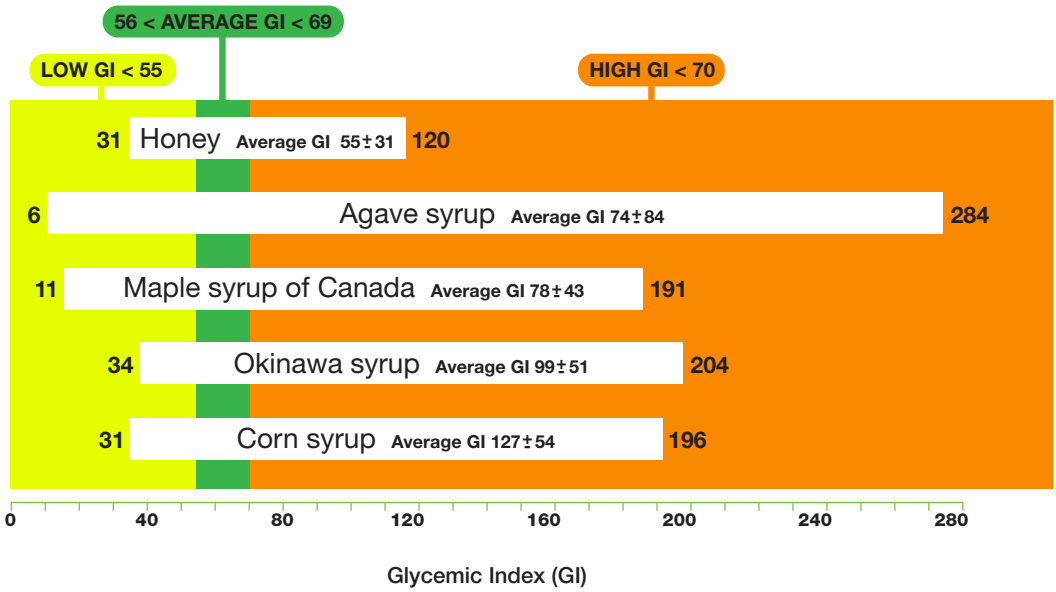
Promotional Spec Sheet: Glycemic Index for maple syrup of Canada

Summary

- Results are presented as a GI value spectrum, taking account of the average value and the rate of variation. The minimum and maximum GI values observed for maple syrup of Canada are 11 and 191 respectively, for an average value of 78 ± 43. The analysis of the results shows that in terms of GI, the sweeteners are equivalent, because their values vary between a low GI and a high GI.

- The high rate of variation in GI for each of the sugars tested (42% and up) is directly related to the metabolism of each individual. This rate of variation in GI is reported in several scientific articles. (Reference: Chlup, R. et al. Biomed. Papers no 148(1), 2004, p. 17-25)

Results



- The clinical study on the glycemic index (GI) of maple syrup of Canada and four other sweeteners performed on 34 healthy individuals at Sainte Justine Hospital in Montreal reveals that the average GI value of maple syrup is 78 ± 43. The other GI values are 55 ± 31 for honey, 74 ± 84 for agave syrup, 99 ± 51 for Okinawa syrup and 127 ± 54 for corn syrup. There is no significant difference between maple syrup from the current year (3 weeks after harvest) and that of the previous year (1 year after harvest).

- This clinical study was carried out using a proven methodology on maple syrup from Canada. The validity of the results is founded on the following measures: validation of the protocol by the research ethics committee of the Sainte Justine Hospital UHC, blood samples taken intravenously, selection of 34 individuals with health checkup, analysis of sugar profile by HPLC, number of repetitions for each sugar tested.

Applications and outlooks

- This clinical study confirms certain prior studies on the wide variability of the GI value from one individual to another.

- All of the sweeteners tested are therefore equivalent in terms of GI, given the spectrum of values obtained.

Collaborators: Ms. Céline Huot, M.D., pediatric endocrinologist and diabetologist at Sainte Justine Hospital in Montreal and member of the board of directors of the Canadian Diabetes Association
Mr. Edgar Delvin, Ph.D., clinical biochemist, Sainte Justine Hospital in Montreal
Ms. Maria Kalergis, Ph.D., nutritionist, Sainte Justine Hospital in Montreal
Mr. Yves Desjardins, Ph.D., plant physiologist, Institute of Nutraceuticals and Functional Foods (INAF) at Université Laval, Quebec City

Promotional Spec Sheet:
Nutritional Value of Maple Products of Canada and Potential Claims

Summary

- Maple syrup of Canada is an excellent source of manganese, riboflavin and zinc, providing 100%, 37% and 18% respectively of the recommended daily value of these nutrients. Plus, magnesium, calcium and potassium make this inimitable sweetener even more healthful.
- Average nutritional values derived from an analysis of more than 600 samples from different regions across Quebec have been added to Health Canada's Canadian Nutrient File.
- Nutritional labels for maple products of Canada are available at **www.siropderable.ca**, showing the minimum nutritional values obtained for the 600 samples analyzed. Based on the minimum values, maple can currently make the following claims in Canada:
  - Excellent source of manganese and Vitamin B2
  - Good source of energy
  - 4 mg of polyphenols per 60 ml

Please refer to the relevant CFIA regulations to incorporate these claims into your packaging.

Results

Per 60 ml portion in %DV*	**Maple syrup of Canada**	Honey	Sugar	Brown sugar
Manganese	**100**	3	0	9
Riboflavin (B_2)	**37**	2	1	0
Zinc	**18**	2	0	1
Magnesium	**7**	1	0	7
Calcium	**5**	0	0	5
Potassium	**5**	1	0	6
Calories	**217**	261	196	211
Sugars (in G)	**54**	71	51	54

Legend : Excellent source of | Good source of | Source of

*DV: The *Daily Value* is the amount deemed sufficient to meet the daily needs of the majority of healthy individuals. Source: Canadian Nutrient File (Health Canada)

According to a study performed on more than 600 samples of maple syrup of Canada from different regions across Quebec, maple products have very interesting nutritional potential compared to other common sweeteners. Maple syrup is an excellent source of manganese, riboflavin and zinc, providing 100%, 37% and 18% respectively of the recommended daily value of these nutrients. Plus, magnesium, calcium and potassium make this inimitable sweetener even more healthful. These average values have been added to the Canadian Nutrient File.

The data from this study has also enabled us to create nutritional labels for maple syrup of Canada and its derivative products. To ensure that all producers and processors follow the CFIA regulations on labelling, the values appearing on nutrition facts labels are the minimum values generated by the 600-sample study. These labels are available to the entire Canadian maple industry at **www.siropderable.ca**.

A second study identified the quantity of polyphenols contained in a portion of maple syrup of Canada. While polyphenols are not recognized as a nutrient that can appear on the nutritional label, the quantity contained in a product can be displayed on the packaging. The average value of polyphenols is 6.6 mg per 60 ml, and the minimum value is 4 mg per 60 ml.

Applications and outlooks

Canada
These results make it possible to make the following claims on maple product of Canada packaging. Please be sure to refer to the relevant CFIA regulations when incorporating these claims into your packaging:

- Excellent source of manganese and Vitamin B2
- Good source of energy
- 4 mg of polyphenols per 60 ml

USA (to be confirmed)

- Potassium : reduction of the risk of hypertension or stroke

Collaborators: Ms. Christine Chénard, Cintech agroalimentaire and Ms. Marie-Claude Poiré, agronomist.

Fédération des producteurs acéricoles du Québec

PRESS RELEASE
For immediate release

Substantial quantities of abscisic acid, a phytohormone recognized by the scientific community for its health benefits, are found in maple water and maple syrup

Montreal, March 4th, 2010 – Thanks to modern science, the closely guarded secrets of one of our nation's most distinctive emblems, maple syrup, are now being revealed. It has recently been reported that maple syrup contains polyphenols and shows ORAC values which compare to commonly eaten fruits and vegetables such as broccoli. Now, further research on maple syrup and its original form, maple water, conducted by Dr Yves Desjardins and his colleagues at the *Institut des neutraceutiques et des aliments fonctionnels,* has revealed that both products contain equally important quantities of terpenes, and in particular, abscisic acid, a phytohormone whose health benefits have only recently been discovered.

Abscisic acid in maple water and maple syrup occurs as a conjugate along with certain metabolites at concentrations that are therapeutic, according to the effective thresholds of abscisic acid (ABA) reported by Dr Guri's group in the US (Guri et al, 2007. Clinical Nutrition 26:107-116). Vegetable physiologists and botanical researchers have known about the physiological properties of abscisic acid in the vegetable kingdom for a long time, but its health benefits for humans has only recently come to light. Along with other effects, it is known to stimulate insulin release through pancreatic cells and to increase sensitivity of fat cells to insulin, which makes it a potent weapon against metabolic syndrome and diabetes. According to Geneviève Béland, Director of Promotion and Market Development for the Federation of Quebec Maple Syrup Producers, "These findings show that maple products contain a whole host of complementary active elements. The sugar molecules which provide the energy and sweetness in maple products are inherently complemented by abscisic acid molecules because they encourage insulin homeostasis. Further studies are obviously needed before we can more accurately understand how eating maple products affects insulin behaviour. Studying maple products is of particular interest to the food science sector when we consider that all the bioactive molecules of the sugar maple are carried in its sap and that these molecules are forty times more concentrated in maple syrup."

The detailed results of the study will be presented by Dr Desjardins at the *Emerging Topics in Health Effects of Fruits and Vegetables* symposium which forms part of the 28th International Horticultural Congress in Portugal, August 22-27, 2010. The study was financed by Agriculture and AgriFood Canada as part of its support programs for science and innovation which are aimed at encouraging collaboration between the agricultural and industrial sectors, the government and universities so that new opportunities for strategic innovation are identified quicker.

Quebec and Canadian maple products will also be in the spotlight in a seminar presented by Dr Navindra Seeram from the University of Rhode Island at the national meeting of the American Chemical Society taking place in San Francisco from March 21-25 of this year. According to Serge Beaulieu, president of the Federation of Quebec Maple Syrup Producers and member of the Canadian Maple Industry Advisory Committee: *"A new era is starting for Quebec and Canadian maple products in which our national emblem will be the pride and joy of this country and abroad because of the vitality that maple represents as one of the world's great products. The Quebec and Canadian maple industry will create an international research network for maple with the best research units dedicated to maple, as well as investing in collaboration with different bodies such as Agriculture and AgriFood Canada so that we can*

add value to the gastronomic and health benefits that maple products bring. With maple being featured at these two scientific conferences it signifies the next stage in a historic strategic step for the industry."

About Dr Yves Desjardins

Dr Yves Desjardins has been professor at the Department of Phytology at Université Laval since 1991. He's an active member of the Centre for Horticultural Research (CRH) where he conducts research work on fruit and market garden horticulture along with more fundamental research on the ecophysiology of in vitro cultures. After heading up the CRH of Université Laval from 1992 to 2002, he now studies the effects of fruits and vegetables on health. As Academic Director of INAF (Institute of Nutraceuticals and Functional Foods), he organized FAVHEALTH 2005 in Quebec, the first international symposium on the effects of fruit and vegetables on health, which brought together researchers from the horticulture and health sectors - nutritionists and clinicians - for the first time. He has recently been appointed President of the Commission Fruits and Vegetables and Health within ISHS, the International Society for Horticultural Science.

Dr Desjardins is an important collaborator in the International Network for Maple Innovation coordinated by the Federation of Quebec Maple Syrup Producers for the Canadian maple industry. He has directed, or is a member of, several Quebec and Canadian high profile networks. His work has resulted in the publication of more than sixty scientific articles and many book contributions, including a recent chapter on the role and physiological function of bioactive molecules in plants.

About the FPAQ

The Federation of Quebec Maple Syrup Producers was founded in 1966 with the mission of defending and promoting the economic, social and moral interests of its 7,400 maple businesses. These men and women are working together to collectively market their products. The quality of their work and their products has made Quebec the producer of close to 80% of today's global maple syrup output.

- 30 -

Source:
Johannie Coiteux
Promotions and Communications Agent
Federation of Quebec Maple Syrup Producers
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From left to right: Mr Serge Beaulieu, president of the Federation of Quebec Maple Syrup Producers (FPAQ), Mrs Genevieve Béland, Promotion director, FPAQ, and Dr Yves Desjardins, Institute of Nutraceuticals and Functional Foods

THE STICKY FACTS

Region

- Canada produces 80% of maple syrup sold in the world, 91% of which is produced in Quebec.
- There are more than 8,600 maple syrup industries in Canada.
- While Quebec is the primary maple-producing region in the world, other regions in Canada such as Ontario, New Brunswick, Prince Edward Island and Nova Scotia also produce syrup.

Taste and Flavor Components

- Quebec maple syrup has a complex flavor profile and classification system, and is graded according to its color, clarity, density and the strength of its maple flavor.
- Categories and Grading of Maple Syrup:

  Grade A: Light amber, medium amber and dark amber coloring
  Grade B: Dark coloring
- Made from sap tapped at the beginning of the season, Grade A syrup is generally clearer and lighter in taste. As the season advances, the syrup becomes darker and more caramelized in flavor.
- Because of its intensity of flavor, Grade B maple syrup is often used in large batch, commercial maple products, such as cereal and granola.
- Because of its more distinctive taste, it is suggested to use darker maple syrup when cooking.

High Quality and Natural Product

- Maple syrup is 100% natural, pure and free of any coloring or additives.
- There are organic and kosher brands of maple syrup and about 12% of all Quebec maple syrup is organic.
- Canadian laws and regulations for maple syrup production ensure the quality and cleanliness of maple syrup production. Each province has its own maple syrup regulations.
- Producers must adhere to strict quality control standards throughout the production process.

Production Process

- Production is closely tied to culture and history in Canada and Quebec, with many farms passed down for generations.
- Syrup is made from the sap of the sugar maple tree and produced in limited capacity only once per year.
- The maple harvest season only lasts for a period of about 12 to 20 days, usually in early March to the end of April.
- In the springtime, the nights are still cold and the water from the soil is naturally absorbed into the tree. During the day, the warmer temperature creates pressure that pushes the water back down to the bottom of the tree, making sap collection possible.
- 40 liters (10.5 gallons) of maple sap make one liter (about .25 gallons or one quart) of maple syrup.

For media inquiries, please contact: Pia Mara Finkell, CRT/tanaka
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Versatility

- Quebec maple syrup can be used as a healthier alternative to sugar in a variety of desserts and baked goods, such as pies and cakes.
- To substitute maple syrup for white sugar, use a one-for-one substitution and reduce the quantity of liquid ingredients in the recipe (water, milk, juice) by about a ¼ of a cup. Maple syrup may also serve as a one-to-one substitution for other liquid sweeteners, such as honey, molasses and corn syrup.
- Maple syrup can add depth and complexity to cooking, as an ingredient in glazes, rubs or barbeque sauces for poultry, meat, seafood or vegetables.
- Maple syrup can also:
  - Add a subtle sweetness and a hint of maple syrup flavor to fresh fruit, cereal and ice cream
  - Sweeten tea, hot chocolate, coffee, eggnog and smoothies
  - Jazz up a cocktail, instead of simple syrup
- By continuing to boil the maple sap, producers can make a variety of maple products, such as maple sugar, maple butter, soft and hard maple candy and maple taffy.

Cultural Connection

- Maple syrup is a key ingredient in Quebec and international cuisine.
- Each spring, many Quebecois go to their local "sugar shack" for a traditional, hearty meal, featuring maple dishes like split pea soup, maple-smoked ham, baked beans, crêpes and a variety of maple desserts and candy.
- Maple taffy, a favorite treat for children, is made by pouring reduced hot maple syrup onto clean snow. Once sufficiently hardened, the soft maple candy can be twirled around a wooden stick and enjoyed.

Health Benefits

**Figures below based on research by the Canadian Nutrient File (Health Canada)*

- Maple syrup contains fewer calories than corn syrup and honey.
- Maple syrup contains various vitamins and minerals.
- Maple syrup contains antioxidants, which can delay or prevent free radical induced diseases, such as diabetes and cancer. A portion of ¼ cup of maple syrup contains as much antioxidant activity as one serving of raw tomato or broccoli.
- A portion of ¼ cup of maple syrup contains 100% of the Daily Value for manganese, which is an important factor in energy production, healthy bone formation and antioxidant defenses and necessary for normal brain and nerve function.
- A portion of ¼ cup of maple syrup contains 34% of the Daily Value of riboflavin, which aids in the metabolic processes and 11% of the Daily Value of zinc, which is essential for normal reproduction and growth, plus a healthy immune system.
- Maple syrup supplies a small amount of potassium. Potassium is essential in electrolyte balance and normal muscle development and aids in the metabolism of protein and carbohydrate.
- Maple syrup does not contain high fructose corn syrup.
- The leading pancake syrup brands contain zero pure maple syrup and rely on high fructose corn syrup as the primary sweetening ingredient, along with additives like artificial flavorings and coloring agents.

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PRODUCING REGIONS IN CANADA

Canadian Maple Syrup

Every spring in Eastern Canada, as the snow melts and animals stir from their winter slumber, the anticipated maple sugaring season begins. Canada produces 80% of the world's maple syrup. With forests brimming with majestic red, black and sugar maples, the country has just the right mix of cold spring nights and warm daytime temperatures to produce the clear-colored sap used to make maple syrup.

Canada's maple syrup producing regions are located in the provinces of Quebec (primary producter), Ontario, New Brunswick and Nova Scotia. There are more than 8,600 maple syrup businesses in Canada, with the largest number of them operating in Quebec. Maple syrup has long been part of Canada's cultural fabric. The country's Amerindian peoples taught the early settlers how to harvest sap and boil it to make maple syrup.

Now enjoyed in 50 countries around the world, Canadian maple syrup products range from traditional maple syrup to maple butter, maple candy and a full range of products containing maple syrup, such as cereals, yogurts and more.

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GRADES AND CATEGORIES

Flavor and Color

Maple syrup is made from maple sap tapped at the beginning of the season and is generally clearer and lighter in taste. As the season advances, the maple syrup becomes darker and more carmelized in flavor. Maple syrup is categorized and graded according to its color, clarity, density and the strength of its maple flavor.

U.S.: Canada:	Grade A: Light Amber (Extra Light)	Grade A: Medium Amber (Light)	Grade A: Dark Amber (Medium)	Grade B (Amber)

Grade A (Light Amber, Medium Amber, Dark Amber)

Maple syrup of a quality grade that is suitable for the table. It has good color, flavor and odor and is practically clear and free of defects.

Grade B (Amber)

Maple syrup of that is suitable for use in cooking or other maple products, such as granola. It has fairly good color, flavor and odor and is moderately clear.

Classifications

Each classification of Canadian maple syrup:

- Must meet the standards of section five of the Maple Products Regulations;
- Must have a clear and uniform color; and
- Must not ferment.

The classification of maple syrup provides consumers and the industry a method for identifying preferred flavors and uses. Grade A syrup is intended for everyday use and is most available to the general consumer. Grade B syrup is mainly used in cooking or by the agri-food processing industry.

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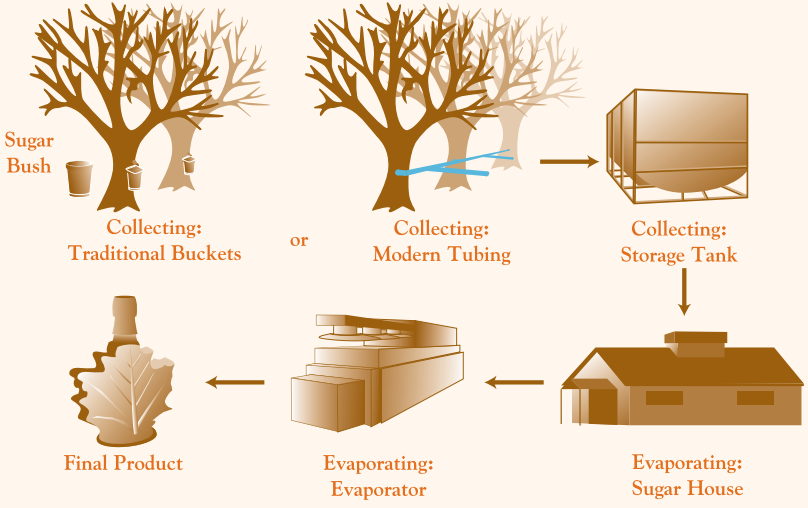
PRODUCTION PROCESS

Beginning

Maple syrup gets its start from one of nature's phenomena. In springtime, when the nights are still cold, the water from the soil is absorbed into the tree. During the day, the warmer temperature creates pressure that pushes the water back down to the bottom of the tree. The sap is collected over a 12 to 20 day period, usually early March to the end of April, according to the region.



Tapping

The number of taps on a single tree is calculated based on the tree's diameter, its health and its growth rate. Any maple tree measuring about 8 inches in diameter or more may be tapped. Larger trees may be tapped more than once (for every additional 20 cm) for a maximum of three taps per tree each season. Regulated tapping does not affect a tree's growth.

Evaporating

A sugar house is where sap is boiled down to maple syrup. During cooking, the sap is fed by pipes from a storage tank to a long and narrow ridged pan called the evaporator. As it boils, the water evaporates; it becomes denser and sweeter. The syrup is boiled until it reaches the density of maple syrup. It takes approximately 40 liters (10.5 gallons) of sap to be boiled down to one liter (about .25 gallons or one quart) of syrup. For other maple products – butter, taffy, or sugar – the maple syrup is further boiled in the evaporator to the temperature necessary to produce each type of product. After the evaporation process, the maple products are bottled or canned.

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FREQUENTLY ASKED QUESTIONS

Q: How do I know if I am buying pure maple syrup or the fake stuff?

A: There are many brands of pure Canadian maple syrup on the U.S. market, all of which are 100% natural and free of any coloring or additives. Leading pancake syrups contain zero pure maple syrup and rely on high fructose corn syrup as the primary sweetening ingredient, along with additives like artificial flavorings and coloring agents. Be sure to check the label. Sometimes imitation syrups list maple syrup as an ingredient, when it only contains 5%. Check the bottle to make sure you're getting 100% of the real thing.

Q: Is maple syrup sustainable in Canada?

A: Yes. Producers must adhere to strict guidelines and standards set forth by Canadian law and the Federation throughout the production process. Each harvest, the sugar trees are tapped in a slightly different area than the previous year, preserving the health of the trees. The Canadian 'Preservation of Agricultural Land and Agricultural Activities' Act (i.e. Loi sur la protection du territoire et des activités agricoles) forbids cutting down an entire maple grove in an agricultural zone.

Q: What are other products made from maple?

A: There is a diverse selection of products, including candies and caramels, cereals, jams and spreads, ketchup and mustard, maple nut snacks, maple vinegar and vinaigrettes, as well as maple beers, wines and other beverages.

Q: What is The Federation of Quebec Maple Syrup Producers?

A: The Federation of Quebec Maple Syrup Producers was founded in 1966 and created to boost the economic, social and ethical interests and enforce safety and environmental standards for the more than 7,400 maple syrup businesses.

Q: How extensive is Canadian production?

A: Canadian maple syrup is exported to approximately 50 countries, and the U.S. is the primary importer. In 2007, Canada produced 67.6 million pounds of maple syrup yet exported 67.7 to the U.S. using reserve supply from previous years to support the growing exportation demand.

For more information, please visit **www.purecanadamaple.com**.

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§ 168.122 Lactose.

(a) Lactose is the carbohydrate normally obtained from whey. It may be anhydrous or contain one molecule of water of crystallization or be a mixture of both forms.

(b) The food shall meet the following specifications:

(1) The lactose content is not less than 98.0 percent, mass over mass (m/m), calculated on a dry basis.

(2) The sulfated ash content is not more than 0.3 percent, m/m, calculated on a dry basis.

(3) The pH of a 10.0-percent m/m solution is not less than 4.5 nor more than 7.5.

(4) The loss on drying for 16 hours at 120 °C is not more than 6.0 percent, m/m.

(c) The name of the food is ''Lactose'' or, alternatively, ''Milk sugar''.

(d) The methods of analysis in paragraphs (d)(1), (d)(2), (d)(3), (d)(4), and (d)(5) of this section are to be used to determine whether the food meets the requirements of paragraphs (b)(1), (b)(2), (b)(3), and (b)(4) of this section. The methods are contained in ''Official Methods of Analysis of the Association of Official Analytical Chemists'', 14th Ed. (1984), including the 4th Supp. (1988), which is incorporated by reference in accordance with 5 U.S.C. 552(a). Copies of the material incorporated by reference may be obtained from the Association of Official Analytical Chemists International, 481 North Frederick Ave., suite 500, Gaithersburg, MD 20877–2504, or may be examined at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC.

(1) Lactose content, sections 31.064 to 31.071, ''Purity of Lactose, Liquid Chromatographic Method,'' First Action, 14th Ed. (1984), pp. 583 and 584.

(2) Lactose content, sections 31.064 to 31.071, ''Purity of Lactose, Liquid Chromatographic Method,'' ''Changes in Official Methods of Analysis,'' 14th Ed., 4th Supp. (1988), p. 212. This reference recognizes the change in status of the method from first action to final action.

(3) Sulfated ash content, section 31.014, ''Ash of Sugars and Sirups,'' Final Action, Sulfated Ash, 14th Ed. (1984), p. 575.

(4) pH, section 14.022, ''pH of Flour, Potentiometric Method,'' Final Action, except that a 10-percent m/m solution of lactose in water is used for the determination, 14th Ed. (1984), p. 252.

(5) Loss on drying at 120 °C, section 31.070, 14th Ed. (1984), p. 584.

[42 FR 14479, Mar. 15, 1977, as amended at 47 FR 11834, Mar. 19, 1982; 49 FR 10103, Mar. 19, 1984; 54 FR 24896, June 12, 1989; 55 FR 8459, Mar. 8, 1990; 63 FR 14035, Mar. 24, 1998]

§ 168.130 Cane sirup.

(a) Cane sirup is the liquid food derived by concentration and heat treatment of the juice of sugarcane (*Saccharum officinarum* L.) or by solution in water of sugarcane concrete made from such juice. It contains not less than 74 percent by weight of soluble solids derived solely from such juice. The concentration may be adjusted with or without added water. It may contain one or more of the optional ingredients provided for in paragraph (b) of this section. All ingredients from which the food is fabricated shall be safe and suitable.

(b) The optional ingredients that may be used in cane sirup are:

(1) Salt.

(2) Preservatives.

(3) Defoaming agents.

(c) The name of the food is ''Cane sirup'' or ''Sugar cane sirup''. Alternatively, the word ''sirup'' may be spelled ''syrup''.

(d) *Label declaration*. Each of the ingredients used in the food shall be declared on the label as required by the applicable sections of parts 101 and 130 of this chapter.

[42 FR 14479, Mar. 15, 1977, as amended at 58 FR 2886, Jan. 6, 1993]

§ 168.140 Maple sirup.

(a) Maple sirup is the liquid food derived by concentration and heat treatment of the sap of the maple tree (*Acer*) or by solution in water of maple sugar (mapel concrete) made from such sap. It contains not less than 66 percent by weight of soluble solids derived solely from such sap. The concentration may be adjusted with or without added water. It may contain one or more of the optional ingredients provided for in

paragraph (b) of this section. All ingredients from which the food is fabricated shall be safe and suitable.

(b) The optional ingredients that may be used in maple sirup are:

(1) Salt.

(2) Chemical preservatives.

(3) Defoaming agents.

(c) The name of the food is ''Maple sirup''. Alternatively, the word ''sirup'' may be spelled ''syrup''.

(d) *Label declaration.* Each of the ingredients used in the food shall be declared on the label as required by the applicable sections of parts 101 and 130 of this chapter.

[42 FR 14479, Mar. 15, 1977, as amended at 58 FR 2896, Jan. 6, 1993]

§ 168.160 Sorghum sirup.

(a) Sorghum sirup is the liquid food derived by concentration and heat treatment of the juice of sorghum cane (sorgos) (*Sorghum vulgare*). It contains not less than 74 percent by weight of soluble solids derived solely from such juice. The concentration may be adjusted with or without added water. It may contain one or more of the optional ingredients provided for in paragraph (b) of this section. All ingredients from which the food is fabricated shall be safe and suitable.

(b) The optional ingredients that may be used in sorghum sirup are:

(1) Salt.

(2) Chemical preservatives.

(3) Defoaming agents.

(4) Enzymes.

(5) Anticrystallizing agents.

(6) Antisolidifying agents.

(c) The name of the food is ''Sorghum sirup'' or ''Sorghum''. Alternatively, the word ''sirup'' may be spelled ''syrup''.

(d) *Label declaration.* Each of the ingredients used in the food shall be declared on the label as required by the applicable sections of parts 101 and 130 of this chapter.

[42 FR 14479, Mar. 15, 1977, as amended at 58 FR 2886, Jan. 6, 1993]

§ 168.180 Table sirup.

(a) Table sirup is the liquid food consisting of one or more of the optional sweetening ingredients provided for in paragraph (b)(1) of this section. The food contains not less than 65 percent soluble sweetener solids by weight and is prepared with or without added water. It may contain one or more of the optional ingredients prescribed in paragraphs (b)(2) through (12) of this section. All ingredients from which the food is fabricated shall be safe and suitable. (Vitamins, minerals, and protein added for nutritional purposes and artificial sweeteners are not considered to be suitable ingredients for this food.)

(b) The optional ingredients that may be used in table sirup are:

(1) One or more of the nutritive carbohydrate sweeteners provided for in this paragraph (b)(1). When a sweetener provided for in paragraph (b)(1)(i) or (ii) of this section is used it shall constitute not less than 2 percent by weight of the finished food.

(i) The sirups identified by §§ 168.130, 168.140, and 168.160, except that the use of any such ingredient is so limited that the finished food does not meet the requirement prescribed for any sirup by §§ 168.130, 168.140, or 168.160.

(ii) Honey.

(iii) Other nutritive carbohydrate sweeteners.

(2) Butter, in a quantity not less than 2 percent by weight of the finished food.

(3) Edible fats and oils, except that, in products designated as ''buttered sirups'', butter as provided for in paragraph (b)(2) of this section is the only fat that may be used.

(4) Emulsifiers or stabilizers or both.

(5) Natural and artificial flavorings, either fruit or nonfruit, alone or in carriers.

(6) Color additives.

(7) Salt.

(8) Chemical preservatives.

(9) Viscosity adjusting agents.

(10) Acidifying, alkalizing, or buffering agents.

(11) Defoaming agents.

(12) Any other ingredient (e.g., shredded coconut, ground orange peel) that is not incompatible with other ingredients in the food.

(c) Except as provided for in this paragraph and in paragraphs (d) (2) and (3) of this section, the name of the food is ''Table sirup'', ''Sirup'', ''Pancake sirup'', ''Waffle sirup'', ''Pancake and waffle sirup'', or ''_____ sirup'', the

Nutritional value for various sweeteners (% of Daily Value)

	Maple Syrup	Corn Syrup	Honey	**Maple Sugar**	Brown Sugar	Sugar
Manganese	100	0	3	29	9	0
Riboflavin	34	0	2	2	0	1
Zinc	11	3	1	5	1	0
Magnesium	5	0	0	3	7	0
Calcium	6	1	1	7	5	0
Potassium	5	0	1	4	6	0
Calories	217	241	258	170	211	194
Sugars (in grams)	54	65	70	41	54	50

Source: Canadian Nutrient File, 2007 (Health Canada) and US Food and Drug Administration.

Nutritional value for various foods (% of Daily Value)

	¼ Cup of Maple Syrup	1 Large Egg	1 Medium Apple	1 (30g) Slice of Bread
Manganese	100	1	2	7
Riboflavin	34	14	2	6
Zinc	11	4	0	1
Magnesium	5	2	2	2
Calcium	6	3	1	5
Potassium	5	2	4	1

Sources: US Department of Agriculture Nutrient Data Laboratory. The Canadian Nutrient File - Health Canada and US Food and Drug Administration.

Antioxidant value for common foods

ORAC Value 100g of fresh product	µmol TE²/100g	ORAC Value per serving		µmol TE/serving
Brocoli, raw	1,362	Banana, raw	1 medium (118g)	1,037
Banana, raw	879	Broccoli, raw	½ cup (46g)	627
Carrot, raw	666	Carrot, raw	1 (72 g)	480
Maple Syrup	600	**Maple Syrup**	¼ cup (60 ml/80 g)	480
Cabbage, raw	508	Tomato, raw	1 medium (123 g)	415
Tomato, raw	337	Cantaloupe	½ cup (85 g)	268
Cantaloupe	315	Cabbage	½ cup (37 g)	188

USDA Database for the Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods. Results showing the antioxidant power of maple syrup were obtained from Brunswick Laboratories, a USDA-certified facility.

DO MORE WITH *Maple*

MAPLE –

FROM TREE TO TABLE

CULINARY

EDUCATION GUIDE

ww.domorewithmaple.com

Authored by Joan Kimball,
Québec Delegation Chicago for
DoMoreWithMaple.com

Table of Contents

OUTCOMES & OBJECTIVES

This culinary education guide provides an overview of the production, grading and flavor of real maple syrup.

Upon completion of this guide and tasting, the student will have a basic understanding of maple syrup production, maple products available and will be able to differentiate real maple syrup from maple-flavored table syrups.

MAPLE PRODUCTION: FROM TREE TO TABLE

Maple syrup is made from the sap of the sugar maple tree.

"Sugaring Off"

Maple sap is the watery fluid that feeds the sugar maple tree's roots, trunk, branches and leaves. The sap will run when the temperatures move above and below freezing. Maple syrup production or the "sugaring off" season occurs once a year for 6 to 8 weeks in mid-February through April.

Tapping Trees

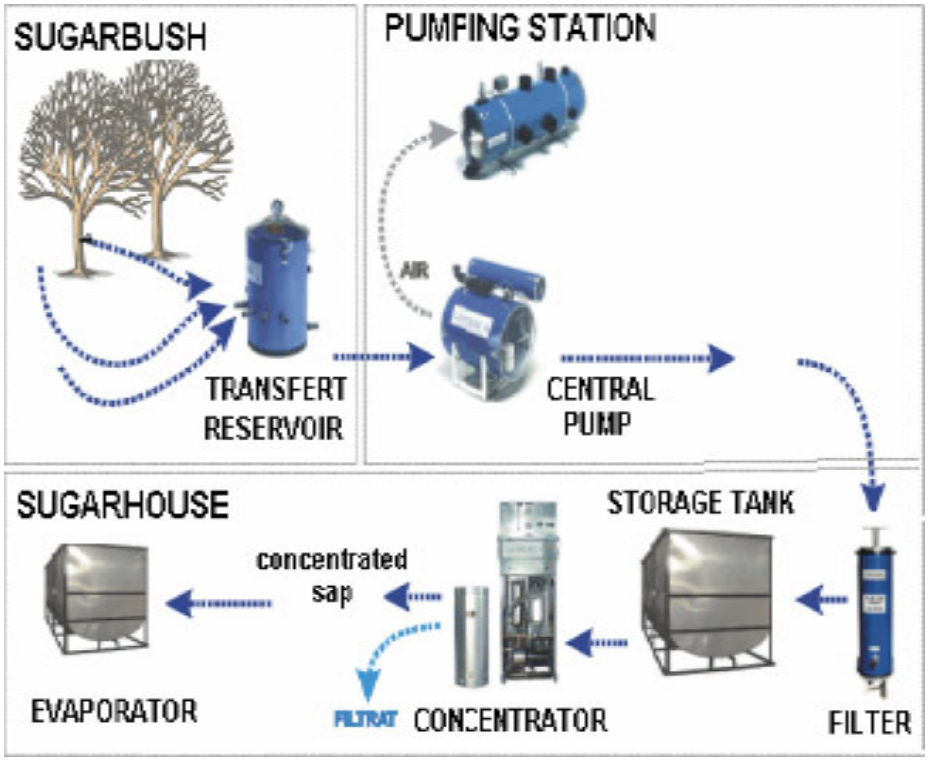
Maple syrup producers insert a tap into the tree to collect the sap. While traditional maple syrup producers relied on buckets and spouts, today's maple syrup producers use gravity-fed plastic tubing and pipelines and in some cases vacuum pumps to collect the sap. The vacuum pumps do not suck sap from the trees but rather help lower the pressure in the pipeline system to allow the sap to flow easily.

It takes about 40 to 60 years for the sugar maple trees to grow large enough for tapping. Holes are typically drilled on an upward angle to a depth of not more than three inches. Tapping will not injure the tree as long as the sugar maple tree is healthy and the number of taps is limited.

Evaporation

The maple sap is boiled and evaporated to make maple syrup. It takes approximately 40 gallons of sap (also called maple water) to produce 1 gallon of maple syrup. Maple syrup is made when the sugar density reaches 66 percent.

Finishing Finished maple syrup is filtered to remove any organic materials found in the sap. The maple syrup is then graded based on color and flavor.



Maple syrup production requires optimal temperatures and sugar maple trees and is made is a small geographic area of northeastern North America. Quebec produces more than 80 percent of the world's maple syrup, equal to 93 percent of Canadian production.

MAPLE PRODUCTION: MAPLE PRODUCTS

In addition to maple syrup, Quebec maple producers have developed several natural maple products made from the sap of the sugar maple tree.

Maple Syrup Maple syrup is produced by the evaporation of the sap of the maple tree. It takes approximately 40 gallons of sap (also called maple water) to produce 1 gallon of maple syrup. Maple syrup has a sugar content of about 66 percent and is graded according to color and flavor. As a general rule, lighter-colored maple syrups have a more delicate flavor and darker-colored maple syrups have a stronger taste. The flavor of maple syrup is also influenced by the growing regions of the sugar maple trees.

Maple Butter Thick and spreadable, maple butter (also known as maple cream or spread) is a whipped version of pure maple syrup.

Clearly Maple Clearly Maple begins as maple syrup and then is altered by the addition of a processing aid that is later removed to create a higher invert sugar content. The result is a honey-like consistency product made of pure maple syrup.

Maple Concentrate Pure maple syrup concentrates are produced by removing nearly half the sucrose content found in pure maple syrup.

Maple Flakes Fine, medium or coarse, maple flakes are made from pure maple syrup dehydrated by a unique and exclusive process.

Maple Jelly Jelly made with pure maple syrup.

Maple Sugar Pure maple syrup dehydrated into granulated sugar crystals. Maple sugar can be substituted 1 to 1 for regular granulated sugar in most recipes and formulas. Various granule sizes are available.

Maple Vinegar Vinegar made from pure maple syrup through alcoholic fermentation and acetic fermentation processes.

MAPLE SYRUP STORAGE

Unopened maple syrup stores easily, unrefrigerated. Prolonged storage may cause the color of maple syrup to darken and the flavor to deteriorate.

After opening maple syrup, store in an air tight container. To slow the natural crystallization process of syrup, keep maple syrup in a refrigerator.

For extended storage (one to three months), it is recommended to store maple syrup in the freezer. The exception is a bag in a box of maple syrup which can be stored at room temperature at all times.

COOKING AND BAKING WITH MAPLE SYRUP

Maple syrup is more than just a topping for pancakes. Maple syrup can be used in diverse menu items. Following are just a few ways that maple syrup can bring a golden touch to menus:

- Use maple syrup to sweeten lemonade, tea, coffee and lattes.
- Glaze sweet potatoes or acorn squash with maple syrup.
- Create a sweet and savory barbecue sauce with maple.
- Add maple vinegar to create a signature salad dressing.
- Drizzle maple syrup on a pear, walnut and gorgonzola pizza.
- Prepare maple-kissed baked goods and desserts.

Substitution Information
When substituting maple syrup for granulated sugar in baked goods, follow these guidelines:

- For each cup of granulated sugar, use 1-1/2 cups of maple syrup.
- Reduce other liquids in the recipe by about one-half.
- Add 1/4 teaspoon baking soda for each cup of maple syrup used
- Decrease oven temperature by 25 degrees to avoid over-browning.

MAPLE SYRUP NUTRITION

Unlike most refined sweeteners, maple syrup contains several vitamins and minerals. Recent studies have also shown that maple syrup is a rich source of antioxidants.

MAPLE SYRUP GRADES/CLASSIFICATIONS

Maple syrup is graded according to its clarity, density and the characteristic taste of maple. The color classifications are based on measuring the amount of light that passes through the maple syrup.

U.S. GRADE	Description	CANADIAN GRADE	QUEBEC GRADE
U.S. Grade A Light Amber/Fancy	Maple syrup produced at the very beginning of the season. Very pale color and delicate taste. Light transmittance over 75 percent.	Canada No. 1 Extra Light	Quebec Grade AA
U.S. Grade A Medium Amber	Maple syrup produced at the beginning of the season. Pale amber in color with a pure, subtle taste. Light transmittance of 61 to 74 percent.	Canada No. 1 Light	Quebec Grade A
U.S. Grade A Dark Amber	Produced in the middle of the season, this maple syrup is the most popular grade available. A rich amber color with a more pronounced flavor. Light transmittance of 44 to 60 percent.	Canada No. 1 Medium	Quebec Grade B
U.S. Grade B Commercial	Maple syrup produced near the end of the season. Strong maple taste and dark color. Light transmittance of 27 to 43 percent.	Canada No. 2 Amber	Quebec Grade C
U.S. Grade B Commercial	Maple syrup produced at the very end of the season. Very dark syrup used primarily as food processing ingredient. Highest mineral content. Light transmittance of 0 to 26 percent.	Canada No. 3 Dark	Quebec Grade D

THE FLAVOR OF REAL MAPLE

Maple Syrup made from maple water tapped at the beginning of the season is generally clearer and lighter in taste. As seasons advance, the maple syrup becomes darker and more caramelized in flavor. The sugar content of maple syrup averages 66.5 BRIX.

According to the scientific literature, the flavor of maple syrup develops during the evaporation process; the taste precursors are part of the sap. In addition to water, minerals and various sugars, maple sap is rich in organic acids, nitrogen compounds and, like red wine, in phenolic compounds and flavonoids. The amount of these compounds in maple sap may vary over the course of the maple syrup season, from one season to the next, according to the area, and from one maple tree to the next.

Among the taste precursors, sugars play an important role. They initiate the caramelization reaction and the Maillard reaction (which gives bread a brown crust) as the water is evaporating. Also, under the effect of the heat, phenols with tasty names such as vanillin and coniferol are released.

Maple syrup is defined by much more than just the degree of caramelization. The richness of the maple syrup flavor is a result of the various reactions of the other compounds in the maple sap. The Maillard reaction (sugar and amino acids) is important. It can be assumed that the phenolic compounds and flavonoids also have an important role in producing the flavor of maple syrup, like in the case of red wine. However, this effect has not yet been confirmed.

MAPLE SYRUP TASTING SENSORY PROFILE

Maple Tasting - Sensory Profile Vocabulary

Dominant Flavors

- Roasted
  - Light – golden sugar, chicory, toast
  - Medium – cooked sugar – caramelized, burnt wood, ground coffee, brown coffee bean, chocolate
  - Strong – burnt sugar, ground black coffee, black coffee bean, smoked

- Confectionery
  - Light - white sugar
  - Medium - corn syrup, light brown sugar
  - Strong - dark brown sugar, molasses, sponge toffee

- Maple*
  - Maple, roasted dandelion root

Variable as a Dominant Flavor

- Woody
  - Firewood, wet wood, softwood (pine, fir, larch, juniper, cedar, etc.)

- Vanilla
  - Marshmallow
  - Vanilla pod

Minor Flavors

- Herbal
  - Fresh Herbs - stem, grassy, shoot, bud
  - Dry Herbs - crushed leaves, nutshells, dry herbs, hay
  - Fermented Herbs – silage

- Plants, Humus, Forest, Cereals
  - Humus, Forest – mushroom, mold, potato
  - Cereals - malt, oat, wheat, rye

- Fruity
  - Nuts - bitter almond, hazelnut, nuts
  - Peach, fruits with pits or seeds

- Milky
  - Fresh - butter, cream, milk
  - Heated - butter, milk

- Floral
  - Flowers
  - Honey

- Spicy
  - Cloves
  - Cinnamon
  - Anis – black licorice

*Important Note: During production, maple syrup is often blended to achieve a consistent flavor.

CLASSROOM ACTIVITY - MAPLE SYRUP TASTING

Have your classroom taste and compare simulated maple sap, various colors of maple syrup and maple-flavored table syrup.

You can mimic the taste of maple sap by combining 1 Tablespoon of granulated sugar and a dash of extra light maple syrup with 1 cup of water.

Compare the sap with various colors of maple. Start with the lightest color of maple syrup and build to the dark amber maple syrup.

Taste maple sugar.

Finally, compare real maple syrup to table syrups with maple flavoring.

Tasting Steps
To taste maple syrup, follow these steps:

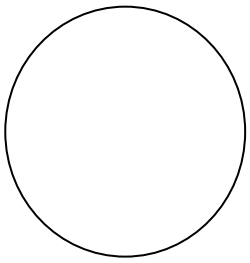
1. Smell the syrup by taking three quick sniffs. Make a mental note of your impression.
2. Take a small sip of the syrup and swirl it around in your mouth. Concentrate on the full range of flavors.
3. Associate the flavor with your own experience (for example, the aroma from a bag of marshmallows
4. Assess the degree of intensity (mild, medium or strong)
5. Share your reaction with others.

Discussion Questions:

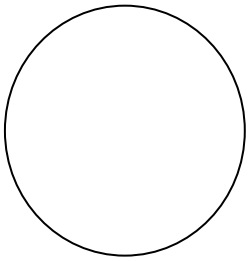
1. How does the flavor of maple syrups vary with color?
2. How does real maple syrup flavor compare to table syrups with maple flavoring?
3. What are the benefits of using real maple syrup in recipes?
4. How does the sweetness and flavor of maple sugar compare with granulated sugar?
5. How could maple syrup and sugar be used in menu items (think beyond a topping for pancakes and waffles)

CLASSROOM ACTIVITY - MAPLE SYRUP TASTING

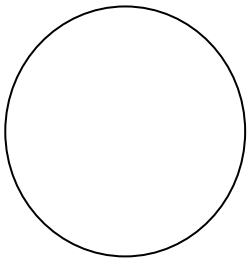
Sap



Extra Light or Light Maple Syrup



Medium Maple Syrup



Amber or Dark Amber Maple Syrup

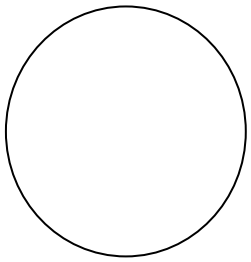
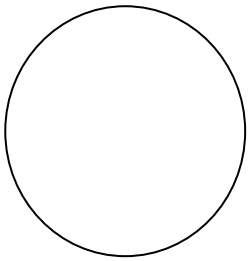
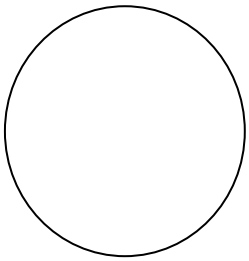


Table Syrup with Maple Flavoring



Maple Sugar or Flakes



RESOURCES

For more information about maple products, visit www.domorewithmaple.com or one of the following websites:

www.citadelle-camp.com

www.decacer.com

www.foodsofquebec.com

www.heritageyamaska.com

www.maplesyrupfederation.com

www.maplesyrupusa.com

Quebec Maple, A Natural, Nutritional Ingredient

A Culinary Curriculum

Developed by Daniel LaGarde, CEC

Executive Chef, ***Do More With Maple!***

American Culinary Federation

Continuing Education Program

ACF 2007 National Conference

Course/Program Outline

- Class Objective
- Maple~where in the world is it?
- Quebec, where maple is King
  - History of Maple Syrup
  - The Tree Defined
- Production process
  - From Tree to bottle
  - Pumping Station
  - Evaporator modern techniques
- Introduction of Maple Syrup
  - Federal/Provincial classification
  - USDA Grades
  - Other Maple products
  - Storage
- Maple Syrup Nutritional Value, compared with other sweeteners (sugar, honey, Brown sugar)
- Five Health Benefits
- More Health Benefits/ Value of Manganese and Zinc
- Maple Products available
- Maple Tasting Techniques & Sensory Profile Vocabulary
- Maple Syrup Tasting
- Resources & Procurement (Citadelle, Decacer, Federation of Quebec Maple Syrup Producers, Heritage Yamaska, Lapierre Maple Farm)
- Cooking tips
- Maple Recipes
- Maple Dessert Demo
- References

Class Objective

- Understand origin and processes of Maple Syrup and maple products
- Discover Maple Syrup as one of the many wonders of the world, the viscous amber liquid with its characteristic earthy sweet taste
- Identify real Maple Syrup from table syrup- taste and nutritional value
- Understand the difference between grades through a maple tasting
- Discover maple depth and complexity as a natural and nutritional culinary ingredient and to “think outside the pancake box” for creative recipe applications

Maple~ Where in the world is it?

- North America is the only part of the world where Maple Syrup is produced.
- Quebec is the largest producer of Maple Syrup in the world, responsible for 93% of Canadian maple production (Ontario 5%, New Brunswick 2%) and 85% of the planet's Maple Syrup production
- In 1984, Quebec produced 21 millions pounds of Maple Syrup, in 2003, 86 millions pounds.
- Quebec is the number one world exporter of Maple Syrup. Quebec Maple Syrup is sent to some 30 countries, of which the USA is the largest importer.

Quebec, where maple is King
History of the Maple Syrup

- The tradition surrounding maple syrup was passed from the Native Americans of North America to the European settlers. Amerindians used their tomahawk, channeling the maple water (sap) towards a bark container, they boiled the sap in clay pots to obtain maple syrup
- Well before the arrival of the European settlers, First Nations peoples knew about and savoured the sap from maple trees and used this "sugared water" to cook game. Much later, in 1702, when war between France and England prevented many basics, including sugar, from being delivered to New France, Agathe de Saint-Père, wife of Pierre Legardeur de Repentigny, of Montréal, initiated the production of maple syrup. When spring came, she and her French and First Nations neighbours tapped the maples and produced sugar from the sap they obtained. Within a few years, Agathe de Saint-Père reported to the King of France that the Montréal colony annually produced 13,600 kg of maple sugar.
- Production in the 19th century, the spout was made of cedar wood, it was called a "reed". Even though horses were used more often than in the previous century, the syrup maker still had to put on his snowshoes to gather the maple water in buckets. When enough water was collected, it was brought to the "sugar house" for boiling.
- From the 20th century to today, wooden buckets were replaced with aluminum ones. The sugar house of the time was also transformed, the heavy kettle was replaced by the evaporator that contains a thermometer, a float to control the level and input of maple water and a hood to evacuate the steam.
- In the mid 70's, technology was introduced into the maple syrup industry with the invention of sap-collection systems. These blue plastic tubes replaced buckets, barrels, horses and tractors. With a vacuum pump, the maple water goes directly from the tree to the maple syrup storage tank. Every spout is connected to this system and the gathering process is automatically activated when the temperature rises enough for the sap to flow.
- In Quebec, the process has become part of the culture. City people often go to Cabanes a Sucre in early spring where lavish meals are served with maple syrup accompaniments. Tire sur la neige is a seasonal treat of thick hot syrup (boiled at 234oF) poured into fresh snow then eaten off sticks as it quickly cools.

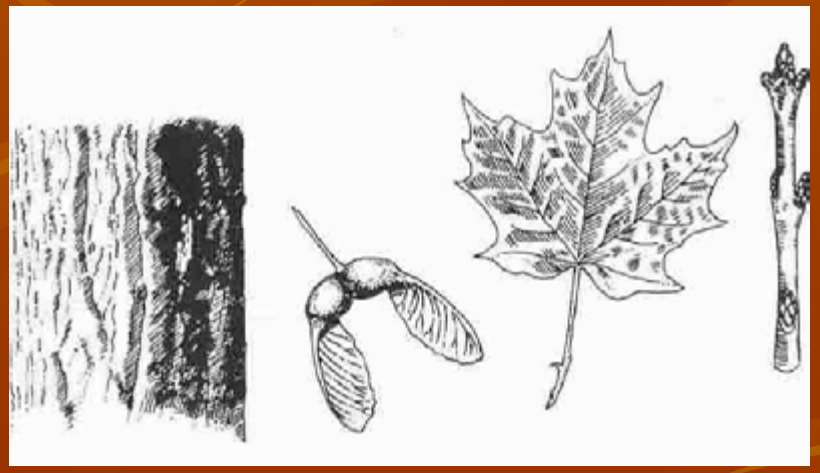
History of the Maple Syrup

History of the Maple Syrup

Quebec, where maple is King
The Tree Defined

- There are four varieties of Sugar Maple Trees. They strive on steep, rich soils and long, bitter winters. The other types of maple trees, namely the Red Maple and Silver Maple, are also used for maple syrup production although their sap has a lower sugar concentration.
- The main maple producing tree is known as the Sugar Maple or Hard Maple which provides the best and highest quality sap. It grows as tall as 100 feet.
- Sap flows naturally when spring comes. Maple syrup making starts from the natural phenomenon. During spring time, when nights are still cold (below freezing), the water from the soil is sucked into the tree via a natural absorption phenomena. During the day time, warmer temperatures create pressure in the tree. The pressure pushes back the water to the bottom of the tree which allows one to collect a part of the sap flowing down.
- To collect maple water from a maple tree, the tree must first be tapped. Any maple tree measuring 10-17 inches in diameter or more may be tapped. Trees18-24 inches in diameter can have no more than two taps. Larger trees over 25 inches may have a maximum of three taps. Tapping should not be done when the bark and wood is frozen.
- Once tapped, the tree releases maple water. Tapping the tree does no permanent damage to the tree.
- Tapholes which are 1-2 inches measured inside bark and 7/16”diameter are deep enough to ensure good sap yields.

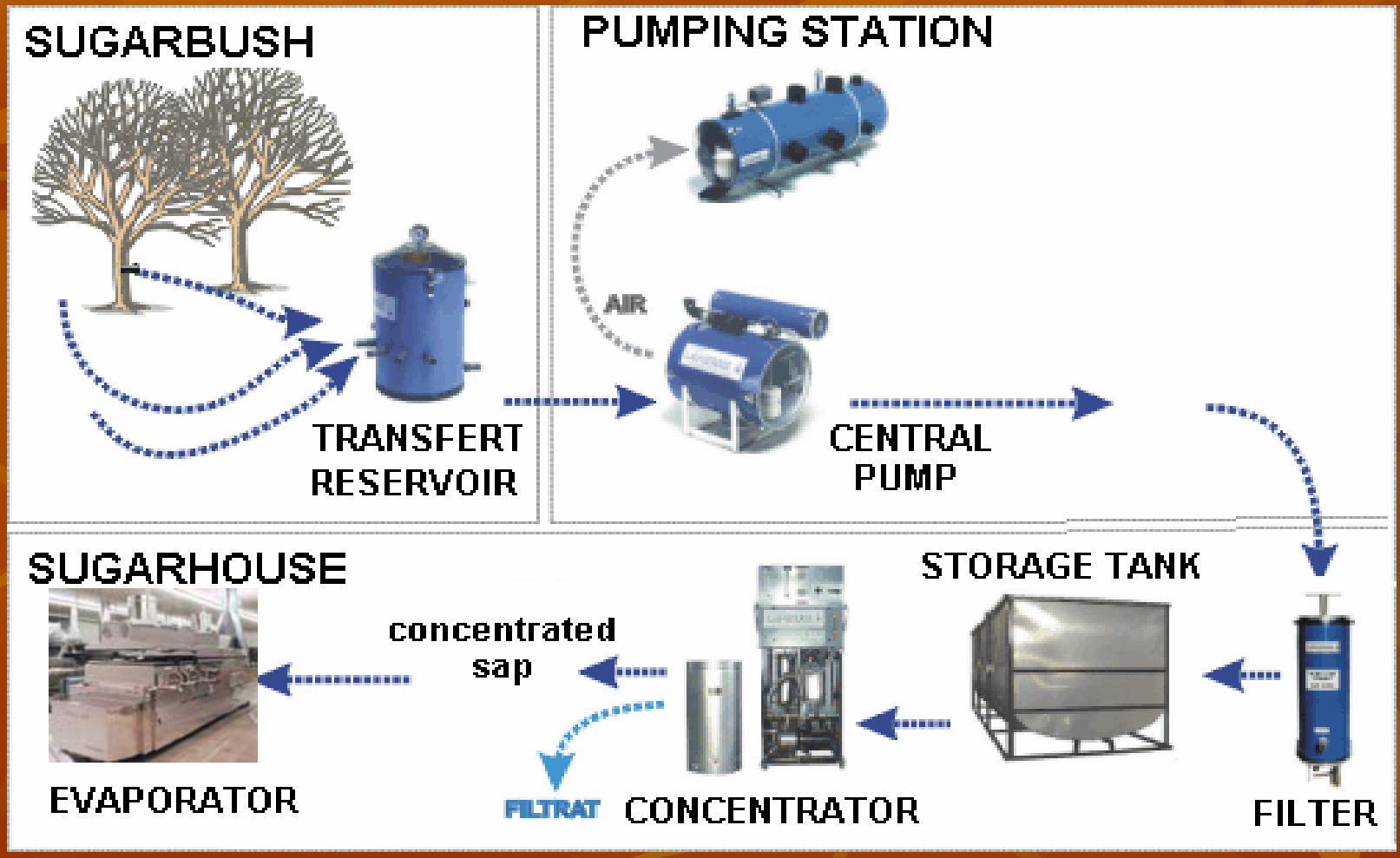
Sugar Maple Tree



Production Process, From Tree to Bottle

- The production is between March and April, Sap is collected over approximately 15 to 20 days, depending on the daily frost and defrost. The sugar content of sap averages 2.5%
- The maple tree will give, drop by drop, about 12 quarts of sap on a warm spring day. An average maple tree will yield between 35-40 quarts of sap (per season), which will produce between 1 -1.5 quarts of pure Maple Syrup, per season
- Maple water is evaporated to produce Maple Syrup
  - The Maple Syrup is ready when it reaches a temperature of ± 219.2° F
  - It takes 32 to 40 gallons of sap to make one gallon of Maple Syrup
  - To produce other maple products such as butter, taffy, soft sugar, hard or crystallized sugar, a maple syrup producer boils the maple in an evaporator to the temperature that is required for each type of desired product.
  - Maple syrup should be packed hot at a temperature of 180oF. After the container is full, the cap is placed on it and the container should be placed on its side in order to sterilize the neck and cap. To prevent an off-flavor call "stack burn", the containers should not be stacked close together until they are cool.
- Variation in color and taste is a natural phenomenon; the color and taste of Maple Syrup vary throughout the harvest season because of the natural composition. Also, the thermal treatment which the maple water undergoes in its transformation into Maple Syrup influences the color and taste of the finished product. Maple Syrup made from maple water tapped at the beginning of the season is generally clearer and lighter in taste. As seasons advance, the Maple Syrup becomes darker and more caramelized in flavor. The sugar content of Maple Syrup averages 66.5 BRIX. (Note: Agriculture Canada defines maple syrup at 66.5 BRIX)

Pumping Process



Evaporator Modern Techniques

There are many ways Maple Syrup producers save energy, hence money, in the evaporation process we will discuss the most common methods used these days.

- Reverse osmosis is probably the process used most by the bigger producers. This method consists of physically removing the water from the sap before passing it thru the evaporator. This saves a lot of energy in heating the excess water to be boiled off. Reverse osmisis is achieved by forcing the sap thru a filter whose pores are big enough to let the water molecules pass but too small for the sugar and other organic materials into the sap. This process can be repeated numerous times until the sap has a high sugar content. Usually, we are able to remove up to 75% of the water, thereby saving much fuel in the heating process.
- Another method which can be used in conjunction with reverse osmosis is preheating. This uses the excess generated heat in the final stage of the evaporator to preheat the incoming sap. This method can save up to 15% of total fuel cost for a sugar bush producer and is quite simple to put into practice.
- A much newer method consists of vapor compression technique similar to the one used for the desalinization of salt water. In the vapor compression process the water in maple sap is evaporated, but unlike open pan evaporators, the heat energy in the steam produced from the evaporating sap is captured and repeatedly reused. Evaporation takes place in an evaporation chamber in which hot sap is sprayed onto an even hotter surface. The result is a vaporization of some of the water molecules which are then pulled out of the chamber under negative pressure. This vapor is compressed, raising its temperature, and is reused to reheat the evaporating surface. Sap is recycled until the desired density is achieved. To increase efficiency, sap is preheated with heat exchangers which absorb heat from the evaporation chamber and finished syrup. External heat energy is required to start the evaporating process but only intermittently thereafter to maintain it.

MAPLE SYRUP
Grades/classification


EXTRA LIGHT


LIGHT


MEDIUM


AMBER


DARK

Introduction of Maple Syrup/Explanation of the different grades, color, flavor Quality Standards, Canada/Quebec Classification

There exists two types of classifications for Maple Syrup in Canada. The classification of the Canadian Federal Government and that of the Quebec Provincial Government. Quebec sugar makers have the choice to conform to one or another of the regulations in force.

- **CANADA FEDERAL GRADES**
- Canada No. 1 (extra clear, clear, medium)
- Canada No. 2 (amber)
- Canada No. 3 (Dark and any of grades above that have a slight taste of bud, sap and caramel)
- Canadian Maple Syrup classification is based on color and authenticity of the maple flavor. As such, maple syrup is categorized and graded according to its color, clearness, density and the strength of its maple flavor.

- **QUEBEC PROVINCIAL GRADES**
- Extra Light (AA)
- Light (A)
- Medium (B)
- Amber (C)
- Dark amber (D)

USDA Grade

- *Grade A Light Amber/Fancy*, (Canada No. 1 Extra Light/Quebec Grade AA): Light transmittance over 75%
- *Grade A Medium Amber,* (Canada No. 1 Light /Quebec Grade A): Light transmittance 61-74%
- *Grade A Dark Amber,* (Canada No. 1 Medium /Quebec Grade B): Light transmittance 44-60%
- *Grade B,* (Canada No. 2 Amber/Quebec Grade C): light transmittance 27-43%

  (Canada No. 3 Dark/Quebec Grade D): Light transmittance 0-26%

Maple Products & Storage

- Maple Products
  - Soft Maple candy- Maple sugar is heated to approximately 244 degrees F, cooled to 155 degrees F, stirred until crystallization starts, and then poured into molds
  - Hard Maple Candy- Maple Syrup is approximately 255 degrees F, it is stirred immediately until crystals form and then poured into molds
  - Maple Butter-Maple Syrup is heated to approximately 236 degrees F, it is cooled rapidly to about 70 degrees F and stirred rapidly to whip air into it also called Maple spread
  - Maple taffy- Maple Syrup is heated to 234 degrees F. The hot syrup is poured into strips on well packed snow and picked up with a fork or stick, crushed ice may be used in place of snow
  - Granulated Maple sugar-Maple Syrup is heated to approximately 238 degrees F and stirred hot, producing a coarse textured sugar
- Storage ~ to enjoy freshness of maple syrup
  - Unopened syrup stores easily, unrefrigerated. However, prolonged storage may cause the color of maple syrup to darken and the flavor to deteriorate. It is recommended to store maple syrup in the freezer, Maple Syrup won't freeze.
  - After opening, store in an air tight container. It should be kept in a refrigerator or freezer, it will slow down the crystallization process brought on by the evaporation of Maple Syrup. After opening and when storing for extended periods (one to three months), it is recommended to store Maple Syrup in the freezer. With the exception of the Bag in the box which can be store at room temperature at all times.

Maple Syrup's Nutritional Value compared with other Sweeteners (Sugar, Honey, Brown Sugar)

Are all the sugars created equal?

NOT REALLY, the table below shows the contribution of various sweeteners to the daily Value (DV*) of various nutrients. No doubt that maple syrup is well ahead of its competitor

*** Per 60 ml (1/4 cup, en % DV1)**

DV1: Daily value is the intake of a given nutrient deemed as to fulfill the daily nutritional needs of most individuals

	Maple Syrup*	Honey	White Sugar	Brown Sugar
Manganese	100	3	0	9
RiboFlavine	34	2	1	0
Zinc	11	1	0	1
Magnesium	5	0	0	7
Calcium	6	1	0	5
Potassium	5	1	0	6

FIVE HEALTH BENEFITS

- **100% NATURAL** The Maple Syrup from which we make taffy, sugar and butter (which doesn't really contain any butter!) is obtained through the concentration of sap of some varieties of maple trees. It contains no coloring agents, artificial flavorings, preservatives or other additives.

- **ESSENTIAL VITAMINS AND MINERALS**. Maple Syrup products are a significant source of several nutrients. For example, on average, a 4 Tbsp serving of Maple Syrup supplies more than 100% of our daily intake of manganese, 37% of riboflavin, 18% of zinc, 7% of magnesium and 5% of calcium and potassium. What other sweetener can beat that!

- **OTHER BENEFICIAL COMPOUNDS.** In addition to carbohydrates, vitamins and minerals, Maple Syrup and its products also contain phenolic compounds which are found in sap.

- **LOW ON THE GLYCEMIC INDEX.** The Glycemic Index (GI) is a scale that ranks foods on how they affect blood glucose levels. By consuming foods with a low GI rating (55 or lower), we can prevent or control heart disease, diabetes and obesity. Maple Syrup (GI 54) compares well with other sweetening products such as sugar (GI58) and honey (GI87).

- **MAPLE PRODUCTS: PARTNERS IN HEALTHY EATING**. In its latest report on dietary reference intakes, Health Canada concluded that current scientific data on the correlation between sugar consumption and the risk of diseases (obesity, cancer, hyperlipidemia and others) did not justify a reduction of sugar intake. There is a difference between foods that have been sweetened which supply nutrients (flavored milk and yogurt, fruit desserts and others) and those (such as pop drinks, candy and pastries) which do not supply much other than calories. In short, Maple Syrup products are completely compatible with a healthy diet.

Health Benefits

Maple syrup is sweet - and we're not just talking flavor.

Maple syrup, as an excellent source of manganese and a good source of zinc, can also be sweet for your health.

- **Sweeten Your Antioxidant Defenses**

  The trace mineral manganese is an essential cofactor in a number of enzymes important in energy production and antioxidant defenses. For example, the key oxidative enzyme *superoxide dismutase*, which disarms free radicals produced within the mitochondria (the energy production factories within our cells), requires manganese. One ounce of maple syrup supplies 22.0% of the daily value for this very important trace mineral.

- **Be Sweet to Your Heart with Maple Syrup**

  Maple syrup is a good sweetener to use if you are trying to protect the health of your heart. The zinc supplied by maple syrup, in addition to acting as an antioxidant, has other functions that can decrease the progression of atherosclerosis. Zinc is needed for the proper function of *endothelial* cells and helps to prevent the *endothelial* damage caused by oxidized LDL cholesterol and other oxidized fats. (The *endothelium* is the inner lining of blood vessels.) *Endothelial* membranes low in zinc are much more prone to injury. Additionally, studies have found that in adults deficient in manganese, the other trace mineral amply supplied in maple syrup, the level of HDL (the "good" cholesterol) is decreased.

- **Sweet Support for Your Immune System**

  Zinc and manganese are important allies in the immune system. Many types of immune cells appear to depend upon zinc for optimal function. Particularly in children, researchers have studied the effects of zinc deficiency (and zinc supplementation) on their immune response and their number of white blood cells, including specific studies on T lymphocytes, macrophages, and B cells (all types of white blood cells important for immune defenses). In these studies, zinc deficiency has been shown to compromise numbers of white blood cell and immune response, while zinc supplementation has been shown to restore conditions to normal. In addition to the role played by zinc, the manganese in maple syrup is important since, as a component of the antioxidant SOD, it helps lessen inflammation, thus supporting healing. In addition, manganese may also act as an immunostimulant.

- **Real Healthy Men Use Maple Syrup**

  Maple syrup may help to support reproductive health and provides special benefits for men. Zinc is concentrated more highly in the prostate than in any other human tissue, and low levels of zinc in this gland relate to a higher risk for prostate cancer. In fact, zinc is a mineral used therapeutically by healthcare practitioners to help reduce prostate size. Manganese may also play a role in supporting men's health since, as a catalyst in the synthesis of fatty acids and cholesterol, it also participates in the production of sex hormones, thus helping to maintain reproductive health.

- **Safety:** Maple syrup in its natural state is not a commonly allergenic food and is not known to contain measurable amounts of goitrogens, oxalates, or purines.

- Source: Asako Aramaki, R.D.

Values of Manganese/Zinc

What can high-manganese foods do for you?

- Help your body utilize several key nutrients such as biotin, thiamin, ascorbic acid, and choline
- Keep your bones strong and healthy
- Help your body synthesize fatty acids and cholestorol
- Maintain normal blood sugar levels
- Promote optimal function of your thyroid gland
- Maintain the health of your nerves
- Protect your cells from free-radical damage

- Source: Asako Aramaki, R.D.

What can high-zinc foods do for you?

- Help balance blood sugar
- Stabilize your metabolic rate
- Prevent a weakened immune system
- Support an optimal sense of smell and taste

Maple Products available

- Maple Syrup
- Maple Butter (maple spread)
- Maple Sugar
- Maple Jelly
- Maple Flakes
- Maple Candy
- Maple Gift Sets
- Flavored Maple Syrup
- Maple Mustard
- Maple Dressing
- Maple Vinegar
- Maple Chocolate
- Maple Peanut Brittle
- Maple Frosted Almonds
- Maple Concentrate

Tasting Maple Syrup

- Although professional tasters require extensive training, you can sharpen your tasting skills by following these steps:
  - First, smell the syrup by taking three quick sniffs. Make a mental note of your impression. Next, take a small sip of the syrup and swirl it around in your mouth. It is a good idea to spit it out if you can. Take about a minute to concentrate on the full range of flavors.
  - Try to associate the flavor with your own experience (for example, the aroma from a bag of marshmallows).
  - If possible, share your reaction with others, as this often helps trigger memory association. Once you have identified what you think characterizes the taste, memorize the sensation and the name for it (for example, vanilla).
  - Finally, try to assess the degree of intensity (e.g.: mild, medium or strong).

Maple Tasting-Sensory Profile Vocabulary

Dominant Flavors

- Roasted
  - Light- Golden Sugar, Chicory, Toast
  - Medium-Cooked Sugar-caramelized, Burnt wood, Ground brown coffee, brown coffee bean, Chocolate
  - Strong- Burnt Sugar, Ground Black Coffee, Black Coffee Bean, Smoke d
- Confectionery
  - Light- White Sugar
  - Medium- Corn Syrup, Light brown sugar
  - Strong- Dark brown sugar, molasses, sponge toffee
- Maple*
  - Maple, Roasted dandelion root

Variable as a dominant flavor

- Woody
  - Firewood, Wet wood, Softwood (pine, fir, larch, juniper, cedar, etc)
- Vanilla
  - Marshmallow
  - Vanilla Pod

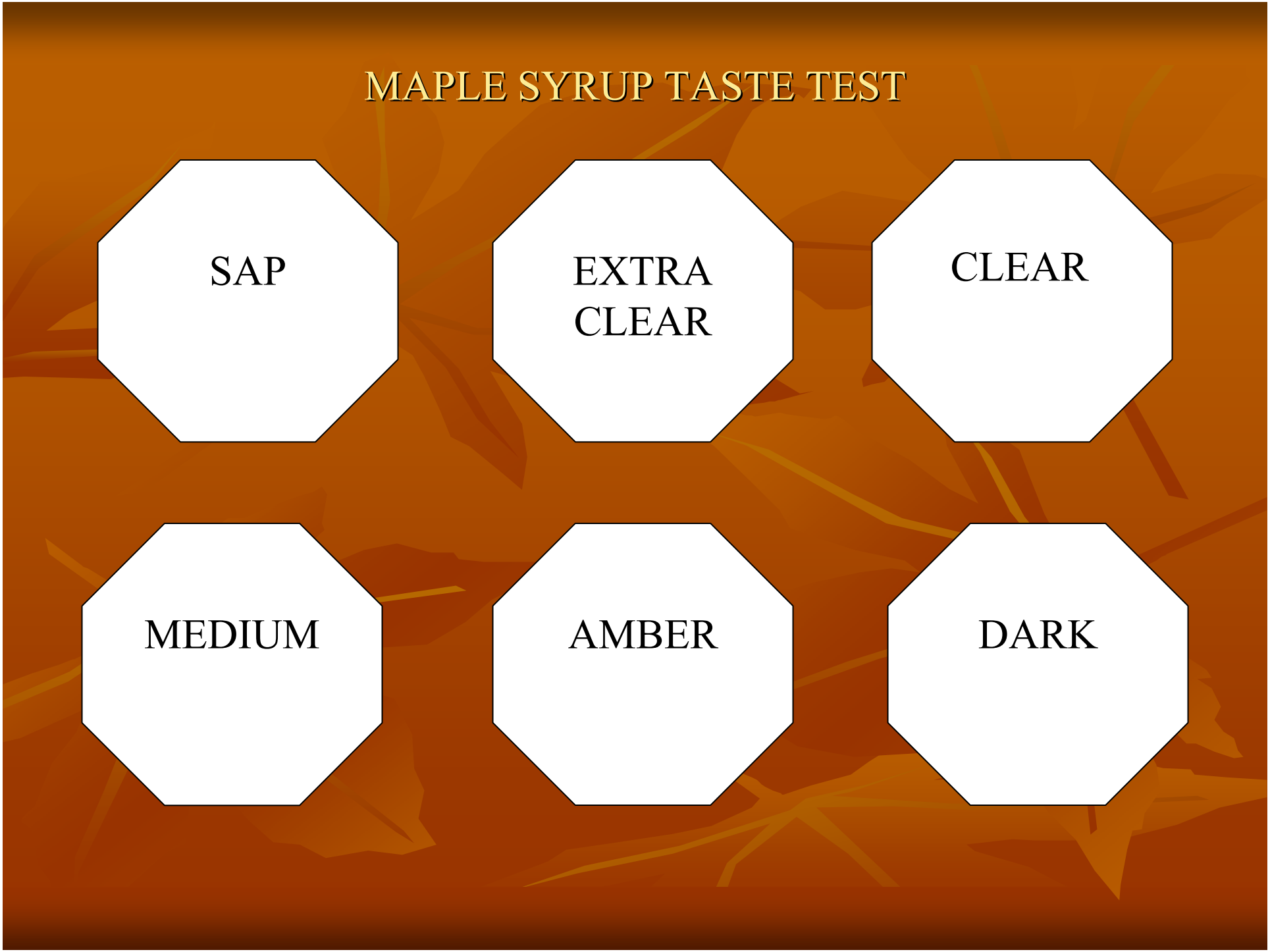
*Important Note:

During production, Maple Syrup is often blended in an effort to obtain perfection of the flavor most often demanded by consumers…that of the flavor of Maple.

Minor Flavors

- Herbal
  - Fresh Herbs- Stem, Grassy, Shoot, Bud
  - Dry Herbs- Crushed leaves, Nutshells, Dry herbs, Hay
  - Fermented Herbs- Silage
- Plants, Humus, Forest, Cereals
  - Humus, Forest- Mushroom, Mould, Potato
  - Cereals-Malt, Oat, Wheat, Rye
- Fruity
  - Nuts- Bitter Almond, Hazelnut, Nuts
  - Peach, fruits with pits or seeds
- Milky
  - Fresh- Butter, Cream, Milk
  - Heated- Butter, Milk
- Floral
  - Flowers
  - Honey
- Spicy
  - Cloves
  - Cinnamon
  - Anis-Black Liquorice

MAPLE SYRUP TASTE TEST



Federation of Quebec Maple Syrup Producers

- **Federation of Québec Maple Syrup Producers** represents 7,000 syrup maker producers from Québec. Québec produces over 80 percent of the worlds Maple Syrup, equal to 93 percent of Canadian production. Its mandate is to create a common resource and provide suitable tools to control the quantity and the preservation of maple products. Since February 28, 2002, the Federation of Québec Maple Syrup Producers has acted as the exclusive sales agency of syrup makers; receiving and marketing maple syrup sold in large containers of more than 5 liters. Through its promotional activities and market development, the Federation of Québec Maple Syrup Producers contributes to the development, knowledge, and consumption of maple products in Québec and around the world.

Federation of Quebec Maple Syrup Producers

www.siropderable.ca

CITADELLE

- **Citadelle Maple Syrup Producers' Cooperative**
  In existence for three-quarters of a century, Citadelle Maple Syrup Producers' Cooperative - the corporate name of the Cooperative since 1996 - has some 2,700 members, or nearly one-third of all Quebec Maple Syrup producers. The Cooperative does business with over 2,000 non-member producers through its division and three subsidiaries in Quebec and New Brunswick.

www.citadelle-camp.com
www.shadymaple.ca
www.mapledelights.com

DECACER

- **Decacer**
  In business for over 6 years, Decacer is a Quebec company with two production centers, one in Saint-Antoine-de-Tilly near Quebec City and another in Dégelis in the Lower St. Lawrence region. Its primary activity is Maple Syrup bottling. Equinox Organic Maple Flakes and Organic Cranberry Maple Flakes are Decacer's latest innovation. These natural sweeteners make healthful eating a lot more flavorful.

www.decacer.com

Heritage Yamaska

- **Heritage Yamaska**
  (La Coulée d'Abbotsford) By 1876, Jean-Baptiste Chagnon was selling his Maple Syrup and sugar products at regional public markets. Through the years, five generations of his descendants have followed in his footsteps, developing a much sought-after expertise in the production of maple products. La Coulee d'Abbotsford was named "Maitre Sucrier" or master sugar maker by the Quebec Department of Agriculture. This prestigious distinction is awarded to the best maple producer and is the hallmark of products of exceptional quality.

www.heritageyamaska.com

Lapierre Maple Farm

- **Lapierre Maple Farm**
  Lapierre Maple Farm is principally located in Milan, Canada and taps roughly 150,000 sugar maple trees each season. About 90% of Lapierre's sugar bush is made up of sugar maples and 10% are a mixture of silver and red maple. In 2002, we provided Metro Inc. stores' with 100% pure Maple Syrup that identified the specific growing region (terroir) or sugar bush, the only producer to do so. We believe that much like wine and olive oil, the flavor profile of maple varies with each growing region. The Canadian Counsel of Food Distribution honored Metro Inc. and Lapierre Maple Farm at the Canadian Grand Prix with a new product award in 2004 for the exceptional quality of their Maple Syrup Sirobec ™ product.

  Lapierre is committed to selling only 100% pure Maple Syrup and to never compromising its quality and the unique flavor identity (terroir) of the maple products derived from our sugarbush.

  Savor and discover 100 % pure Lapierre Maple Syrup ! A unique gift of nature….shared with you…from our sugarbush to your table!

  www.maplesyrupusa.com

Cooking Tips

- **Use as a sugar substitute**
  In general, Maple Syrup can be substituted for granular sugar in baked goods by following these rules of thumb:
  For each cup of granulated sugar, use 1-1/2 cups of Maple Syrup.
  Reduce other liquids in the recipe by about one-half.
  Add 1/4 teaspoon baking soda for each cup of Maple Syrup.
  Decrease oven temperature by 25 degrees F.

A Few Quick Serving Ideas

- Maple syrup, used in place of table sugar as a sweetener, gives tea and coffee a unique taste.
- Pour some maple syrup on oatmeal topped with walnuts and raisins.
- Add maple syrup and cinnamon to puréed cooked sweet potatoes.
- Combine maple syrup with orange juice and tamari and use as a marinade for baked tofu
- Spread peanut butter on a piece of whole wheat toast, top with sliced bananas and then drizzle maple syrup on top for a sweet treat.

MAPLE RECIPES

- For Delicious Maple Recipes visit: http://www.domorewithmaple.com/maplesyrupprecipes.html

Bon appétit!

- If you have any questions on the recipes or the curriculum, please contact Chef LaGarde via email at cheflagarde@domorewithmaple.com

References

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- http://laws.justice.gc.ca/en/showtdm/cr/C.R.C.-c.289?noCookie
- Agriculture & Agri-Food Canada
- Federation of Quebec Maple Syrup Producers
- The Maple Syrup book (Janet Eagleson & Rosemary Hasner
- A taste of Maple Book (Micheline Mongrain-Dontigny)

Canadian Nutrient File (CNF)

Sweets, syrups, maple, bulk
Food Code : 4326

Nutrient name	Unit	30ml / 41 g	100ml / 136 g	250ml / 340 g
Proximates				
Moisture	g	13.13	43.75	109.39
Ash	g	0.291	0.970	2.426
Protein	g	0.00	0.00	0.00
Total Fat	g	0.10	0.33	0.83
Carbohydrate	g	27.31	91.03	227.58
Alcohol	g	0.0	0.0	0.0
Energy (kcal)	kCal	107	355	888
Energy (kJ)	kJ	446	1488	3719
Other Carbohydrates				
Fibre, total dietary	g	0.0	0.0	0.0
Glucose	g	0.27	0.89	2.21
Fructose	g	0.14	0.46	1.14
Galactose	g	0.00	0.00	0.00
Lactose	g	0.00	0.00	0.00
Sucrose	g	24.06	80.20	200.50
Sugars, total	g	24.46	81.54	203.86
Minerals				
Calcium, Ca	mg	44	148	370
Iron, Fe	mg	0.49	1.63	4.08
Magnesium, Mg	mg	9	28	71
Phosphorus, P	mg	1	3	7
Potassium, K	mg	92	306	765
Sodium, Na	mg	4	12	31
Zinc, Zn	mg	0.29	0.95	2.38
Copper, Cu	mg	0.030	0.101	0.252
Manganese, Mn	mg	0.939	3.130	7.825
Selenium, Se	µg	0.2	0.8	2.0
Vitamins				
Beta carotene	µg	0	0	0
Alpha carotene	µg	0	0	0
Retinol	µg	0	0	0
Retinol activity equivalents, RAE	µg	0	0	0
Folacin, total	µg	0	0	0
Folic acid, synthetic form	µg	0	0	0
Folate, naturally occurring	µg	0	0	0
Dietary folate equivalents, DFE	µg	0	0	0
Niacin	mg	0.033	0.110	0.276
Niacin equivalents	NE	0.033	0.110	0.276
Pantothenic acid	mg	0.012	0.041	0.102

Riboflavin	mg	0.519	1.731	4.328
Thiamin	mg	0.027	0.090	0.225
Vitamin B-6	mg	0.001	0.003	0.007
Vitamin B-12	µg	0.00	0.00	0.00
Choline, total	mg	0.7	2.2	5.4
Vitamin C	mg	0.0	0.0	0.0
Vitamin D	µg	0.000	0.000	0.000
Vitamin K	µg	0.0	0.0	0.0
Tocopherol, alpha	mg	0.00	0.00	0.00
Amino Acids				
Tryptophan	g	0.000	0.000	0.000
Threonine	g	0.000	0.000	0.000
Isoleucine	g	0.000	0.000	0.000
Leucine	g	0.000	0.000	0.000
Lysine	g	0.000	0.000	0.000
Methionine	g	0.000	0.000	0.000
Cystine	g	0.000	0.000	0.000
Phenylalanine	g	0.000	0.000	0.000
Tyrosine	g	0.000	0.000	0.000
Valine	g	0.000	0.000	0.000
Arginine	g	0.000	0.000	0.000
Histidine	g	0.000	0.000	0.000
Alanine	g	0.000	0.000	0.000
Aspartic acid	g	0.000	0.000	0.000
Glutamic acid	g	0.000	0.000	0.000
Glycine	g	0.000	0.000	0.000
Proline	g	0.000	0.000	0.000
Serine	g	0.000	0.000	0.000
Hydroxyproline	g	0.000	0.000	0.000
Lipids				
Fatty acids, saturated, total	g	0.015	0.049	0.122
4:0	g	0.000	0.000	0.000
6:0	g	0.000	0.000	0.000
8:0	g	0.000	0.000	0.000
10:0	g	0.000	0.000	0.000
12:0	g	0.000	0.000	0.000
14:0	g	0.000	0.000	0.000
16:0	g	0.015	0.049	0.122
18:0	g	0.002	0.005	0.014
Fatty acids, monounsaturated, total	g	0.026	0.087	0.218
16:1	g	0.000	0.000	0.000
18:1	g	0.026	0.087	0.218
20:1	g	0.000	0.000	0.000
22:1	g	0.000	0.000	0.000
Fatty acids, polyunsaturated, total	g	0.041	0.136	0.340
18:2	g	0.041	0.136	0.340
18:3	g	0.000	0.000	0.000
18:3n3cccn-3	g	0.000	0.000	0.000
18:3n6cccn-6	g	0.000	0.000	0.000

18:4	g	0.000	0.000	0.000
20:3	g	0.000	0.000	0.000
20:3n-3	g	0.000	0.000	0.000
20:3n-6	g	0.000	0.000	0.000
20:4	g	0.000	0.000	0.000
20:5n-3	g	0.000	0.000	0.000
22:5n-3	g	0.000	0.000	0.000
22:6n-6	g	0.000	0.000	0.000
Cholesterol	mg	0	0	0
Other components				
Caffeine	mg	0	0	0
Theobromine	mg	0	0	0
Lutein and zeaxanthin	µg	0	0	0
Lycopene	µg	0	0	0
Beta cryptozanthin	µg	0	0	0

Canadian Nutrient File, 2010

(CFG) - Refers to the serving size based on Eating Well with Canada's Food Guide

Date : Apr 13, 2011

Canadian Nutrient File (CNF)

sweets, syrups, maple, prepackaged
Food Code : 6175

Nutrient name	Unit	30ml / 40 g	100ml / 130 g
Proximates			
Moisture	g	12.84	41.72
Ash	g	0.218	0.708
Protein	g	0.00	0.00
Total Fat	g	0.06	0.19
Carbohydrate	g	26.89	87.38
Alcohol	g	0.0	0.0
Energy (kcal)	kCal	108	351
Energy (kJ)	kJ	452	1468
Other Carbohydrates			
Fibre, total dietary	g	0.0	0.0
Glucose	g	0.24	0.77
Fructose	g	0.13	0.42
Galactose	g	0.00	0.00
Lactose	g	0.00	0.00
Sucrose	g	23.06	74.94
Sugars, total	g	23.44	76.17
Minerals			
Calcium, Ca	mg	29	95
Iron, Fe	mg	0.48	1.56
Magnesium, Mg	mg	9	30
Phosphorus, P	mg	1	3
Potassium, K	mg	86	279
Sodium, Na	mg	5	15
Zinc, Zn	mg	0.80	2.58
Manganese, Mn	mg	1.538	5.000
Selenium, Se	µg	0.2	0.8
Vitamins			
Beta carotene	µg	0	0
Alpha carotene	µg	0	0
Retinol	µg	0	0
Retinol activity equivalents, RAE	µg	0	0
Folacin, total	µg	0	0
Folic acid, synthetic form	µg	0	0
Folate, naturally occurring	µg	0	0
Dietary folate equivalents, DFE	µg	0	0
Niacin	mg	0.066	0.213
Niacin equivalents	NE	0.066	0.213
Pantothenic acid	mg	0.014	0.047
Riboflavin	mg	0.293	0.952
Thiamin	mg	0.007	0.023

Vitamin B-6	mg	0.001	0.003
Vitamin B-12	µg	0.00	0.00
Vitamin C	mg	0.0	0.0
Vitamin D	µg	0.000	0.000
Vitamin K	µg	0.0	0.0
Tocopherol, alpha	mg	0.00	0.00
Amino Acids			
Tryptophan	g	0.000	0.000
Threonine	g	0.000	0.000
Isoleucine	g	0.000	0.000
Leucine	g	0.000	0.000
Lysine	g	0.000	0.000
Methionine	g	0.000	0.000
Cystine	g	0.000	0.000
Phenylalanine	g	0.000	0.000
Tyrosine	g	0.000	0.000
Valine	g	0.000	0.000
Arginine	g	0.000	0.000
Histidine	g	0.000	0.000
Alanine	g	0.000	0.000
Aspartic acid	g	0.000	0.000
Glutamic acid	g	0.000	0.000
Glycine	g	0.000	0.000
Proline	g	0.000	0.000
Serine	g	0.000	0.000
Hydroxyproline	g	0.000	0.000
Lipids			
Fatty acids, saturated, total	g	0.014	0.047
4:0	g	0.000	0.000
6:0	g	0.000	0.000
8:0	g	0.000	0.000
10:0	g	0.000	0.000
12:0	g	0.000	0.000
14:0	g	0.000	0.000
16:0	g	0.014	0.047
18:0	g	0.000	0.001
Fatty acids, monounsaturated, total	g	0.026	0.083
16:1	g	0.000	0.000
18:1	g	0.026	0.083
20:1	g	0.000	0.000
22:1	g	0.000	0.000
Fatty acids, polyunsaturated, total	g	0.040	0.130
18:2	g	0.040	0.130
18:3	g	0.000	0.000
18:3n3cccn-3	g	0.000	0.000
18:3n6cccn-6	g	0.000	0.000
18:4	g	0.000	0.000
20:3	g	0.000	0.000
20:3n-3	g	0.000	0.000
20:3n-6	g	0.000	0.000
20:4	g	0.000	0.000

20:5n-3	g	0.000	0.000
22:5n-3	g	0.000	0.000
22:6n-6	g	0.000	0.000
Cholesterol	mg	0	0
Other components			
Caffeine	mg	0	0
Theobromine	mg	0	0
Lutein and zeaxanthin	µg	0	0
Lycopene	µg	0	0
Beta cryptozanthin	µg	0	0

Canadian Nutrient File, 2010
(CFG) - Refers to the serving size based on Eating Well with Canada's Food Guide

Date : Apr 13, 2011

Syrups, maple

Refuse: 0%
NDB No: 19353 (Nutrient values and weights are for edible portion)

Nutrient	Units	Value per 100 grams	Number of Data Points	Std. Error	1.00 X 1 ------- 315g	1.00 X 1 tbsp ------- 20g
Proximates						
Water	g	32.39	12	0.357	102.03	6.48
Energy	kcal	260	0	0	819	52
Energy	kJ	1088	0	0	3427	218
Protein	g	0.04	8	0.037	0.13	0.01
Total lipid (fat)	g	0.06	8	0.058	0.19	0.01
Ash	g	0.47	6	0	1.48	0.09
Carbohydrate, by difference	g	67.04	0	0	211.18	13.41
Fiber, total dietary	g	0	1	0	0	0
Sugars, total	g	60.44	9	5.089	190.39	12.09
Sucrose	g	58.32	25	3.685	183.71	11.66
Glucose (dextrose)	g	1.6	22	0.58	5.04	0.32
Fructose	g	0.52	25	0.218	1.64	0.1
Lactose	g	0	6	0	0	0
Maltose	g	0	6	0	0	0
Galactose	g	0	6	0	0	0
Minerals						
Calcium, Ca	mg	102	16	6.652	321	20
Iron, Fe	mg	0.11	8	0.003	0.35	0.02
Magnesium, Mg	mg	21	16	0.203	66	4
Phosphorus, P	mg	2	3	0.929	6	0
Potassium, K	mg	212	16	12.41	668	42
Sodium, Na	mg	12	16	4.228	38	2
Zinc, Zn	mg	1.47	16	0.772	4.63	0.29
Copper, Cu	mg	0.018	6	0	0.057	0.004
Manganese, Mn	mg	2.908	16	0.608	9.16	0.582
Selenium, Se	mcg	0.6	0	0	1.9	0.1

Vitamins						
Vitamin C, total ascorbic acid	mg	0	1	0	0	0
Thiamin	mg	0.066	5	0.027	0.208	0.013
Riboflavin	mg	1.27	5	0.201	4	0.254
Niacin	mg	0.081	5	0.01	0.255	0.016
Pantothenic acid	mg	0.036	3	0.003	0.113	0.007
Vitamin B-6	mg	0.002	3	0.002	0.006	0
Folate, total	mcg	0	3	0.167	0	0
Folic acid	mcg	0	0	0	0	0
Folate, food	mcg	0	3	0.167	0	0
Folate, DFE	mcg_DFE	0	0	0	0	0
Choline, total	mg	1.6	0	0	5	0.3
Vitamin B-12	mcg	0	1	0	0	0
Vitamin B-12, added	mcg	0	0	0	0	0
Vitamin A, RAE	mcg_RAE	0	0	0	0	0
Retinol	mcg	0	0	0	0	0
Carotene, beta	mcg	0	0	0	0	0
Carotene, alpha	mcg	0	0	0	0	0
Cryptoxanthin, beta	mcg	0	0	0	0	0
Vitamin A, IU	IU	0	1	0	0	0
Lycopene	mcg	0	0	0	0	0
Lutein + zeaxanthin	mcg	0	0	0	0	0
Vitamin E (alpha-tocopherol)	mg	0	0	0	0	0
Vitamin E, added	mg	0	0	0	0	0
Vitamin D (D2 + D3)	mcg	0	0	0	0	0
Vitamin D	IU	0	0	0	0	0
Vitamin K (phylloquinone)	mcg	0	0	0	0	0
Lipids						
Fatty acids, total saturated	g	0.007	0	0	0.022	0.001
4:0	g	0	0	0	0	0
6:0	g	0	0	0	0	0
8:0	g	0	0	0	0	0
10:0	g	0	0	0	0	0
12:0	g	0	0	0	0	0
14:0	g	0	0	0	0	0

16:0	g	0.006	0	0	0.019	0.001
18:0	g	0.001	0	0	0.003	0
Fatty acids, total monounsaturated	g	0.011	0	0	0.035	0.002
16:1 undifferentiated	g	0	0	0	0	0
18:1 undifferentiated	g	0.011	0	0	0.035	0.002
20:1	g	0	0	0	0	0
22:1 undifferentiated	g	0	0	0	0	0
Fatty acids, total polyunsaturated	g	0.017	0	0	0.054	0.003
18:2 undifferentiated	g	0.017	0	0	0.054	0.003
18:3 undifferentiated	g	0	0	0	0	0
18:4	g	0	0	0	0	0
20:4 undifferentiated	g	0	0	0	0	0
20:5 n-3 (EPA)	g	0	0	0	0	0
22:5 n-3 (DPA)	g	0	0	0	0	0
22:6 n-3 (DHA)	g	0	0	0	0	0
Cholesterol	mg	0	1	0	0	0
Other						
Alcohol, ethyl	g	0	0	0	0	0
Caffeine	mg	0	0	0	0	0
Theobromine	mg	0	0	0	0	0

High-Performance Liquid Chromatography Characterization and Identification of Antioxidant Polyphenols in Maple Syrup*

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ABSTRACT

Maple syrup of four grades (extra-light, light, medium, and dark) of the 2007 crop was provided by three local (St. Joseph's Island, Ontario, Canada) producers. Twenty-four phenolic compounds were isolated from a medium-grade syrup and identified on the basis of spectral and chemical evidence. They were (a) benzoic acid and several hydroxylated and methoxylated derivatives (gallic acid, 1-O.-galloyl-β-d-glucose, γ-resorcylic acid); (b) cinnamic acid derivatives (p.-coumaric acid, 4-methoxycinnamic acid, caffeic acid, ferulic acid, sinapic acid, and the ester chlorogenic acid); (c) flavonoids, the flavanols catechin and epicatechin, and the flavonols kaempferol and its 3-O.-β-d-glucoside, 3-O.-β-d-galactoside, quercetin and its 3-O.-β-d-glucoside, 3-O.-β-L-rhamnoside and 3-O.-rhamnoglucoside (rutin). Traces obtained at 280 and 350 nm in HPLC runs of the ethyl acetate–soluble fractions of eight samples indicated the presence of many more phenolic substances, most at very low concentration with some varibilities in peak heights, but not in retention times, among the syrups. In view of the well-established antioxidant activity these substances possess, it is suggested that it is the complexity of the mixture rather than any one compound that may serve to counter the unhealthful presence of the high concentration of sugars in the syrup.
Keywords: Acer, Aceraceae, A. saccharum. Marsh, ellagic acid, flavonols, gallic acid and/or gallates, phenolics

Introduction

Maples (Acer. spp., family Aceraceae) are among the most important hardwood species in North America (van Gelderen et al., 1994). Of the approximately 160 known species

of the genus, only six are native to eastern Canada (Farrar, 1995). Of these, A. rubrum. L. (red maple) and A. saccharum. Marsh (sugar maple) are responsible for most of the red to orange autumnal coloration of Northeastern American forests. Sugar maple, found only in North America, is one of the most valuable commercial hardwoods in the Maritime Provinces and in the southern parts of Ontario and Quebec and is the main source of maple syrup for which Canada is so well-known. Syrup is made from sap that the sugar maple tree produces in great abundance in late winter, when daytime temperatures exceed the freezing point and nighttime temperatures fall below it. The colorless, watery sap is collected by drilling a hole into the new wood of the trunk and inserting a collection device (called a spile). It is composed of approximately 97–98% water, thus requiring about 40 L of sap to produce 1 L of syrup. The original process used to effect concentration by the aboriginal peoples who invented it is simple heating. In the modern sugar shanty, large volumes of sap are concentrated by using continuous-feed evaporators heated by wood or oil stoves. The shallow evaporation pan with a large surface area is designed so that it allows for continuous addition of unprocessed sap at one end of its surface and the continual removal of the syrup at the other end. The more recent introduction of the process of reverse osmosis reduces the need for fuel. Excess water (75%) is removed from the sap by forcing it under pressure across membranes, leaving behind the sugars and other organic substances. Repeated passes of the sap results in additional water loss. By thus concentrating the sap before it enters the evaporator, the time that it is processed at elevated temperatures is greatly shortened. During the heating process, the typical flavor and brown color of maple syrup develops due to caramelization of the sugars and oxidation of the phenolic constituents. Some Maillard reaction products may also be formed (Kermasha et al., 1995) if nitrogenous compounds are present. Typically, the color becomes deeper as the season progresses, and the syrups are graded as “light” (in color), “medium” and “amber.” Sap concentrated in a rotary evaporator at low temperature (> 30°C) has neither the color nor the flavor of maple syrup (personal observation).

Recently, there has been considerable interest in the evaluation of phenolics from plant sources owing to their antioxidant properties. Phenolics are hydrophilic substances; a common origin is the aromatic shikimic acid. We have begun a series of investigations to increase our understanding of the role that phenolics play in forestry, and our studies have included several species of maples. We have shown that the most likely reason that red maple (A. rubrum.) is highly resistant to forest tent caterpillar is because of the presence of high amounts of ethyl m.-digallate (Abou-Zaid et al., 2001). It has both the highest insect antifeedant activity and concentration of any compound in red maple but is not present in the susceptible sugar maple (A. saccharum.), lending support to the suggestion that it is the major resistance factor in red maple leaves. Red maple leaves also contain a rare galloyl sugar, galloyl rhamnose (Abou-Zaid & Nozzolillo, 1999), and both red and sugar maples contain small amounts of methyl gallate (Abou-Zaid et al., 2007).

The current study investigates phenolics in finished maple syrup produced on St. Joseph Island near Sault Ste. Marie, Ontario, Canada; two of the producers used the conventional boiling method, and one used reverse osmosis. Kermasha et al. (1995) and Theriault et al. (2006) identified several phenolic compounds in maple syrup from Quebec and the sap

from which it was made. Thériault et al. (2006) reported on the antioxidant activities of the phenolic mixture present in the Quebec maple products and compared activities of samples produced at early and later times in the short late-winter season. Our results include isolation and identification from syrup produced by reverse osmosis of 23 phenolic compounds, including seven flavonoids. The high-performance liquid chromatography (HPLC) traces show notable differences in the amounts at individual phenolic peaks that can be more closely tied to the color grade rather than to mode of production.

Materials and Methods

Maple syrup was obtained from three different producers located on St. Joseph Island, Ontario, Canada (lat. 46.25 N, long. 83.88 W): (1) produced by conventional concentration: (a) Irwin's Maple Products, Canada No. 1 Light and Canada No. 1 Medium; and (b) Thompson's Maple Products, Canada No. 1 Light and Canada No. 1 Medium; (2) produced by reverse osmosis: Gilbertson's Maple Products, Canada No.1 Extra Light, Canada No. 1 Light, Canada No. 1 Medium, and Amber (dark).
HPLC conditions

An Agilent Technology 1200 Liquid Chromatograph equipped with a computer and Chem Station software (Chem 32), a binary pump SL (G1312B), a high-performance autosampler SL (G1367C), and an autoscan photodiode array spectrophotometer detector (DAD; Agilent Technology G1315C) were used. An Agilent Technology Eclipse Plus C-18, 5 μ m (4.6 × 150 mm i.d.) reverse-phase analytical column was also used. A linear gradient chromatographic technique was used at room temperature with the following solvent system: solvent A = MeOH/acetonitrile (95:5); solvent B = 0.05% aqueous HCOOH; starting at 85% A:15% B and ending 42 min later with 5% A:95% B and a flow rate set at 1 mL/min. Two fixed detection wavelengths were used, at 280 and 350 nm, and the resolved peaks were scanned by the photodiode array detector from 190 to 450 nm. Dilute solutions (10 mg/mL) of the ethyl acetate extracts were passed through a 13-mm GHP 0.45-μ m Minispike filters (Waters, EDGE) and 10-μ L aliquots were used for injection onto an HPLC column with and without spiking with standards. Peaks were identified on the basis of retention times and UV spectra where possible.
Preparation of extracts
For analysis by HPLC

Samples (10 mL) of each kind of syrup were extracted at room temperature by liquid-liquid extraction using ethyl acetate three times (3 × 5 mL). The combined ethyl acetate extracts were evaporated under nitrogen to dryness to yield the following: from Gilbertson's Maple Products, 15 mg of extra-light, 21 mg of light, and 24 mg of medium; from Irwin's Maple Products, 19 mg of light and 15 mg of medium; and from Thompson's Maple Products, 23 mg of light and 24 mg of medium. The residues were dissolved in methanol/water 85:15 at a concentration of 10 mg/mL and passed through 13-mm GHP 0.45-μ m Minispike filters (Waters, EDGE) prior to HPLC analysis.
For isolation and identification of compounds

A sample (2 L) of medium-grade (Gilbertson's Maple Products) was evaporated under reduced pressure until most of the water had been removed. The residue was freeze-dried and weighed to obtain 1878 g.

Fractionation of bulk extract

The freeze-dried maple syrup (200 g) was dissolved in 100 mL distilled water and adsorbed onto polyvinylpolypyrrolidone (PVPP) powder (Sigma) packed in a Buchner funnel (2 L). Elution was carried out at a slow rate initially with water to wash out sugars and other nonadsorbed substances followed by aliquots of increasing concentrations (20%, 50%, 70%, and 100%) of methanol to produce five fractions. Each fraction was concentrated under vacuum and chromatographed one-dimensionally on Whatman No. 1 chromatography paper using either BAW (n.-butanol–acetic acid–water, 4:1:5, upper phase), water, or acetic acid–water (15:85). Bands detected by absorbance/fluorescence under short-wave light (254 nm) and long-wave light (366 nm) were eluted with methanol and placed onto a PVPP column from which they were eluted with the following solvent systems sequentially: (1) CH2Cl2-EtOH-MeCOEt-Me2CO (1:1:1:1), (2) EtOH-MeCOEt-Me2CO-H2O (1:1:1:1), and (3) EtOH-H2O (1:1). Purification was achieved with the aid of a low-pressure liquid chromatograph (Chemco low-prep pump, model 9 1-M-8R, with 6-port valve, max. 80 mL/min). Final clean-up of the compounds was achieved on a Sephadex LH-20 column (1 cm × 50 cm), using methanol as the eluting solvent, a step essential to obtaining good spectra of purified compounds.

Identification of the isolated compounds

UV spectra were recorded on a UV-Vis Beckman DU series 800 spectrophotometer. 1H NMR and 13C NMR spectra were recorded on a Bruker AMX-500 spectrometer at 500 MHz and 125 MHz, respectively; samples were dissolved in DMSO-d.6 with TMS as an internal standard. Structures of purified compounds were determined according to standard methods (Dey & Harborne, 1989; Fossen & Anderson, 2006): acid hydrolysis in 2 M and 0.1M HCl (mild hydrolysis) at 100°C for 60 min; enzymatic hydrolysis with β-glucosidase (Sigma) using an acetate buffer (pH 5); hydrogen peroxide oxidation; UV spectroscopy; 1H NMR; 13C NMR and FAB–mass spectroscopy (positive and negative), and by comparison with authentic samples where available. The glycosides and aglycones obtained by hydrolysis of isolated compounds were identified by co-chromatography with authentic samples (Apiin and Extrasynthese) using PC, TLC, and HPLC. Sugars released by hydrolysis were identified by PC and TLC using standards.

Results and Discussion

Phenolic compounds isolated from maple syrup in the current study and identified by physical analysis and comparison with standards where available are listed in Table 1 with structures shown in Figure 1. They are (a) benzoic acid and its hydroxylated and methoxylated derivatives: gallic acid, 1-O.-galloyl-β-d-glucose, γ resorcylic acid, protocatechuic acid, vanillic acid, gentisic acid, syringic acid; (b) cinnamic acids (p.-

coumaric acid, 4-methoxycinnamic acid, caffeic acid, ferulic acid, sinapic acid, and the ester chlorogenic acid; (c) flavan 3-ols: catechin and epicatechin; (d) flavonol glycosides: kaempferol and its 3-O.-β-d-glucoside, and galactoside and quercetin and its 3-O.-β-d-glucoside, 3-O.-β-l-rhamnoside, and 3-O.-rhamnoglucoside (rutin). Vanillic, syringic, ρ-coumaric, ferulic, and sinapic acids were also identified by Kermasha et al. (1995) in maple syrup from Quebec sources and in addition they found coniferyl alcohol, coniferyl aldehyde, and homovanillic, vanillin, and syringaldehyde. Theriault et al. (2006) further identify hydroxybenzoic and an unnamed flavonol. That the 23 compounds isolated and identified represent only a fraction of the total numbers present is further indicated by the many 280-nm peaks in the HPLC traces (Fig. 2). Most of the peaks are very small, less than 10 mAU of a maximum possible of 100 mAU, and few of them contain only one compound as shown by the spectra provided by the diode array. As a result, it is impossible to estimate the amounts of identified compounds in the traces because in many instances one or more unidentified compounds are also present with them and in amounts that are often overwhelming. It is also possible that some of the isolated compounds are present in the extracts as conjugates, which, owing to hydrolysis, oxidation, and other chemical changes occurring during the lengthy isolation process, are removed to leave only the parent compound. For example, there is no quercetin spectrum in the peak eluting at 34 min where quercetin is expected (Table 1).

Changes in the phenolic constituents with the season, as was also reported by Theriault et al. (2006), are evident from comparison of the extracts of light and medium syrups. The former were produced early in the season, the latter at a later time. Many of the most prominent peaks in the 280-nm traces contain compounds with spectra typical of proanthocyanidins (peak 280 nm) or gallates (peak 275 nm), and these tend to be lower in the syrups produced later in the season. HPLC traces of a Gilbertson dark syrup produced late in the season are dominated by two compounds eluting in the first 5 min (data not shown).

A point of interest in the results of the current study is the presence of flavonoids in the syrup. Theriault et al. (2006) identified a flavonol peak in the HPLC trace of a syrup extract, but isolation of flavonols and catechins and evidence of the presence of the latter and of their polymers in the HPLC traces is new to the current study. As Kermasha et al. (1995) stated, the presence of phenolic acids can be explained by their key role in lignin synthesis with breakdown products of lignin or its precursors an important part of the phenolic mixture. Lignin biosynthesis requires its precursors to be transported outside the plasma membrane of the living cell, thus exposing them to the potential of being carried away in the flow of sap in the xylem vessels. Flavonoid glycosides, on the other hand, are normally stored in the vacuole of the cell and would not be expected to be available to the sap unless the cell dies. Such is the fate, of course, of cells destined to become tracheids or vessels. Assuming that the xylem precursors produced by the cambium contain flavonoids in their vacuoles, these flavonoids could be swept away by the sap from the dying cell. A second potential source of flavonoids might be living cells of the cambium, phloem, and cortex damaged by the tapping process (i.e., when the trunk of the tree is drilled to provide the hole in which to insert the sap collection device). Flavonoids, both

glycosides and aglycones, are also important antioxidant compounds (Rice-Evans & Packer, 2003; Clifford & Brown, 2006; Wijeratne et al. 2006).

In any case, total phenolic content of the syrup is very small in proportion to the sugar content as shown by the weights of the ethyl acetate extracts reported in "Materials and Methods" (about 20 ± 5 mg/10 mL of syrup (dry wt. = 9.4 g, i.e., 0.2% by weight). Nevertheless, the biological activity shown by Theriault et al. (2006) may aid in overcoming any negative effects of the high sugar content of the syrup on humans, thus, indulging a sweet tooth, and is most likely, as suggested by Theriault et al. (2006), not related so much to any particular compound as to the overall complexity of the mixture.

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Characterization of the Pyrazines Formed during the Processing of Maple Syrup

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

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

INTRODUCTION

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

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

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

Although the presence of pyrazines has been reported in maple syrup (Alli et al., 1990; Akochi-K et al., 199 1994), as far as the authors are aware, the presence of these compounds has not been investigated. In the present work, the levels of sucrose, glucose, and fructose were found in maple sap and heated maple sap to be 9, 16, and 65.5% total soluble solids. The formation of pyrazine compounds was monitored during the conversion of maple sap to syrup.

MATERIAL AND METHODS

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

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

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

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

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

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

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

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

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

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

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

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

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

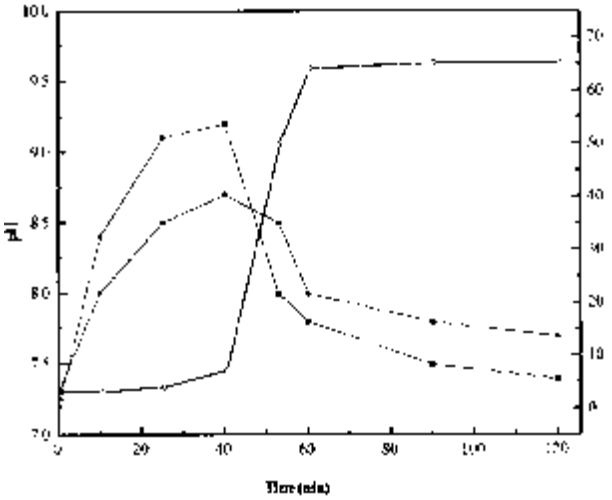

Figure 1. Monitoring of pH values and total soluble solid contents during the heating of maple sap: changes in pH (open boiling and (●) under reflux boiling, and (○) changes total soluble solid contents.

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

RESULTS AND DISCUSSION

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

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

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

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

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

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

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

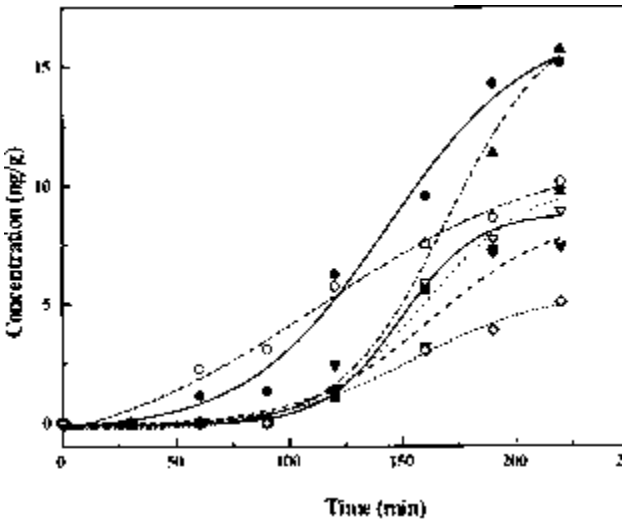

Figure 2. Accumulation of pyrazines during the boiling of maple sap: methyl- (■), 2,5-dimethyl- (●), 2,6-dimethyl (▲ ethyl- (▼), 2,3-dimethyl- (◇), trimethyl- (○) , and 2-ethyl-3-methyl pyrazine (▽).

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

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

time[b] (min)	pyrazine (ng/g)[a] methyl-	2,5-dimethyl-	2,6-dimethyl-	ethyl-	2,3-dimethyl-	trimethyl-	2-ethyl-3-methyl-	total
30	*c*	*c*	*c*	*c*	*c*	*c*	*c*	*c*
60	*c*	1.15 (0.03)[e]	*c*	*c*	*c*	2.27 (0.15)[e]	*c*	3.4
90	*c*	1.33 (0.01)[e]	*c*	*c*	*c*	3.11 (0.22)[e]	*c*	4.4
120	1.18 (0.05)[e]	6.25 (0.32)[e]	1.01 (0.00)[e]	2.40 (0.11)[e]	1.35 (0.01)[e]	5.75 (0.73)[e]	1.22 (0.08)[e]	19.1
160	5.60 (0.12)[e]	9.57 (1.01)[e]	7.53 (0.09)[e]	3.21 (0.15)[e]	3.05 (0.00)[e]	7.55 (1.05)[e]	5.90 (0.00)[e]	42.4
190	7.25 (0.19)[e]	14.30 (1.21)[e]	11.35 (1.00)[e]	7.17 (1.02)[e]	3.90 (0.05)[e]	8.65 (0.16)[e]	7.75 (0.93)[e]	60.3
220	9.77 (0.65)[e]	15.20 (0.00)[e]	15.73 (0.55)[e]	7.42 (0.00)[e]	5.10 (0.37)[e]	10.17 (1.23)[e]	8.93 (0.82)[e]	72.3
%[d]	*13.51*	*21.02*	*21.75*	*10.26*	*7.05*	*14.06*	*12.35*	

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

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

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

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

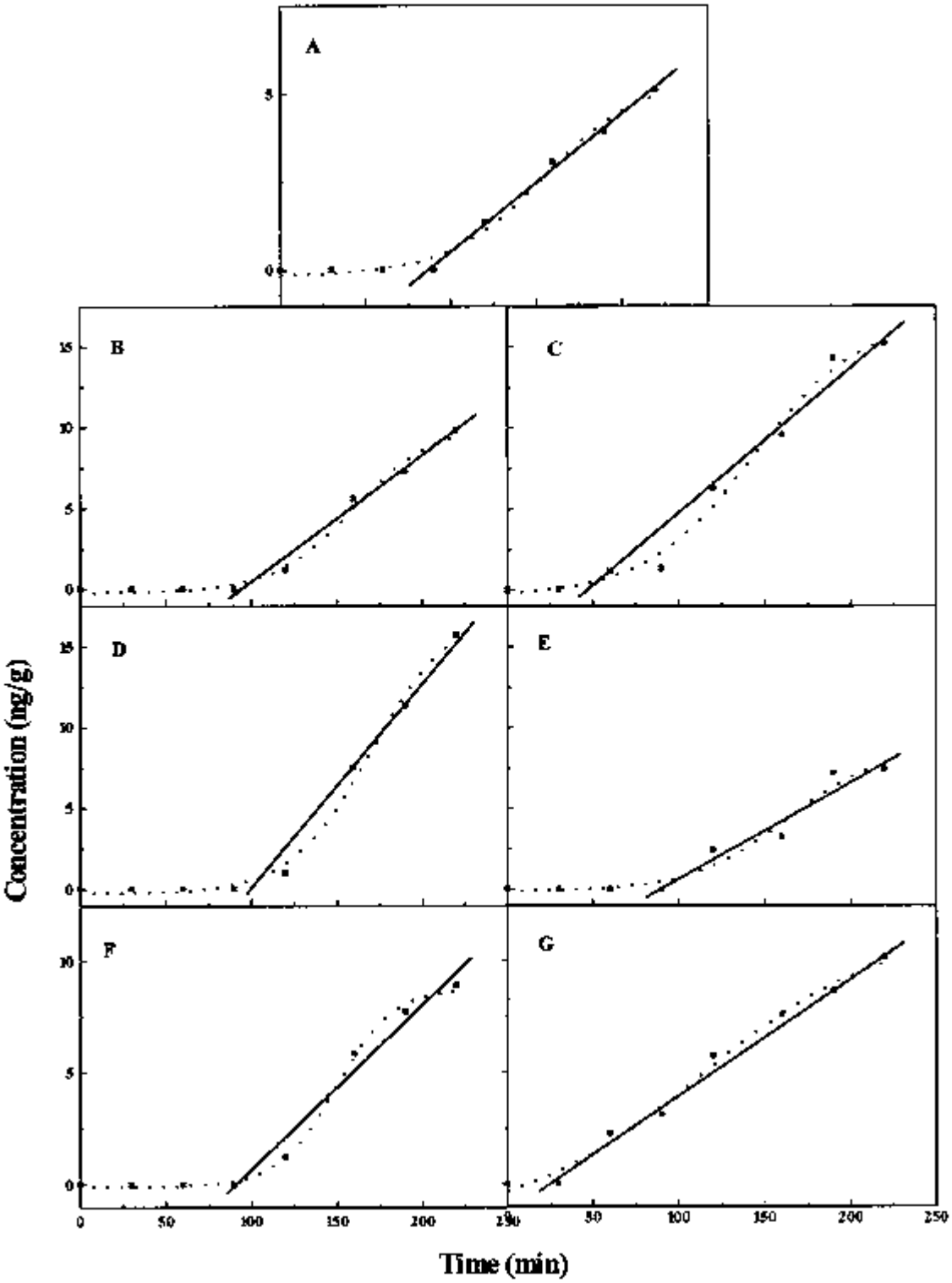

Figure 3. Accumulation of pyrazines during the boiling of maple sap: 2-ethyl-3-methyl- (A), methyl- (B), 2,5-dimethyl- (C), 2,6-dimethyl- (D), ethyl- (E), 2,3-dimethyl-(F), and trimethylpyrazine (G).

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

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

Table 5. Rate Constants for Pyrazine Formation in Maple Syrup

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

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

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

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

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

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

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

CONCLUSION

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

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The Chemical Composition of Maple Syrup

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No one is quite sure how it was discovered that the sap of most maple trees can be concentrated into a sweet, delectable syrup. However, by the time European settlers arrived in North America, native Americans had already learned to slash the bark of maple trees in late winter or early spring, collect the near-clear sap that came out, and boil the sap into a thick, sweet product. Indeed, maple syrup production is one of the few agricultural processes native to North America and not introduced by outside settlers *(1)*.

Maple syrup and its drier cousin, maple sugar, were the dominant food sweeteners in the United States until after the U.S. Civil War when improvements in production and transportation made cane sugar the preferred sweetener for nonfarmers *(2)*. Today, about 7.5 million gallons of pure maple syrup are produced, mostly in eastern Canada and, albeit to a much lesser extent, the northeastern United States. At $30 per gallon (which is a conservative estimate), the industry has a $225 million economic impact. Many consumers, however, only consume a corn syrup-based imitation syrup (many of which do not even claim "maple" on their labels!), which is part of an $11 billion industry *(3)*.

This article is not meant to be a primer on how to tap trees and boil sap to make syrup; many such primers are available (see, for example, ref *1*). Instead, this article is meant to be an introduction to the chemistry of maple sap and syrup: in particular, what makes this sweet liquid maple syrup instead of just a concentrated sugar solution? The types of sugars, the trace ingredients, and the mineral content make maple syrup more than just plain sugar water.

Maple syrup is one of only three syrups derived from tree sap. Another is birch syrup, which comes from the boiled sap of paper birch (*Betula papyrifera*) or Alaska birch (*B. neoalaskana*) trees. Produced in Alaska, Canada, Russia, and Scandinavia, birch syrup is distinctive in flavor but differs from maple syrup in that its sugar content is due to fructose and glucose, rather than sucrose. Birch sap is only half as concentrated as maple sap, so a greater concentration of sap is needed to produce birch syrup. A syrup can also be made from black walnut tree (*Juglans nigra*) sap *(4)*; however, we will not consider the latter syrups further here *(3)*.

Sap

Most maple trees can be tapped and the collected sap can be concentrated (either by boiling or by reverse osmosis followed by boiling) to make maple syrup. However, of the thirteen species of the genus *Acer* in North America, the sugar maple (*A. saccharum*), the black maple (*A. nigrum*), and the red maple (*A. rubrum*) provide most of the sap for syrup production. There are two reasons for this. First, the sugar content of their sap is typically higher than other species, at 2.0–2.5%. Second, the annual growth spurt of these species occurs later in the spring than other maple species, increasing the length of the sap-collecting season. Both of these characteristics tend to produce a superior syrup, although syrup made from the sap of other species of maple tree still has the characteristic maple taste and smell *(1)*.

The organic components of maple sap, not including water, are listed in Table 1 *(1)*. To estimate the actual concentrations in raw sap, the numbers in the second column of the table should be divided by 40–50, which has been done in the last column of the table. Note that almost all of the organic content is sucrose. If present at all, glucose has a concentration of well less than 1% of the organic content and only about 0.004% of raw sap. The commanding presence of sucrose is interesting because the two saccharides in sucrose (glucose and fructose) are joined by an alpha glycosidic bond; cellulose, a major structural component of plants, is formed by joining monosaccharides using a beta glycosidic bond, as shown in Figure 1. At some point in the tree's cells, the sucrose in sap must be broken into its two constituent saccharides before being reassembled into cellulose.

Table 1. Typical Organic Components of Maple Sap

Component	Fraction of Total Organic Content[a,b]	Actual Concentration in Sap
Sucrose	98.0–100%	2–2.5%
Glucose	0–0.17%	0–0.004%
Phenolic compounds	0–4.55 ppm	0–0.1 ppm
Primary amines	0.5–36.1 ppm	0.01–0.9 ppm
Peptides	0.4–18.6 ppm	0.01–0.41 ppm
Amino acids	0–11.3 ppm	0–0.25 ppm
Protein	0–50.9 ppm	0–1.2 ppm
Other organic acids	0–45 ppm	0–1 ppm

[a]The total solids in the sap are 1.0–5.4% and the pH of the sap is 3.9 – 7.9. [b]The data are from ref *1*, Appendix 2 and are used with permission.

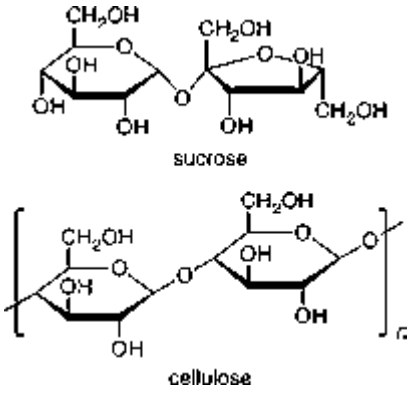

Figure 1. General structures of sucrose and cellulose. The bonds joining the monomers in sucrose have a different orientation from the bonds joining the monomers in cellulose.

Table 2. Average Compositions of Maple Syrup

Component	Quantity
Sucrose	68.0%
Glucose	0.43%
Fructose	0.30%
Water	31.7%
Malic acid	0.47%
Fumaric acid	0.004
Calcium	775 mg/L
Magnesium	167 mg/L
Potassium	2026 mg/L

NOTE: The data are from ref *10* and used with permission. The pH of the maple syrup is 6.7.

Table 3. Mineral Content of Finished Syrup

Mineral	Concentration (ppm)
Potassium	1300–3900
Calcium	400–2800
Magnesium	12–360
Manganese	2–220
Sodium	0–6
Phosphorus	79–183
Iron	0–36
Zinc	0–90
Copper	0–2
Tin	0–33

NOTE: The data are from ref *10* and used with permission.

Maple sap is slightly acidic owing to the presence of several organic acids: oxalic, succinic, fumaric, malic, tartaric, citric, and aconitic (1-propene-1,2,3-tricarboxylic acid) acids. The total quantity of acid in sap starts low, around 8 ppm, then rises to over 45 ppm as the season progresses. Although oxalic acid has the lowest pK_a (1.27) *(5),* there is about 500 times as much malic acid in sap as there is oxalic acid (45 ppm of malic acid vs 0.1 ppm for oxalic acid). Most sap has a pH ranging from 3.9–7.9 *(1).*

Sap has detectable quantities of amino acids, some in trace quantities. Amino acids found in sap include glycine, alanine, asparagine, threonine, leucine, isoleucine, valine, and methionine. The quantities and types of amino acids vary over time, with the largest variety of amino acids present near the end of the annual sap running season *(1).*

Sap also contains minerals but at low concentrations. The two most common minerals in sap are potassium and calcium, found at concentrations of 26–75 and 8–56 ppm, respectively. Sap also contains trace (< 10 ppm) of magnesium, manganese, sodium, phosphorus, zinc, and copper *(1).* Because these minerals are nonvolatile, they concentrate as sap is processed into maple syrup. This can sometimes cause problems, as the mineral salts of the organic acids present in sap may not be soluble in finished syrup, causing precipitation.

Sap into Syrup

Honey is harvested as a concentrated solution *(6),* but human intervention is necessary to generate maple syrup. About 98% of the water in sap must be removed to make syrup; it takes 40–50 gallons of sap to make one gallon of maple syrup. This is done by either heat-induced evaporation or by reverse osmosis followed by evaporation *(7).* It is estimated that it takes about 2600 kJ to evaporate 1 L of sap into syrup. Knowing the enthalpy of methane combustion (890.8 kJ/mol; ref *5*), we can also estimate that it takes the combustion of 65 L of natural gas to generate about 20 mL of syrup—about 1.5 tablespoons. Clearly, the production of significant volumes of syrup is an energy-intensive process. Even if reverse osmosis is used, it is only used to remove about 75% of the water; the remaining water is removed using evaporation.

Two important processes occur as sap is transformed into syrup: first, the concentrations of solutes rise, and second, chemical reactions occur between the chemicals dissolved in the sap. While increasing the complexity of the final product, these reactions also give maple syrup its characteristic color, odor, and flavor. Indeed, from the author's own personal observation, if a small quantity of sap were to evaporate to dryness naturally, the remaining solid residue would be white.

Maple Syrup

Sap becomes syrup when the liquid reaches 66–67 degrees Brix (abbreviated °Bx; the Brix scale is used to express the concentrations of sugar solutions, such as honey, maple syrup, and frozen concentrated orange juice. It is defined as the number of grams of sucrose per 100 grams of solution; ref *8*). At this point, the syrup is 66–67% sucrose and 33–34% water. With these concentration, the syrup boils about 4.3 °C (7.1 °F) higher than pure water. At higher concentrations sugar will begin to precipitate from the syrup, while at lower concentrations microbes can grow in the syrup, spoiling it. There are also other trace compounds present in the syrup; Table 2 lists the approximate composition of maple syrups from the United States and Canada *(9).* The dominant component is sucrose, with only small quantities of glucose and fructose present.

The finished syrup has a wide range of minerals, concentrated from the sap. The average mineral content of maple syrup is given in Table 3. Note that, like honey *(6),* maple syrup can be considered a low- or zero-sodium food. The tin content may be due to the use of tin-plated buckets to collect the sap.

Despite the fact that most maple syrups are graded based on their color, the components that determine the color and flavor of maple syrup are still not completely understood *(10).* There are three possible sources for the color of maple syrup: Maillard reactions between amino acids and reducing sugars, caramelization of sugars, and formation of polycarbonyl compounds *(11, 12).* In any case, it is clear that chemical reactions are occurring in the sap to develop the syrup's color and flavor, as normally evaporated sap dries to a white solid.

Table 4. Compounds Identified in Maple Syrup Thought to Contribute to Its Flavor

Phenolic compounds
Vanillin
Syringaldehyde
Dehydroconiferyl alcohol
Syringoyl methyl ketone
2,6-Dimethoxyphenol
Pyrazine compounds
Methylpyrazine
2,3-Dimethylpyrazine
2,5-Dimethylpyrazine
2,6-Dimethylpyrazine
Ethylpyrazine
2-Ethyl-6-methylpyrazine
2,5-Dimethyl-3,6-diisobutylpyrazine
Butylpyrazine
5-Isopropyl-2,3-dimethypyrazine
Other compounds
2-Ethyl-1-hexanol
2-Hydroxymethylcyclopenten-2-enol
2-Furanmethanol
2-Ethyl-1-hexanoic acid
n-Hexanoic acid
n-Nonanoic acid
Carbonyl compounds
2-Hydroxymethylcyclopent-2-en-1-one
2-Hydroxy-3-methyl-2-cyclopenten-1-one
2-Methyl-2-cyclopenten-1-one
2-Methyl-2,5-cyclohexadien-1,4-dione
2,3-Dihydro-3,5-dihydroxy-6-methyl-(4*H*)-pyran-4-one
2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone
3-Methyl-3-buten-2-one
3-Methyl-2,5-furandione
3-Methyl-2-cyclopenten-2-ol-1-one
3-Hydroxybutanone
3-Hydroxy-2-pyranone
3-Hydroxy-4-methyl-5-ethyl-2(5*H*)-furanone
Propionaldehyde

NOTE: Data are from ref *12* and used with permission.

As for flavor, more seems to be known about what causes maple syrup to taste bad than taste good. Excess sodium leads to a salty flavor, and relatively high quantities of amino acids are responsible for an off flavor known as "buddy" (i.e., bud-like) *(10)*. This is typical of late-season sap; as the tree begins the budding process, the relative concentrations of the various amino acids in sap increase dramatically.

Alli and coworkers *(12)* list several classes of known volatile chemicals in maple syrup (Table 4); however, they admit that the compounds that contribute to the characteristic flavor of maple syrup are not yet established. They suggest that it is likely that these compounds are formed during the evaporation process, as many of these compounds are not present in maple sap. The phenolic compounds are likely due to degradation of lignin components in sap, while the other compounds are formed by reactions between other chemicals found in the sap.

As hot sap is evaporated into syrup, solubility of various salts remains high, but when the finished product is cooled to room temperature, the solubility of certain salts drops below the saturation limit, and crystals precipitate from the syrup. These crystals are called sugar sand *(13)*. Sugar sand can amount to as much as 1.5% of the finished syrup. Sugar sand has a variable composition, but is mostly a combination of small sugar crystals (34–86%) and calcium malate ($CaC_4H_4O_5$; 1–50%). The calcium malate results from the relatively high calcium and malic acid concentrations in the syrup and is one of the least soluble salts in the concentrated syrup. Other components of sugar sand include potassium, magnesium, manganese, phosphorus, and iron. A small percentage (< 3%) of other organic acids may also be present. Most federal and state guidelines involving the sale of pure maple syrup require that the product be clarified *(13)*, so the sugar sand is filtered off from the final product before sale.

Most people actually use imitation maple syrup. Imitation maple syrup is based on corn syrup with added artificial colorings and flavorings. The flavorings include extracts of fenugreek (a spice) or lovage (an herb) and cyclotene (3-methyl-2-cyclopenten-2-ol-1-one, $C_6H_8O_2$), methylcyclopentenol ($C_6H_{10}O$), or a variety of alkyl hydroxyfuranones. Labeling laws usually prohibit the use of the word "maple" unless the product actually contains real maple syrup *(14)*. Most tasters agree that the real product is much tastier than the imitation product, but note that it is also much more expensive!

Conclusion

Maple syrup, native to North America, is much more than a concentrated sugar solution. It contains organic acids, amino acids, minerals, and a wide variety of unidentified chemicals formed during the evaporation process that contribute to its color, odor, and characteristic taste. Derived from the sap collected from trees of the genus *Acer*, it is one of only three syrups derived from tree sap, the others being the less common birch and black walnut syrups. Although the use of corn syrup-based artificial products dwarfs that of real maple syrup in the United States, most people would consider the flavor of real maple syrup superior to any substitute.

Acknowledgment

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DETERMINATION OF THE GLYCAEMIC INDEX OF SELECTED FOODS (WHITE BREAD AND CEREAL BARS) IN HEALTHY PERSONS

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The glycaemic index (GI) is a measure of the food power to raise blood glucose (B-glucose) concentration after a meal. For healthy eating, foods with low GI are recommended.However, for many foods in the European Union the GI has not been defined yet. The aims of this prospective open-label study were: (1) to determine the GI of white bread and juicy cereal bars FIT (Úsovsko, Czech Republic) by means of the glucometer Optium (Abbott/Medisense); (2) to compare the GI of tested foods determined in the morning and in the evening hours; (3) to compare the GI of tested foods in men and women and (4) to assess the variability of the GI. Methods: To determine the GI, measured portions of food containing 50 g of carbohydrates were eaten by 11 healthy volunteers. B-glucose curves were constructed from B-glucose values at time 0, 15, 30, 45, 60, 60, 120 min after the meal. The GI was calculated by dividing the incremental area under the curve (IAUC) for the tested food by that for the standard food (IAUCS). In each volunteer each food was tested 5 times so that 5 GI's was obtained and the average was calculated. The GI for each tested food was calculated as the mean from the respective average GI's of the 11 volunteers. MS Excel and the statistical program SPSS v. 10.1 were used to analyze the data. Results: (1) The mean values of the GI for white bread was 70.3 % and for juicy cereal bars was 101.0 %, as determined in a total of 139 tests in the whole group of 11 volunteers. There was a difference when comparing white bread vs. glucose ($p = 0.012$) and white bread vs. cereal bars ($p = 0.026$) but no difference between glucose and cereal bars. (2) There was no significant difference between the GI determined in the morning and in the evening hours either for the total of 139 tests or for the individual tested foods. (3) No significant difference could be seen between the GI in men and women when comparing glucose, cereal bars and white bread. (4) There was a wide variability of GI in all tested foods: the standard deviation of GI for white bread was 30.7 %, for juicy cereal bars 38.0 %. Conclusions: The GI's for white bread and juicy cereal bars were determined. There was no difference either between the GI values determined in the morning vs. the evening hours or between the values in men vs. women. The results show wide variability. An accurate standard method for the determination of GI needs to be defined, carefully used and re-evaluated to enable a comparison of the results with various methods of other working groups.

INTRODUCTION

The glycaemic index (GI) is an important parameter of food quality which compares the hyperglycaemic effect of a tested meal with pure glucose (or of another defined standard food). The GI is a measure of the food power to raise B-glucose concentration after a meal. The GI is defined as relation of the incremental area under the B-glucose response curve (IAUC) of a tested meal containing 50 g of digestible carbohydrates and the incremental area under the B-glucose response curve of the standard food, i.e. 50 g pure glucose (IAUCS). Carbohydrates that breakdown quickly during digestion have a high GI because their B-glucose response is fast and high. Carbohydrates that breakdown slowly have a low GI[11].

For healthy eating, particularly in persons with diabetes, obesity and insulin resistance, foods with low GI are recommended as they may help keep the euglycaemia and the normal spectrum of lipoproteins[3, 4, 8, 12, 22]. These effects result in decreased cardiovascular danger and probably also in reduced risk for colon and breast cancer[1].

On the other hand, the GI values have a large inter- and intraindividual variability[18, 19, 24]. For many foods in the European Union the glycaemic index has not been defined yet. Even the methods for defining the GI are not standardized[13]. Therefore, any effort to enable the

determination and practical use of GI may support establishing optimum dietary recommendations and good eating habits.

AIMS

Aims of this prospective open-label study were:

1. to determine the GI of white bread and cereal bars by means the glucometer system Optium;
2. to compare the GI of tested foods determined in the morning and in the evening hours;
3. to compare the GI of tested foods in men and women;
4. to assess the variability of the GI.

METHODS

Determination of the glycaemic index

a) Getting basic data

To determine the GI, measured portions of tested food containing 50 g of carbohydrates were eaten by each of the 11 healthy volunteers (Table 1) after an overnight fast; the same approach was used after an afternoon fast. Finger-prick blood samples were investigated at 15–30 minute intervals over the next two hours after the meal (at times 0, 15, 30, 45, 60, 90, 120 min; the beginning of the food intake was time 0).

Each volunteer measured his/her B-glucose concentrations by means of a glucometer Optium. At the end of the one-week test period the B-glucose values were transferred from the memory of the glucometer into a PC for further analysis.

b) Construction of B-glucose response curves.

The averages of the respective B-glucose concentrations after the meal were used to draw a B-glucose response curve for the two-hour period. The values at times 75 and 105 min were obtained by extrapolation.

c) Exclusion of disturbed tests.

For the purpose of statistical evaluation, all tests that were not complete and all tests where the first (i.e. fasting) B-glucose concentration was 7.0 mmol/l or higher were excluded.

d) Calculations of individual GI values in every volunteer.

The incremental area under the curve (IAUC) was calculated for each meal in every volunteer separately (as the sum of the surface of triangles and trapezoids between the B-glucose curve and horizontal baseline going parallel to x-axis from the beginning of B-glucose curve at time 0 to the point at time 120 min) to reflect the total rise in B-glucose concentration after eating the tested food.

The IAUCS for the standard reference food (i.e. 50 g of pure glucose) was obtained similarly to the mean from the first three independent $IAUCS_1$, $IAUCS_2$, $IAUCS_3$ in the same volunteer.

In the IAUC/IAUCS calculations, all B-glucose values in the course of the test lower than the first value (at time 0) were equalized to the respective first value.

In each volunteer, the GI (%) was calculated by dividing the IAUC for the tested food by the IAUCS for the standard food and multiplying by 100. The following formula was used:

$$GI = \frac{IAUC}{\frac{1}{3}\left(IAUCS_1 + IAUCS_2 + IAUCS_3\right)} \times 100\left[\%\right]$$

IAUC – Incremental Area Under the blood glucose response Curve for the tested meal

IAUCS – Incremental Area Under the blood glucose response Curve for the standard meal

e) Working out the average of GI's for tested food in each volunteer.

In each volunteer each food item was tested 5 times so that 5 GI's was obtained and (after the exclusion of disturbed tests) the average was calculated.

f) Final calculation of the GI for each tested food.

The GI for each tested food was calculated as the mean from the respective average GI's of the 11 volunteers.

g) The variability of GI for each tested food was assessed according to standard deviation of the mean; histograms of GI values demontrated the frequency and range of results.

Healthy volunteers

The participants in this study were healthy persons recruited from the nursing staff, laboratory assistants and students. Thorough clinical and laboratory investigations were performed to establish that the volunteers were healthy. (Table 1).

Tested foods

Three different foods (A–C) with a known content of nutrients were tested:

A. pure glucose, one serving 50 g;
B. white bread (Vodová veka Penam, Olomouc, Czech Republic); composition: carbohydrates 59.0 %, protein 9.0 %, fat 1.2 %, energy 1190 kJ / 100g; one serving 85 g (equal 50.0 g of carbohydrates);
C. juicy cereal bar (šťavnatá tyčinka FIT, Úsovsko, Czech Republic); composition: carbohydrates 68 %, proteins 5.9 %, fat 13 %, energy 1740 kJ/100g; one serving 75 g (equal 50.0 g of carbohydrates).

The food was professionaly prepared in the expected quality and quantity; the portions were packed and marked with a set sign. Each serving contained 50 g of digestible carbohydrates. Glucose was dissolved in 300 ml of tea, coffee or water before drinking.

Study design

1. Each volunteer received a glucometer Optium and 100 strips (Lot No 51322); everyone was trained in selfmonitoring and instructed how to keep to the principles of the study protocol:
   - to consume the tested and the standard food

Table 1. Characteristics of healthy volunteers in the study.

Parameter	Mean ± SE	Reference range
n	11	
men/women	5/6	
Age [years]	24.0 ± 2.02	
BMI [kg/m^2]	22.8 ± 0.96	< 25
Puls rate [min^{-1}]	76.4 ± 3.16	60 – 90
Blood pressure systolic [torr]	121.4 ± 3.28	< 130
Blood pressure diastolic [torr]	74.1 ± 2.21	< 85
HbA1c [%]	5.5 ± 0.07	4.4. – 6.0
T3 [nmol/l]	1.6 ± 0.07	1.2 – 3.1
T4 [nmol/l]	90.9 ± 6.73	58 – 142
TSH [mU/l]	1.9 ± 0.39	0.35 – 4.4
STH [ng/ml]	1.0 ± 0.38	0 – 4.1
Cortisol [nmol/l]	454.3 ± 72.81	330 – 710
C-peptid [nmol/l]	0.7 ± 0.05	0.59 – 1.30
Insulin [mIU/l]	9.4 ± 1.05	7.1 – 15.6
Cholesterol [mmol/l]	4.6 ± 0.30	3.5 – 5.2
LDL-cholesterol [mmol/l]	2.9 ± 0.20	1.20 – 2.60
HDL-cholesterol [mmol/l]	1.5 ± 0.10	1.20 – 2.30
Triacylglycerols [mmol/l]	1.1 ± 0.15	0.8 – 1.7
C-reactive protein [mg/l]	11.1 ± 1.64	< 5.0
Total protein [g/l]	72.6 ± 1.13	60 – 80
Albumin [g/l]	45.6 ± 0.60	35 – 50
Glucose [mmol/l]	4.5 ± 0.12	3.5 – 6.0
Na [mmol/l]	142.2 ± 0.49	135 – 146
K [mmol/l]	4.3 ± 0.08	3.7 – 5.2
Cl [mmol/l]	105.3 ± 0.53	96 – 110
Ca [mmol/l]	2.5 ± 0.03	2.2 – 2.65
P [mmol/l]	1.4 ± 0.12	0.7 – 1.6
Mg [mmol/l]	1.0 ± 0.05	0.65 – 1.15
Urea [mmol/l]	4.3 ± 0.24	2.6 – 8.5
Creatinin [µmol/l]	85.1 ± 3.08	55 – 95
Uric acid [µmol/l]	322.0 ± 21.8	180 – 370
Bilirubin [µmol/l]	11.2 ± 0.92	< 20.0
ALT [µkat/l]	0.55 ± 0.15	< 0.56
AST [µkat/l]	0.47 ± 0.06	< 0.52
GMT [µkat/l]	0.35 ± 0.05	< 0.63
ALP [µkat/l]	1.8 ± 0.68	< 2.10

Table 2. The time schedule for individual tests with food A (glucose), B (white bread) and C (juicy cereal bars) used by each volunteer.

Day	Mo	Tue	Wed	Thu	Fri	Sat	Sun	Mo
Breakfast	-	B1	A2	C2	B3	A4	C4	B5
Dinner	A1	C1	B2	A3	C3	B4	A5	C5

daily for breakfast (6:00–7:00 h) and for dinner (18:00–19:00 h) according to the given schedule (Table 2); no other food was allowed for breakfast and dinner;
- to consume no food from dinner until breakfast and from lunch to dinner; drinking water, mineral water, tea and coffee without sugar was allowed;
- to keep to the same extent of physical exercise during the whole one-week test period;
- to consume no alcohol and not to smoke.

2. Each volunteer received 15 servings of standard and tested foods with an exact marking when to eat which serving.
3. Each volunteer kept a diary on food intake, exercise and results of B-glucose selfmonitoring.
4. The PC Link was used to transfer the data from glucometer Optium to a PC. MS Excel and statistical program SPSS v. 10.1 were used to analyze the data.

RESULTS

Blood glucose curves

A total of 164 tests (with each food 55 tests, one test with white bread was omitted) were performed.

The mean 2-hour B-glucose curves for pure glucose, white bread and cereal bars are drawn in Fig. 1.

The mean B-glucose curves in the morning (n = 77) and in the evening (n = 87) for all kinds of tested foods are shown in Fig. 2 and for the individual tested foods in Fig. 3–5.

The mean B-glucose curves for 5 men (n =74 and 6 women (n = 90) comparing all kinds of food together are demonstrated in Fig. 6 and curves comparing individual foods in Fig. 7–9.

Statistical evaluation of GI values for tested foods

Due to incomplete number of B-glucose estimations or due to high B-glucose concentration at start (≥ 7.0 mmol/l), 25 tests (15.1 %) had to be excluded from the statistical analysis.

Table 3. Glycaemic index GI of juicy cereal bars and white bread in 11 volunteers (5 men, 6 women); mean ± SD [%].

Group	GI for cereal bars	GI for white bread
Whole group	101.0[b] ± 30.74	70.3[a, b] ± 37.98
Men	77.5[a] ± 17.89	61.9[a] ± 27.18
Women	120.7[b] ± 40.13	77.3[b] ± 34.20
Breakfast	85.3 ± 53.83	74.6 ± 34.69
Dinner	114.7[b] ± 42.53	68.9[a, b] ± 30.56

$p < 0.05$ [a] tested food vs. glucose, [b] cereal bars vs. white bread

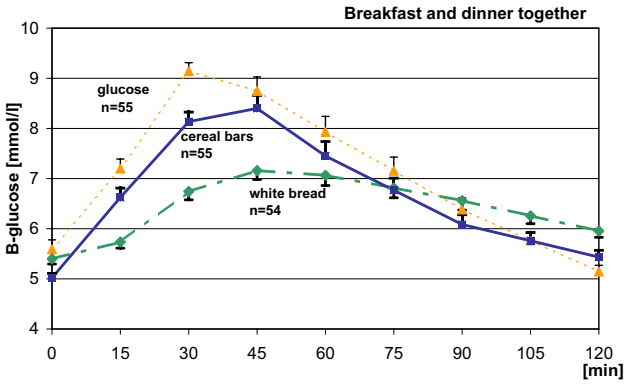

Fig. 1. Mean B-glucose curves (glucometer Optium) for glucose, white bread and cereal bars; each meal contained 50 g of carbohydrates; in all 11 volunteers a total of 164 tests were performed (in every volunteer 5 tests with glucose, 5 tests with white bread and 5 tests with cereal bars); one test with bread was omitted; $\bar{x}$ ± SEM

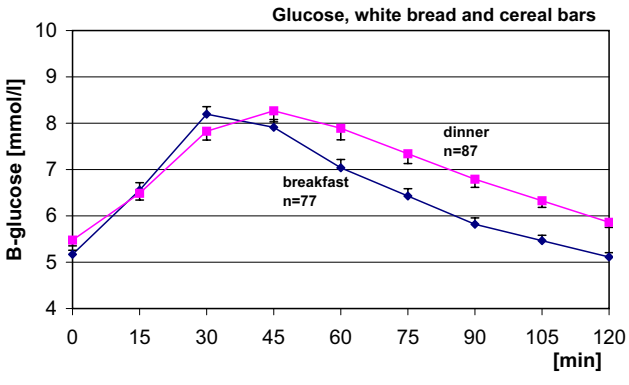

Fig. 2. Mean B-glucose curves (glucometer Optium) after consumption of 50 g of carbohydrates for breakfast and for dinner: in every volunteer 7 tests for breakfast (3 tests with glucose, 2 tests with bread, 2 tests with cereal bars) and 8 tests for dinner (2 tests with glucose, 3 tests with bread, 3 tests with cereal bars) were performed; one test with bread was omitted; $\bar{x}$ ± SEM

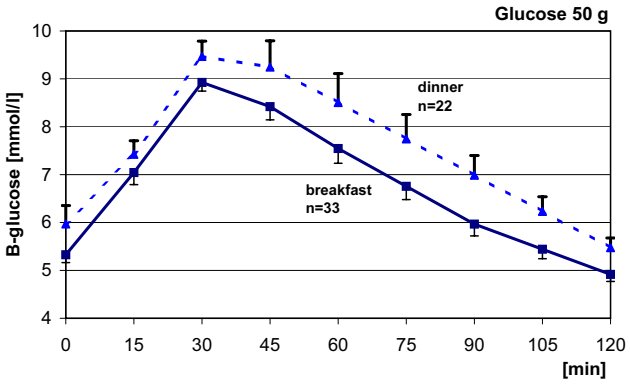

Fig. 3. Mean B-glucose curves (glucometer Optium) after consumption of 50 g of glucose for breakfast and for dinner: in every volunteer 3 tests for breakfast and 2 tests for dinner were perfomed; $\bar{x}$ ± SEM

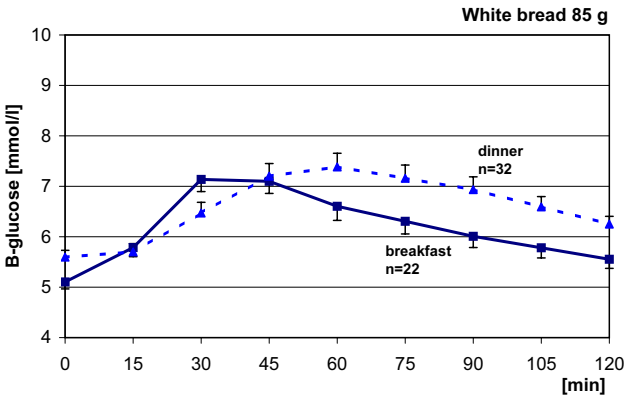

Fig. 4. Mean B-glucose curves (glucometer Optium) after consumption of 85 g of white bread for breakfast and for dinner: in every volunteer 2 tests for breakfast and 3 tests for dinner were performed; $\bar{x}$ ± SEM

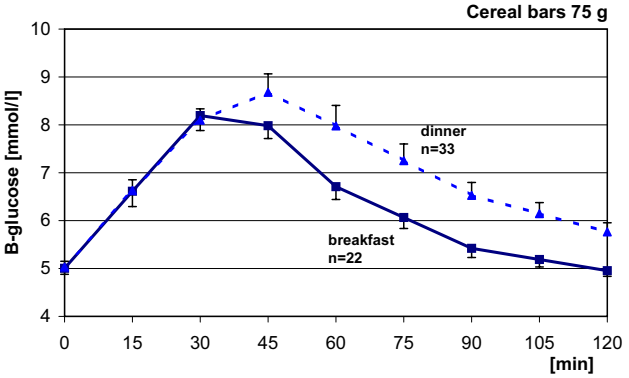

Fig. 5. Mean B-glucose curves (glucometer Optium) after consumption of 75 g of cereal bars for breakfast and for dinner: in every volunteer 2 tests for breakfast and 3 tests for dinner were performed; $\bar{x}$ ± SEM

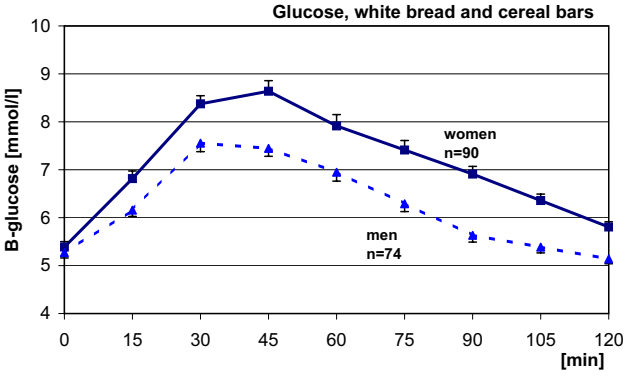

Fig. 6. Mean B-glucose curves (glucometer Optium) after consumption of 50 g of carbohydrates (glucose, white bread, cereal bar): a total of 74 tests in 5 men and a total of 90 tests in 6 women were performed; one test with bread was omitted; $\bar{x}$ ± SEM

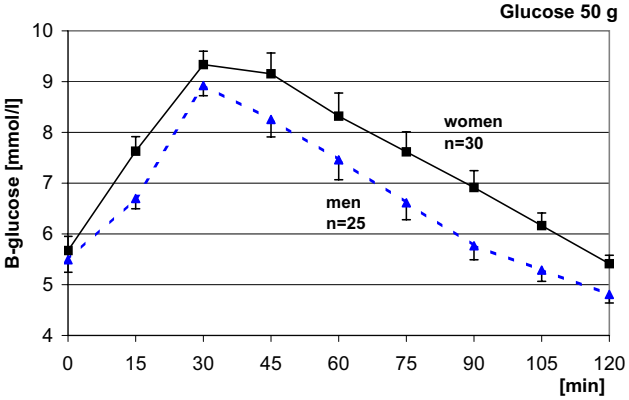

Fig. 7. Mean B-glucose curves (glucometer Optium) after consumption of 50 g of glucose in 5 men (25 tests) and in 6 women (30 tests) were performed; $\bar{x}$ ± SEM

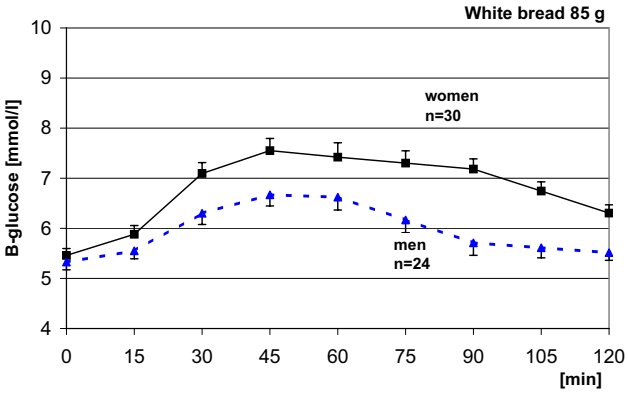

Fig. 8. Mean B-glucose curves (glucometer Optium) after consumption of 85 g of white bread in 5 men (24 tests) and in 6 women (30 tests) were performed; one test was omitted; $\bar{x}$ ± SEM

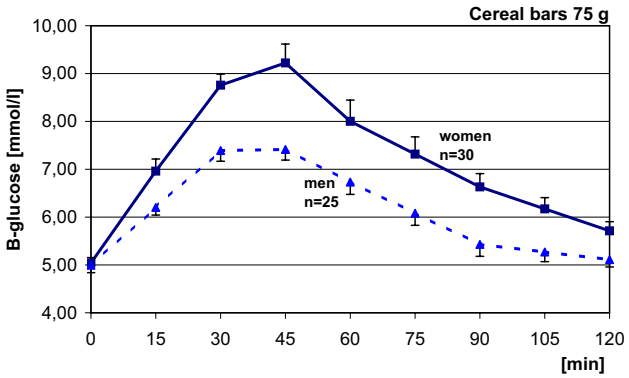

Fig. 9. Mean B-glucose curves (glucometer Optium) after consumption of 75 g of cereal bars in 5 men (25 tests) and in 6 women (30 tests) were performed; $\bar{x} \pm SEM$

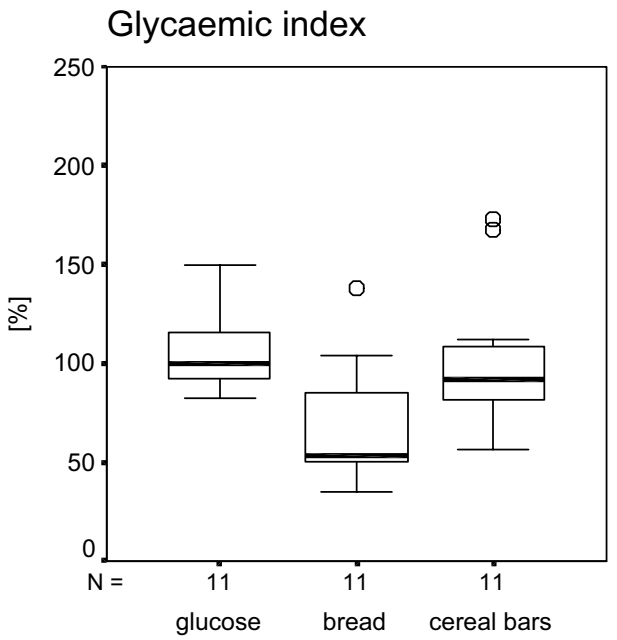

Fig. 10. Glycaemic index for glucose (n = 43), white bread (n = 47) an cereal bars (n = 49) in the 11 volunteers; median, quartiles and outliers;

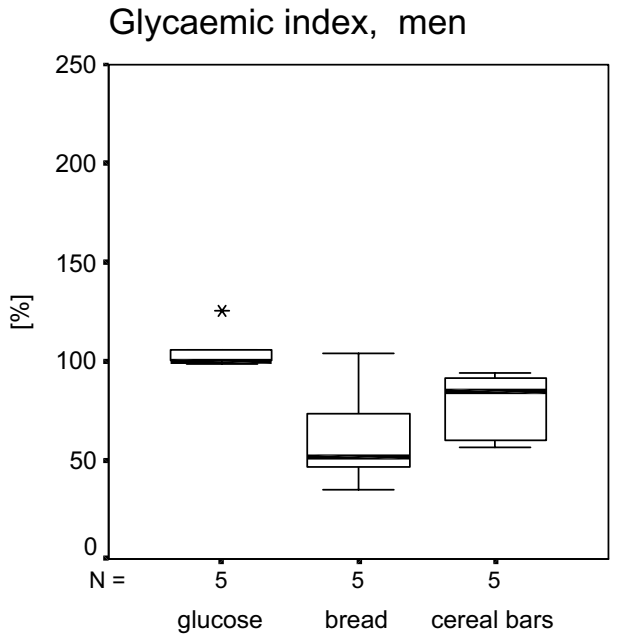

Fig. 11. Glycaemic index for glucose, white bread and cereal bars in 5 men: median, quartiles, outlier

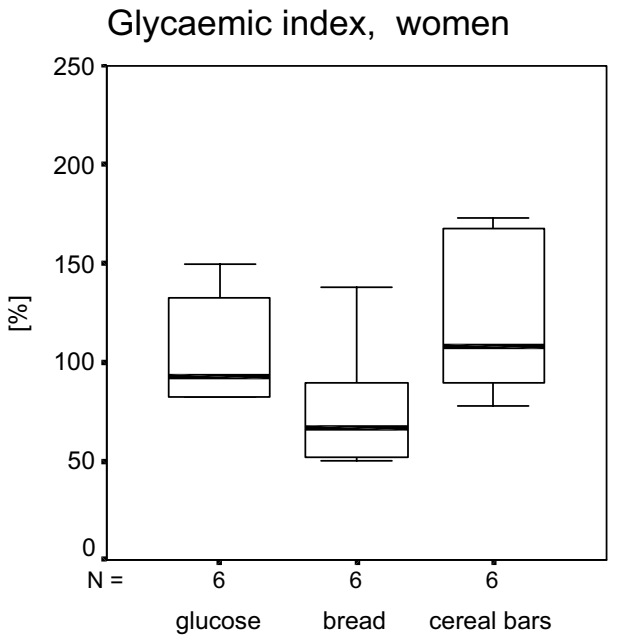

Fig. 12. Glycaemic index for glucose, white bread and cereal bars in 6 women: median, quartiles

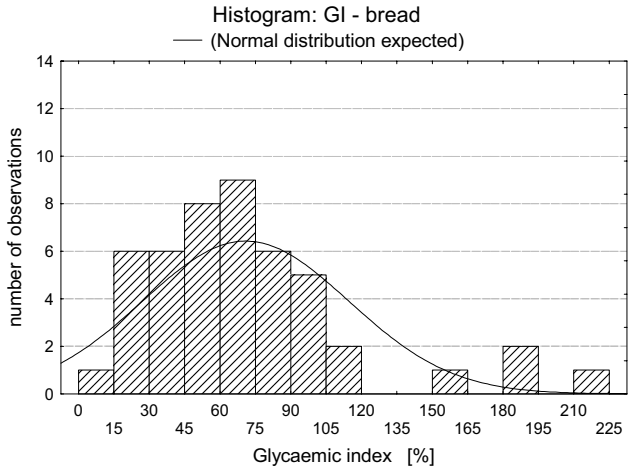

Fig. 13. Histogram of glycaemic indexes (n = 47) for white bread

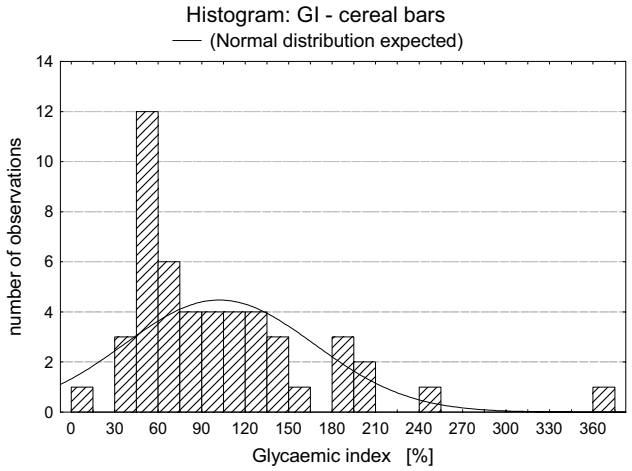

Fig. 14. Histogram of glycaemic indexes (n = 49) for cereal bars

The mean values of the GI for glucose, cereal bars and white bread determined in a total of 139 tests in the whole group of 11 volunteers are shown in Table 3. There was a significant difference when comparing white bread vs. glucose (p = 0.012) and white bread vs. cereal bars (p = 0.026) but no difference between glucose and cereal bars (p = 0.732) could be seen (Fig. 10).

There was no significant difference between the GI determined in the morning and in the evening hours neither for the total of 139 tests nor for the individual tested foods.

No significant difference could be seen between the GI in 5 men and the GI in 6 women when comparing glucose, cereal bars and white bread. However, in men, there was a significant difference between the GI for white bread vs. glucose (p = 0.004) and cereal bars vs. glucose (p = 0.044) – see Fig. 11; in women, the only significant difference was between white bread vs. cereal bars (p = 0.047) – see Fig. 12.

Variability of GI

The standard deviations of GI see Table 3. The histograms (Fig. 13–14) demonstrate the frequency of GI values in individual tested foods.

DISCUSSION

The concept of the glycaemic index of foods has been developed in the course of the last thirty years without having reached its final version[22, 25].

Recent studies from Harvard School of Public Health indicate that the risks of diseases such as type 2 diabetes and coronary heart disease are strongly related to the GI of the overall diet. In 1999, the World Health Organisation (WHO) and Food and Agriculture Organisation (FAO) recommended that people in industrialised countries base their diets on low-GI foods in order to prevent the most common diseases, such as coronary heart disease, diabetes and obesity[5, 7, 9, 10, 14, 15, 17, 23].

Some foods on the world market already show their GI rating on the nutrition information panel. Terms such as complex carbohydrates and sugars, which commonly appear on food labels, are now recognised as having little nutritional or physiological significance. The WHO/FAO recommend that these terms be replaced with the total carbohydrate content of the food and its GI value.

In the Czech Republic, however, we were not able to find any food product containing the nutritional lable with GI value.

International Tables of Glycaemic Index were published by the American Journal of Clinical Nutrition in 1995 and 2002[6]. Tables of the GI values contain about 600 different foods. According to GI, foods may be divided into three groups: foods with low GI (GI = 55 % or less), foods with medium GI (GI = 56–69 %) and foods with high GI (GI = 70 % or more).

The GI values of foods must be measured using valid scientific methods[16, 20, 21]. The accuracy of the measurements of the GI is influenced particularly by the following factors[2]:

1. method for calculating IAUC;
2. method for measuring the B-glucose;
3. defining the amount of the tested food which contains 50 g of hyperglycaemic (i.e. absorbable, digestive) carbohydrates;
4. the usage of the standard food (defining the amount and the kind of the white bread that contains 50 g of digestive carbohydrates);
5. tested individuals;
6. the glycaemia variability from day to day;
7. time of the day when the test is carried out.

In this study, the glucometers Optium were used by 11 trained volunteers. Optium system enabled a reliable registration of all B-glucose values including exact times of measurement even though it was not possible to keep an eye on the performance of the test. Over 50 tests carried out with each tested food in order to investigate the GI exceeds the usual amount of tests used by other working

groups. The tests were performed not only in the morning but also in the evening hours which does not correspond to the recommended method of GI investigation. Nevertheless, no significant difference of GI was found between breakfast and dinner times.

Defining the amount of tested food could be a potential source of mistakes. We have used the declared content of carbohydrates in each food to calculate the amount of food for the tests but we were not able to check the accuracy and precision of these declared data (except the pure glucose which we weighed alone).

A wide range of GI values for individual foods demonstrated by the respective standard deviations and histograms corresponds to the experience of others[2, 19, 24]. Attention must be paid to the standardization of methods of investigation and their comparison with the methods of other laboratories.

Currently, only a few nutrition research groups around the world provide a legitimate testing service. E.g. in Australia, Sydney University GI Research Service (SUGiRS) was established in 1995 to provide a reliable commercial GI testing laboratory for the local and international food industry. Foods are tested in healthy volunteers according to standardised methods that have been validated against laboratories overseas. Insulin, satiety, hunger and other parameters can be assessed simultaneously. Foods that meet nutrition guidelines and have been GI tested can carry the GI symbol.

The research group at the Human Nutrition Unit at Sydney University has determined the GI values of more than 400 foods using the following method:

1. The GI value of a food is determined by feeding 10 or more healthy people a portion of the food containing 50 grams of digestible (available) carbohydrate and then measuring the effect on their B-glucose concentrations over the next two hours. For each person, the incremental area under their two-hour B-glucose response (IAUC) for this food is then calculated.
2. On another occasion, the same 10 people consume an equal-carbohydrate portion of glucose (the reference food) and their two-hour B-glucose response is also measured.
3. A GI value for the test food is then calculated for each person by dividing their B-glucose IAUC for the test food by their B-glucose IAUC for the reference food.
4. The final GI value for the test food is the average GI value for the 10 people.

It would be certainly worthwhile to compare the GI values obtained by this simple Australian method with the method used in this study.

CONCLUSIONS

1. The GI's for white bread and juicy cereal bars were determined.
2. There was no difference between the GI values determined in the morning vs. the evening hours.
3. There was no difference between the GI values determined in men vs. women.
4. The GI values show wide variability.

An accurate standard method for the determination of GI needs to be defined, carefully used and re-evaluated to enable comparison of results with various methods of other working groups. Increased interest in GI determination may be expected. Hence, adequate facilities and equipment should be made available to fulfil the demands.

ACKNOWLEDGEMENT

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JCE Featured Molecules

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Molecular Models of Compounds in Maple Syrup

October Featured Molecules

This month's issue of *J. Chem. Educ.* includes articles by David Ball dealing with the chemical composition of honey *(1)* and maple syrup *(2)*. The *JCE* Featured Molecules for this month are drawn from those papers. In prior months we have included sucrose, glucose, and fructose *(3),* and all of the naturally occurring amino acids *(4)* in the molecule collection. This month we add the molecules identified in Table 4 of ref *2* as probable contributors to the taste of maple syrup. This group of molecules could serve easily as a starting point for a variety of student activities in the area of taste. Students in non-majors courses could be asked to identify structural similarities and differences among the various molecules and could be introduced to functional groups. Students could look for other foods in which some of these molecules are found, and could begin to develop a list of molecules contributing to flavor. In the penultimate paragraph of the maple syrup paper there is a list of substances used as flavoring agents in artificial "maple" syrup. What molecules are in fenugreek and lovage that might be important in flavoring? What are the structures of the other molecules in that paragraph and what, if any, structural features do they have in common with the featured molecules? Students in organic or biochemistry courses could begin to explore the chemistry of taste in more detail. Good starting points for this work are *The Chemistry of Taste: Mechanisms, Behaviors, and Mimics* by Peter Given and Dulce Paredes *(5)* and the *Chemical and Engineering News* Web site *(6)*, which includes a number of articles on this subject.

Students can examine the structures of compounds in maple syrup in Jmol or Chime, along with other molecules in the collection, at the *JCE* Digital Library Web site:

http://www.JCE.DivCHED.org/JCEWWW/Features/MonthlyMolecules/2007/Oct/

2,3-dimethylpyrazine

syringaldehyde

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Determination of Phenolic Compound Profiles in Maple Products by High-Performance Liquid Chromatography

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A high-performance liquid chromatography method, using ultraviolet and electrochemical detectors, was developed for the analyses of phenolic and furfural compounds in maple products. The concentrations of compounds were calculated using external standards that conformed to linear behavior. Most of compounds identified in saps, concentrates, and syrups were related to lignin derivatives. Statistical analyses of data showed that 5-(hydroxymethyl)-2-furaldehyde (HMF) concentrations and phenolic profiles were significantly different as related to harvest time and maple products. Although HMF concentrations were not significantly different as related to the producers, a highly significant difference was observed for phenolic profiles. An increase in the relative proportion of phenolic acids and a decrease in that of aldehydes and alcohols were observed during the reverse osmosis of maple sap. The thermal evaporation resulted in an increase in the amount of HMF, ferulic acid, vanillin, and syringyl aldehyde with a concomitant drastic decrease in sinapic acid.

Keywords: *Maple products; HPLC; UV-diode array; electrochemical detector; ANOVA*

INTRODUCTION

Maple syrup is one of the most important plant product in Québec, Canada, and represents 72% of the world production (Dumont et al., 1993). The distinctive flavor of maple syrup has kept this product selling at premium prices for many years. Maple syrup is the characteristic product resulting from thermal processing of maple sap, the exudate tapped from the trunk of mature sugar maple trees (*Acer saccharum* Marsh). The initial maple sap represents a solution in which sucrose is the major component (Naghski and Willits, 1957). In addition, minor quantities of reducing sugars (Jones and Alli., 1987; Kallio, 1988), organic acids (Mollica and Morselli, 1986; Kallio, 1988), minerals (Kuentz et al., 1976), and nitrogenous compounds (Morselli and Whalen, 1986) have been reported to be present in maple sap.

Phenolic compounds are widely distributed in plants, many being essential metabolites, and contribute to the sensory properties associated with food quality such as color and aroma (Macheix et al., 1990a). In addition, Huang and Ferraro (1992) reported that some phenolic compounds may have potential health benefits, including the reduction of cancer risk. Filipic and Underwood (1964) reported the presence of phenolic-related compounds such as vanillin, coumarin, syringaldehyde, coniferaldehyde, and 2,6-dimethoxybenzoquinone at concentrations lower than 1 ppm in chloroform extracts of maple sap as well as an ether insoluble lignin. Recently, Potter and Fagerson (1992) reported on the identification of phenolic lignin monomers and related flavor compounds in dichloromethane extracts of maple syrup. The source of vanillin and syringaldehyde in maple syrup has been suggested by Underwood and Filipic (1964) to be lignin or lignin fragment. Bound vanillin was also reported to be present in maple sap as a precursor of vanillin in maple syrup (Belford et al., 1992).

The separation and quantitative analyses of phenolic compounds in plant extract were achieved by high-performance liquid chromatography (HPLC) equipped with an ultraviolet (UV) detector (Wilson, 1981; Spanos et al., 1990) as well as an electrochemical (EC) detector (Nagels and Creten, 1985; Roston and Kissinger, 1981; Joerg and Sontag, 1993). The literature indicated that the limit of detection of some phenolic compounds is higher with the electrochemical detector compared to that of UV detector (Hayes et al., 1987; Galetti et al., 1990; Kermasha et al., 1994).

The objectives of this study were to develop a HPLC analytical method for the separation and characterization of phenolic and furfural compounds in maple products extracts, using a combination of UV diode array and EC detectors as well as to analyze the effects of harvest time, technological processes, and producers on the profiles of these compounds in maple products.

MATERIAL AND METHODS

Reagents and Standards. All chemicals used throughout this study were of ACS reagent grade or better. Phenolic standards (*p*-coumaric and ferulic acids) were purchased from Sigma Chemical Co. (St. Louis, MO). Sinapic (3,5-dimethoxy-4-hydroxycinnamic acid), syringic (4-hydroxy-3,5-dimethoxybenzoic acid), coumaric (4-hydroxycinnamic acid), vanillic (4-hydroxy-3-methoxybenzoic acid), and homovanillic (4-hydroxy-3-methoxyphenyl)acetic acid) acids, coniferol (4-hydroxy-3-methoxycinnamyl alcohol), coniferal (4-hydroxy-3-methoxycinnamyl aldehyde), syringal (4-hydroxy-3,5-dimethoxybenzaldehyde), vanillin (4-hydroxy-3-dimethoxybenzaldehyde), and 5-(hydroxymethyl)-2-furaldehyde (HMF) were obtained from Aldrich Chemical Co. (Milwaukee, WI). Chemical structures of phenolic compounds are reported in Figure 1.

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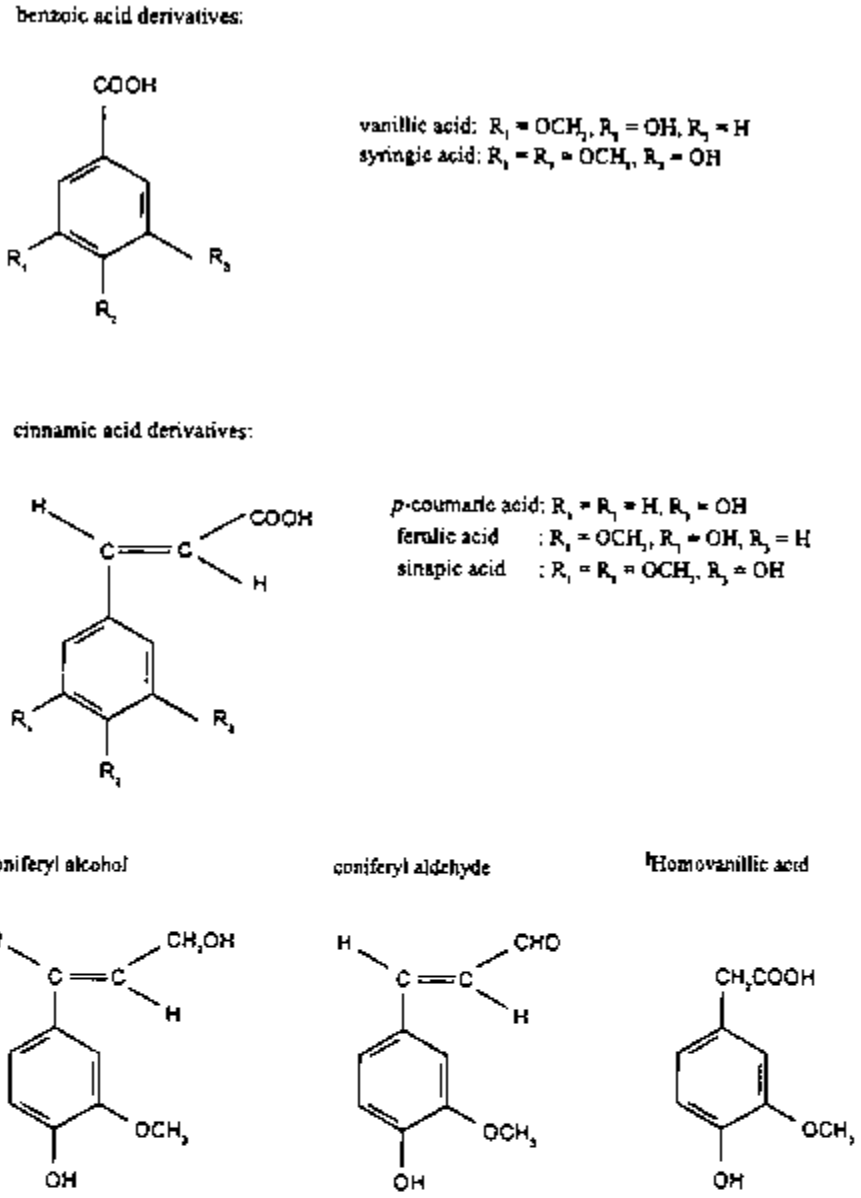

Figure 1. Structures of phenolic compounds studied.

Maple Products Samples. Maple products were obtained from three different Quebec producers identified as ML, AT, and LL. Maple saps, reverse osmosis concentrated saps, and syrups were provided by both producers ML and AT, whereas producer LL provided only saps and syrups.

Reverse osmosis concentration was performed by producers ML and AT at 10 °C using, respectively, a Lapierre system (St. Ephrem, Québec) equipped with two membranes Filmtech (Filmtech Corp., Minneapolis, MN) and a Dominion Grim system (Montréal, Québec) equipped with a Seprotec high-performance membrane (Ottawa, Ontario) both set at 3000 KPa and 2700 L/h. Thermal evaporation was performed by producers ML, LL, and AT at boiling point of the solution until 66 °Brix, using, respectively, an oil burner evaporator 1.65 m × 4 m (Small Brothers, Durham, Québec), a wood-heated evaporator 2.0 m × 5.3 m (Dominion Grim, Montréal, Québec), and an oil burner evaporator 2 m × 4 m (Waterloo, Waterloo, Québec).

Maple saps, concentrates, and syrups were sampled in triplicate for each harvest day over the season 1993. The pH and Brix degree (°Brix) values were determined for each sample. The degree Brix was defined as the refractometric dry substance at 20 °C. In accordance with Québec regulations (Gouvernement du Québec, 1983), maple syrups had 66% of refractometric dry substance at 20 °C.

Extraction of Phenolic and Furfural Compounds. Different methods of extraction of phenolic and furfural compounds were developed. The standard solution, containing 1 μg/mL of each phenolic and furfural standard compounds, was concentrated 25 times by all methods of extraction.

Lyophilization. The standard solution (25 mL) was concentrated by lyophilization using a Labconco (Labconco, Kansas City, MO) freeze dryer set at −50 °C with a vacuum of 10 μm of Hg. The resulting residue was redissolved in 1 mL of methanol and filtered throughout a 0.20 μm filter. The filtrate was subjected to HPLC analyses.

Ethyl Acetate Extraction. The extraction of phenolic compounds was carried out according to a modification of the method of Mahler et al. (1988). The standard solution (25 mL) was adjusted to pH 2 with 6 N HCl and the compounds of interest were extracted successively with 60, 30, and 30 mL of ethyl acetate. The fractions were then pooled and dried with anhydrous Na_2SO_4, and the solvent was removed at room temperature under a gentle stream of N_2. The resultant residue was dissolved in 1 mL of methanol.

Diethyl Ether Extraction. The extraction of phenolic compounds was performed according to a modification of the method described by Krygier et al. (1982). The extracted phenolic and furfural compounds were prepared as described above.

Sep-Pak. The extraction of phenolic and furfural compounds using Sep-Pak C18 column (Millipore, Bedford, MA) was performed according to the method described by Jaworski et

al. (1987). The extracted phenolic and furfural compounds were prepared as described above.

Supelclean. The extraction of phenolic and furfural compounds using Supelclean column (Envichrom-P-SPE, Supelco, Oakville, Ontario) was performed according to the method described previously (Anonymous, 1993). The extracted phenolic and furfural compounds were prepared as described above.

The best recovery was obtained with the ethyl acetate extraction of phenolic and furfural compounds; this method was used for the extraction of saps and concentrates as well as syrups diluted 40 times with distilled water.

High-Performance Liquid Chromatography Analyses of Phenolic and Furfural Compounds. The extracted phenolic and furfural compounds were separated by a gradient elution using a HPLC system (Beckman Model 126, Beckman Instruments Inc., San Ramon, CA) equipped with a UV diode array (UV) detector (Beckman, Model 168) and an electrochemical (EC) detector (Coulochem II, Esa Inc., Bedford, MA) assembled in series and computerized integration with data handling. A Beckman analog interface Model 406 was used to transfer data from the EC detector to the HPLC system. The UV detection was performed at two different wavelengths, 280 and 320 nm. Scanning from 200 to 400 nm was monitored at 1 s interval. The EC detector was set at an output of 1 V, and the detection was performed at 200 and 600 mV, at 10 μA. Automatic injection (Varian, Autosampler 9095, Varian Associates, Inc., Walnut Creek, CA) was carried out with a 20 μL loop onto an Econosil C18 column (150 × 4.6 mm i.d., pore size 5 μm) (Alltech Associates, Inc., Deerfield, IL). The elution (47.5 min) was performed at room temperature and at a flow rate of 0.75 mL/min, using methanol (Omnisolv grade, BDH Inc., Poole, United Kingdom) as solvent A and an aqueous solution of 0.2% trifluoroacetic acid as solvent B, with a linear gradient of 2 to 40% solvent A.

Identification and Quantitation. Initial identity assignments of phenolic and furfural compounds were based on comparison retention data obtained with UV and EC detectors for standard compounds and sample components. Comparison of spectral characteristics (scans from 200 to 400 nm) of standards and sample components provided confirmation of the initial identity assignment. Additional confirmation was provided by the comparison of EC characteristics of standards and sample components.

Calculation of concentrations of compounds of interest was based on the external standard method. Dilutions of aqueous solutions containing 50 ng/mL of all standards were used to fit a standard curve (area versus concentration in nanograms per milliliter), with a linear regression for each compound. Concentration (C) of each compound was calculated from peak area (A) by using the equation

$$C = \alpha + \beta A$$

where α is the curve intercept and β is the curve slope.

The concentrations of phenolic and furfural compounds in maple products were determined in triplicate and the average concentrations were standardized per degree Brix of initial solution and expressed as nanogram per milliliter per degree Brix of solution.

Statistical Analyses. Statistical analyses were performed using StatGraphics software version 5.2 (STSC, Inc., Rockville, MD). Analyses of variance (ANOVA) of variable "HMF concentrations" (147 samples) were performed with three factors, i.e., day of harvest, maple products, and producers. ANOVA of variable "phenolics concentrations" (1470 samples) were performed with four factors, i.e., type of phenolic compound, day of harvest, maple products, and producers. Hypothesis of a nonsignificant effect was made for all ANOVA.

RESULTS AND DISCUSSION

Extraction of Phenolic and Furfural Compounds. The results (Table 1) indicate that the mean percentage of recovery for all phenolic and furfural compounds, using different methods of extraction, was obtained in a decreasing order and as follows: ethyl acetate (87.6%) > Sep-Pak (82.2%) > lyophilization (62.9%) > ether (44.3%) > Supelclean (41.8%). Additional work on ethyl acetate extraction (data not shown) indicated that the mean standard of deviation for all phenolic and furfural compounds of 10 replicates of extraction was 3.081 with a mean coefficient of variation of 7.7%; these findings may indicate a very good reproducibility. On the basis of these results, the ethyl acetate method of extraction was used throughout this study.

Table 1. Percentage of Recovery of 5-(Hydroxymethyl)-2-furaldehyde and Phenolic Compounds Using Different Methods of Extraction

	recovery[a] (%)				
compound	lyophilization	diethyl ether	ethyl acetate	Sep-Pak	Supelclean
5-(hydroxymethyl)-2-furaldehyde	48.6	64.7	95.8	85.7	28.6
vanillic acid	86.0	53.9	97.1	110.8	22.1
syringic acid	0.0	43.9	84.1	104.5	43.0
homovanillic acid	84.6	49.8	85.5	99.1	19.7
coniferyl alcohol	91.3	0.0	87.2	14.6	63.2
vanillin	0.0	58.6	115.8	90.3	44.2
p-coumaric acid	80.7	58.9	88.7	95.7	52.3
syringaldehyde	78.3	45.4	99.1	94.7	52.3
sinapic acid	92.4	0.0	36.0	51.3	58.2
ferulic acid	78	45.2	97.1	86.0	53.6
coniferylaldehyde	52	66.4	87.3	71.6	22.4
av recovery	*62.9*	*44.3*	*87.6*	*82.2*	*41.8*

[a] The relative recovery was calculated as percentage of mean of peak area obtained from triplicate HPLC injections of extract of standard compounds divided by the mean of peak area obtained from triplicate HPLC injections of standard compounds but without extraction. Coefficients of variation of the values reported were from 0.9 to 2.0%.

Optimization of HPLC Analyses. Preliminary trials, carried out for the optimization of HPLC analyses, indicated that the retention times of standard phenolic acids were mostly pH dependent, whereas those of furfural compounds were mostly dependent on acetonitrile concentration. Hence, a gradient elution solvent system, consisted of 2−40% acetonitrile and 98−60% of an aqueous solution of 0.2% trifluoroacetic acid, was developed to provide a chromatogram of well-separated and high-resolution peaks (Figure 2). Scan analyses of standard compounds indicated that the detection of phenolic and furfural compounds was optimum at 280 and 320 nm. Preliminary work for the optimization and selection of the most appropriate potential values for setting the electrode of EC detector indicated that both sensitivity and stable baseline were obtained for the analyses of phenolic compounds at 200 and 600 mV. Typical retention times are reported in Table 2.

The EC analyses provided a dramatic increase in the limits of detection of all phenolic compounds compared with those obtained by UV analyses. The results (Table 2) demonstrate that the limits of detection obtained with EC analyses were 100 (coniferol and homovanillic acid), 50 (vanillin and sinapic acid), 40 (vanillic acid), and 20 (syringic, *p*-coumaric, ferulic, and coniferal) times higher than those obtained with UV analyses. The detection limits (Table 2) are of the order of previous work on UV/EC comparison (Hayes et al., 1987; Galetti et al., 1990).

Identification of Phenolic and Furfural Compounds in Maple Products. Typical chromatograms of HPLC analyses of phenolic and furfural compounds are reported for maple sap (Figure 3), concentrate (Figure 4), and syrup (Figure 5). The literature indi-

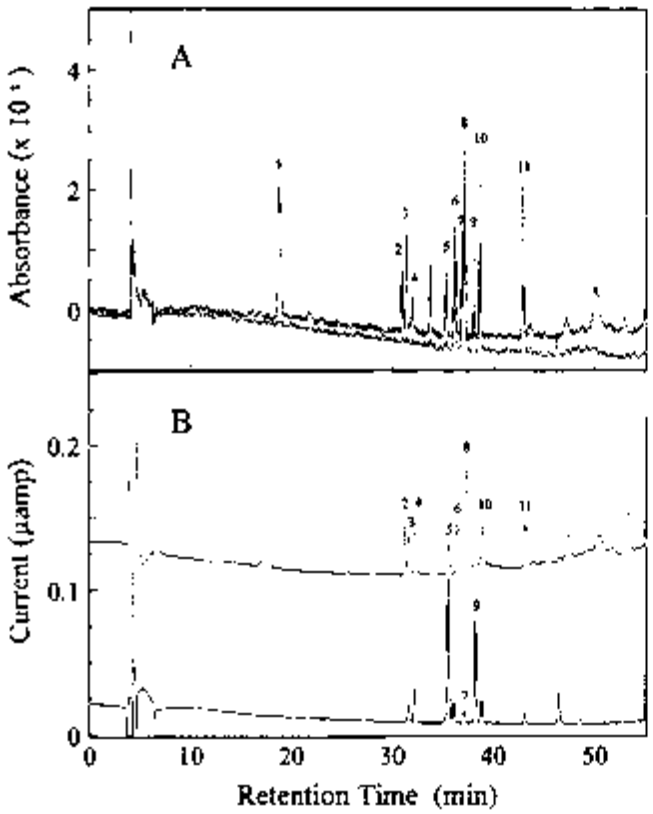

Figure 2. Chromatograms of HPLC analyses of a mixture of standard phenolic and furfural compounds using (A) ultraviolet detection at 280 (upper, solid) and 320 nm (lower, dashed) and (B) electrochemical detection at 200 (lower, solid) and 600 mV (upper, dashed). Peaks are indicated as follows: (1) 5-(hydroxymethyl)-2-furaldehyde, (2) vanillic acid, (3) syringic acid, (4) homovanillic acid, (5) coniferol, (6) vanillin, (7) syringal, (8) *p*-coumaric acid, (9) sinapic acid, (10) ferulic acid, and (11) coniferal.

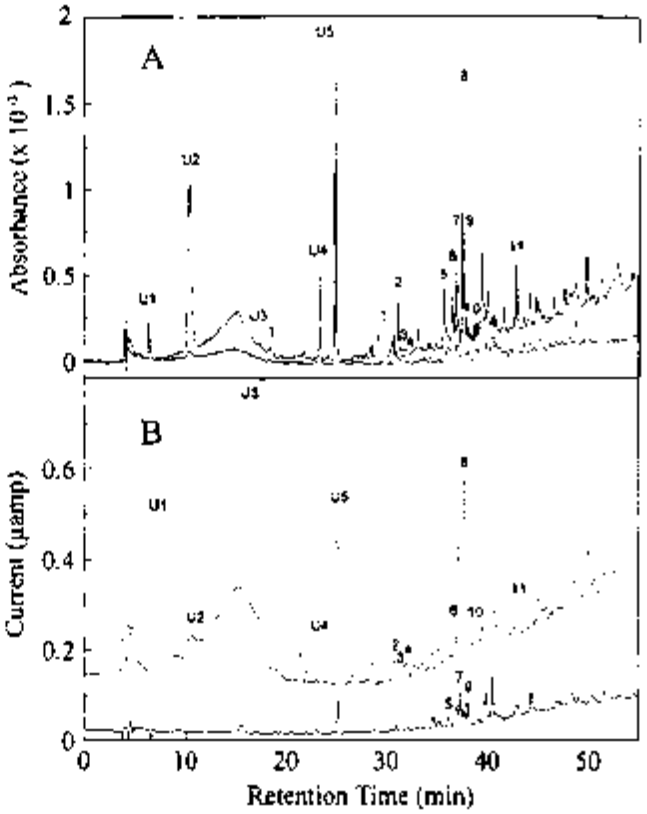

Figure 3. Chromatograms of HPLC analyses of maple sap ethyl acetate extract using (A) ultraviolet detection at 280 (upper, solid) and 320 nm (lower, dashed) and (B) electrochemical detection at 200 (lower, solid) and 600 mV (upper, dashed). Peaks are indicated as follows: (1) 5-(hydroxymethyl)-2-furaldehyde, (2) vanillic acid, (3) syringic acid, (4) homovanillic acid, (5) coniferol, (6) vanillin, (7) syringal, (8) *p*-coumaric acid, (9) sinapic acid, (10) ferulic acid, and (11) coniferal, (U1, U2, U3, U4, U5) unknown compounds.

Table 2. Limit of Detection and Retention Times of 5-(Hydroxymethyl)-2-furaldehyde and Phenolic Compounds Using Ultraviolet and Electrochemical Detectors

		detection limit[a] (ng/mL)			
		ultraviolet diode array		electrochemical	
compound	retention time (min)	280 nm	320 nm	200 mV	600 mV
HMF[b]	18.5	5.00	50.00	c	c
vanillic acid	30.7	10.00	c	50.00	0.25
syringic acid	31.3	5.00	750.00	1.00	0.25
homovanillic acid	31.8	25.00	c	1.00	0.25
coniferyl alcohol	35.0	10.00	50.00	0.10	5.00
vanillin	35.7	5.00	5.00	1.00	0.10
p-coumaric acid	37.2	5.00	5.00	c	0.25
syringaldehyde	36.7	25.00	5.00	1.00	5.00
sinapic acid	37.9	25.00	5.00	0.10	5.00
ferulic acid	38.3	5.00	5.00	1.00	0.25
coniferylaldehyde	43.0	25.00	5.00	5.00	0.25

[a] Detection limit is the minimum detectable concentration of phenolic and furfural compounds calculated on the basis of a 3:1 of signal/noise ratio and expressed as nanograms of standard compound per milliliter. [b] 5-(Hydroxymethyl)-2-furaldehyde. [c] Not detectable at 750 ng/mL.

cates that spectral (Bartolomé et al., 1993) and EC (Joerg and Sontag, 1993) characteristics could be used to assign standard compounds to unknown sample components. Roston and Kissinger (1981) indicated that the comparison of EC responses of standards and sample components could provide a confirmation of the initial identity assignment, obtained with retention time and UV data. Hence, by matching retention time data and spectral and electrochemical characteristics of the corresponding peaks in maple products HPLC analyses with those of standards, the results (Figures 3–5) indicate that peaks 2, 3, 4, 8, 9, and 10 correspond, respectively, to vanillic, syringic, homovanillic, *p*-coumaric, sinapic, and ferulic acids. Similarly, the presence in maple products, of HMF (peak 1), coniferol (peak 5), vanillin (peak 6), syringal (peak 7), and coniferal (peak 12) were confirmed by retention times and spectral and electrochemical data. Our results are in agreement with those reported by Potter and Fagerson (1992) who identified the presence of vanillin, homovanillic, syringic, and vanillic acids as well as coniferal and coniferol in maple syrup.

Spectral characteristics of five major unknown peaks U_1, U_2, U_3, U_4, and U_5 (Figures 3–5) did not allow the identification of these compounds. In order to identify these major peaks, preparative purification was performed. A maple sap sample (100 mL) was extracted and subjected to preparative HPLC, using the same conditions as for the analytical analyses. The five separated fractions exhibited a positive response with the 0.2 N Folin–Ciocalteu reagent (Sigma), a specific test of phenolic compounds (Singleton and Rossi, 1965); hence peaks U_1 to U_5 were tentatively identified as phenolic-related compounds.

Effect of Harvest Time, Processing, and Producer on Concentration of HMF in Maple Products. The results (data not shown) indicate that HMF (0–155.52 ng/mL/°Brix) was detected in the majority

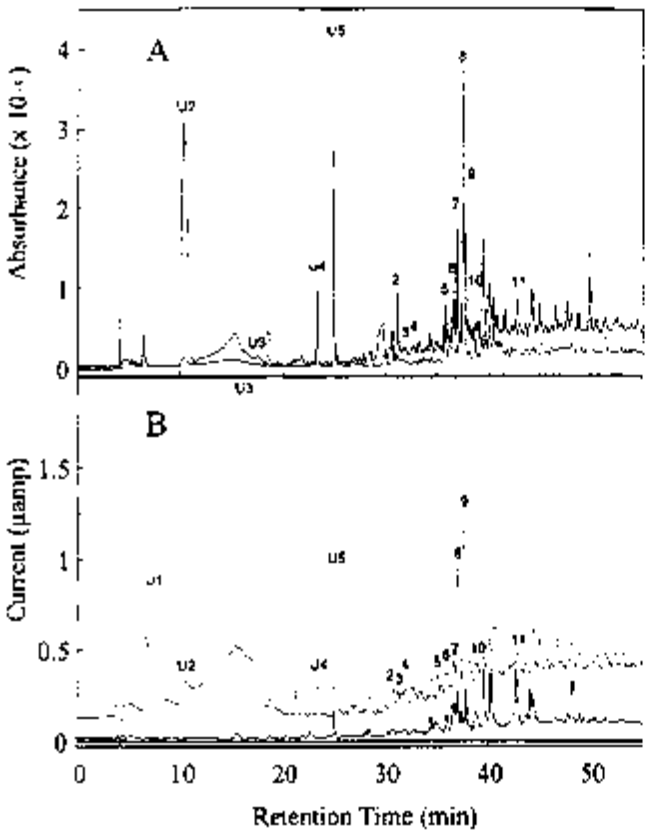

Figure 4. Chromatograms of HPLC analyses of maple concentrate ethyl acetate extract using (A) ultraviolet detection at 280 (upper, solid) and 320 nm (lower, dashed) and (B) electrochemical detection at 200 (lower, solid) and 600 mV (upper, dashed). Peaks: see Figure 2.

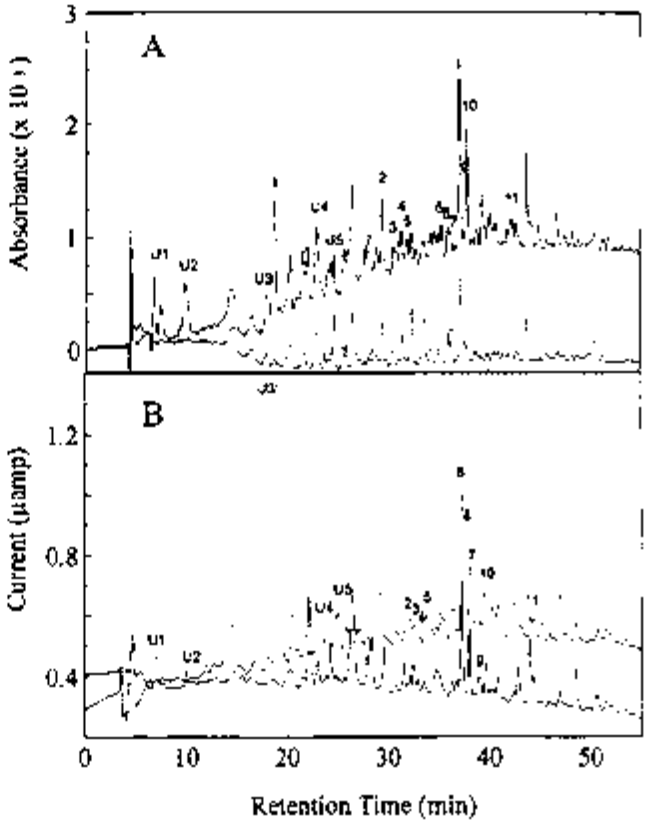

Figure 5. Chromatograms of HPLC analyses of maple syrup ethyl acetate extract using (A) ultraviolet detection at 280 (upper, solid) and 320 nm (lower, dashed) and (B) electrochemical detection at 200 (lower, solid) and 600 mV (upper, dashed). Peaks: see Figure 2.

of maple products. An ANOVA at three factors of the variable "HMF concentrations" in maple products was performed. The results (Table 3) indicate a significant day effect at the level of 0.05, a highly significant maple product effect, and a nonsignificant producer effect.

Graphic representations of ANOVA, i.e., means plots, are reported on Figure 6. The results (Figure 6A) indicate a trend toward a slight seasonal increase of HMF in maple products, that may be related to the increase of the temperature during the season.

The results (Figure 6B) also indicate that the syrups exhibited the highest concentration of HMF, compared to that present in saps and concentrates. Alfonso et al. (1980) reported that the most common product of dehydration of ketopentose, particularly in acid or high-temperature environments, was HMF. The presence of HMF in maple saps and concentrates, with mean pH values of, respectively, 7.06 ± 0.48 and 7.02 ± 0.58, may suggest that the formation of HMF could occurred in neutral or slightly basic conditions. The drastic increase of HMF during heating could be related to the thermal processing, in agreement with the study of Underwood (1971) who reported an increase of HMF peak height by 250% and 800% after heating times of 1.5 and 4 h, respectively.

Although the ANOVA indicate that there is no significant difference in HMF concentration between the producers, the results (Figure 6C) suggest that the concentration of HMF in maple products of producer LL is higher than that of the two other producers.

The concentration of HMF in maple syrups (data not shown) up to 10.26 ppm can be related to the implication of HMF in maple syrup flavor. HMF has been described by Filipic et al. (1969) to be a major constituent of high-flavored maple syrup.

Effect of Harvest Time, Processing, and Producer on Phenolic Compound Profiles in Maple Products. An ANOVA at four factors of the concentrations of 10 phenolic compounds in maple products was performed. The results (Table 4) indicate a highly significant phenolic compound effect, a harvest time effect at the level of 0.05, a highly significant maple product effect, and a highly significant producer effect. Corresponding means plots are reported on Figure 7.

Phenolic Compounds Effect. The intervals of factor means for the level of phenolic compounds in all samples (Figure 7A) show the presence of four different homogeneous groups: group 1 with coniferyl alcohol at the lowest concentration, group 2 with vanillic and syringic acids, group 3 with homovanillic, coumaric and ferulic acids, as well as vanillin, syringaldehyde and coniferaldehyde, and group 4 with sinapic acid with the highest concentration.

Harvest Time Effect. The periods of harvest were from March 26 to April 16, March 27 to April 19, and March 24 to April 11 for the producers ML, AT, and LL, respectively. The results (Table 4) demonstrate the significant effect of harvest time on the concentration of total phenolic compounds present in saps, concentrates, and syrups.

Figure 7B indicates a trend toward a slight seasonal increase of phenolic compounds in maple products. It appears that the highest contents of phenolic compounds occurred at the end of harvest time. In addition a seasonal increase of unknown phenolic-related compounds U_1, U_2, U_3, U_4, and U_5 was observed (data not

Table 3. Analysis of Variance of Concentrations of (Hydroxymethyl)furfural in Maple Products

source of variation	sum of squares	degrees of freedom	mean square	*F* ratio[a]	significant level[b]
main effects					
A: concentration[c]/day[d]	1254.43	22	57.02	1.73*	0.0321
B: concentration[c]/maple product[e]	6644.78	2	3322.39	101.01**	0.0000
C: concentration[c]/producer[f]	24.28	2	12.14	0.37	0.6921
residual	3946.94	120	32.89		
total	11870.43	146			

[a] All *F* ratios are based on the residual mean square error. [b] Effect A is significant at the level 0.05, effect B is nonsignificant, and effect C is highly significant at the level 0.01. [c] Concentrations of (hydroxymethyl)furfural are expressed as ng/mL/°Brix. [d] Concentrations of (hydroxymethyl)furfural were determined each day during the harvest time. [e] Concentrations of (hydroxymethyl)furfural were determined in maple saps, concentrates, and syrups. [f] Concentrations of (hydroxymethyl)furfural were determined for three different producers. *Significant at the 0.05 level **Significant at the 0.01 level.

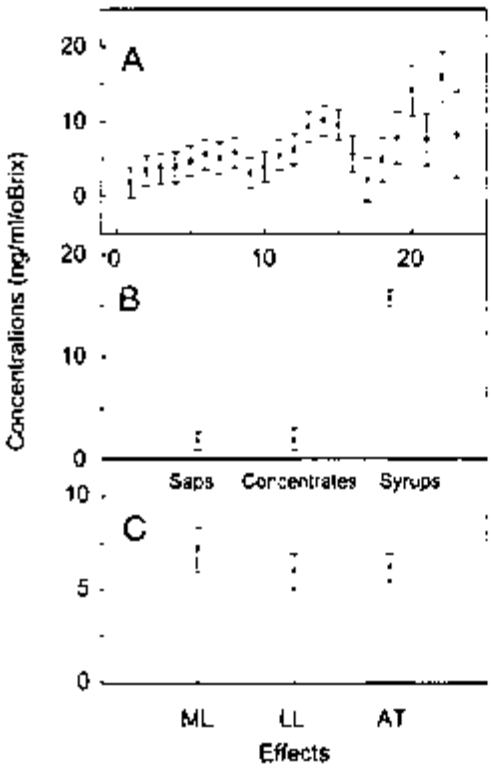

Figure 8. Means plots with 95% confidence for the variable concentration of (hydroxymethyl)furfural concentrations (A) day effect, (B) maple product effect, and (C) producer effect.

shown). These results are in agreement with those of Laing et al. (1971) who reported a slight seasonal increase of phenol-reacting compounds in maple saps. It is probable that different factors, including genetics and climatic and soil conditions, combined to provide variations in qualitative and quantitative profile of phenolic compounds in maple products (Belford et al., 1992); these authors reported that variations in vanillin glycosides concentrations were associated with harvest time during the season of collection.

Maple Products Effect. The results (Figure 7C) indicate that the saps exhibited the lowest concentration of phenolic compounds, whereas there were no significant differences in phenolic concentrations between concentrates and syrups.

Analyses of the interaction between phenolic compound and maple product sources of variations (Table 4) show a highly significant effect. These results indicate that the proportion of each phenolic compound is different as related to the maple products. ANOVA for saps, concentrates, and syrups were performed separately and the results (data not shown) demonstrate that there were significant differences between phenolic compound concentrations for each ANOVA. Corresponding mean plots are reported in Figure 8. The results (Figure 8) show that the relative importance of each phenolic compound is different for saps, concentrates, and syrups. The results (Figure 8) show the presence of four different homogenous groups in the decreasing importance order for saps (group 1, sinapic acid; group 2, vanillic, homovanillic, and *p*-coumaric acids, coniferal, syringal, and vanillin; group 3, syringic and ferulic acids; group 4, coniferol), concentrates (group 1, sinapic acid; group 2, homovanillic acid and coniferal; group 3, vanillic, syringic, and *p*-coumaric acids, syringal, and vanillin; group 4, ferulic acid and coniferol), and syrups (group 1, ferulic acid; group 2, syringal and vanillin; group 3, syringic, homovanillic, *p*-coumaric, and sinapic acids and coniferal; group 4, vanillic acid and coniferol). Thus, sinapic acid is the major phenolic compound identified in saps, and sinapic and homovanillic acids and coniferal are the major phenolics in concentrates, whereas ferulic acid, syringal, and vanillin are the major phenolic compounds identified in maple syrup.

The analyses of the relative proportion of each phenolic in percentages of total phenolics results (Figure 9) indicate that the effect of concentration by reverse osmosis of maple sap has the same trend on the relative composition of phenolic compounds for the producers ML and AT which is an increase of the relative proportions of phenolic acids and a decrease of the relative proportions of aldehyde and alcohol. The loss of aldehydes could be related to the oxidation of the sap in the reverse osmosis system. Chou et al. (1991) reported a substantial reverse osmosis processing loss of aldehyde compounds in apple juices due to the evaporation and membrane capture. In addition, Sheu and Wiley (1983) showed that the retention of some apple juice aldehyde components was dependent on the type of membrane used.

The results (Figure 9) also indicate that the thermal evaporation process resulted in a dramatic increase of ferulic acid and moderate increases of vanillin and syringal, with a concomitant drastic decrease of sinapic acid.

Vanillin and syringal have been previously reported and ascribed to degradation of ligneous material present in maple sap (Underwood et al., 1964). Recently, Belford et al. (1992) reported the presence of a bound vanillin fraction that could be hydrolyzed by β-glucosidases. Macheix et al. (1990b) reported that ferulic acid can be found in plants linked by ester bonds to various polymers, such as lignin derivatives. Hence, the increase in the concentrations of vanillin and ferulic acid during the thermal evaporation process (Figure 9) could be related to the hydrolysis of bound forms of these phenolic compounds.

Table 4. Analysis of Variance of Concentrations of Phenolic Compounds in Maple Products

source of variation	sum of squares	degree of freedom	mean square	*F* ratio[a]	significant level[b]
main effects					
A: concentration[c]/phenolics[d]	5164.77	9	5738.64	48.61**	0.0000
B: concentration/day[e]	10274.42	22	487.47	4.13**	0.0000
C: concentration/maple product[f]	2979.69	2	1489.85	12.62**	0.0000
D: concentration/producer[g]	6773.62	2	3386.81	28.69**	0.0000
interactions					
A/C	60161.51	18	3342.31	28.31**	0.0000
A/D	21.34	18	1185.67	10.04**	0.0000
residual	165048.70	1398	118.06		
total	318677.75	1469			

[a] All *F* ratios are based on the residual mean square error. [b] All effects and interactions are highly significant with a the level 0.01. [c] Concentrations of phenolic compounds are expressed as ng/mL°Brix. [d] Concentrations of 10 phenolic compounds were determined. [e] Concentrations of phenolic compounds were determined each day during the harvest time. [f] Concentrations of phenolic compounds were determined in maple saps, concentrates, and syrups. [g] Concentrations of phenolic compounds were determined for three different producers. **Significant at the 0.01 level.

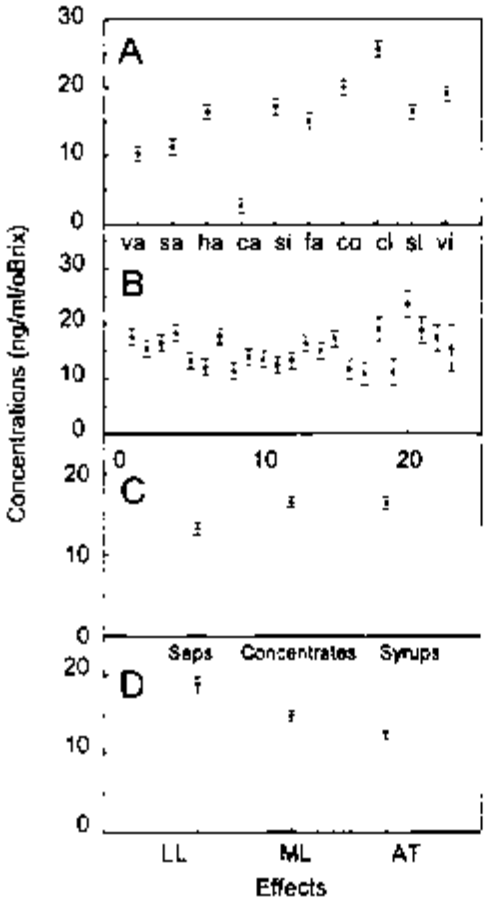

Figure 7. Means plots with 95% confidence for the variable concentration of phenolic compounds (A) phenolic compound effect (vanillic acid, va; syringic acid, sa; homovanillic acid, ha; *p*-coumaric acid, ca; sinapic acid, si; ferulic acid, fa; coniferol, co; coniferal, cl; syringal, sl; vanillin, vi), (B) day effect, (C) maple product effect, and (D) producer effect.

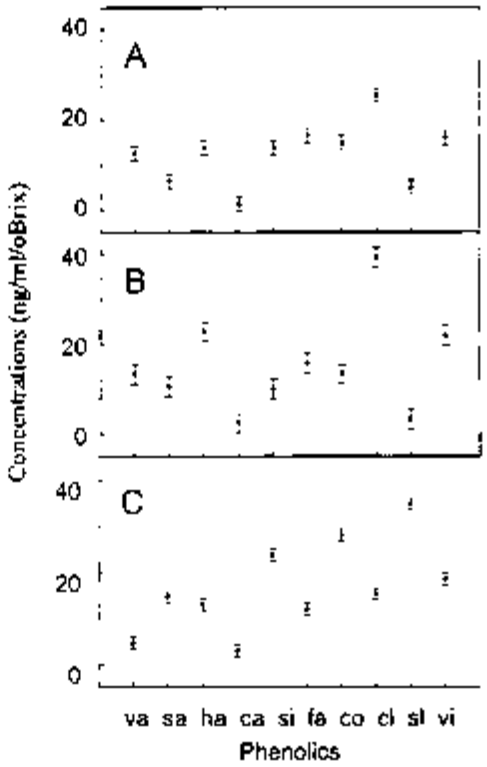

Figure 8. Means plots with 95% confidence for the variable concentration of phenolic compounds (see Figure 7) for the analyses of variance as related to maple products (A: saps, (B) concentrates, and (C) syrups.

The results (data not shown) indicate that vanillin is present in maple syrups at concentrations from 11.05 to 62.02 ng/mL/°Brix which correspond, for syrups of 66 °Brix, to 0.73 to 4.09 ppm, respectively. Vanillin has been described as the most important compound derived from ligneous material with respect to flavor contribution in maple syrup (Filipic et al., 1969). Vanillin is known to have extremely low flavor threshold of 0.69 ppm (Fazzalari, 1978). The results (data not shown) indicate concentrations of ferulic acid in maple syrups from 1.61 to 2.80 ppm. Fazzalari (1978) reported that the flavor threshold of ferulic acid is 90 ppm; in addition, Huang and Ferraro (1992) suggested an anticarcinogenic effect of ferulic acid.

Although there was an evidence of some variations in identified phenolic compound concentrations of maple products, there was not a pronounced variation in unknown phenolic related compounds U_1, U_2, U_3, U_4, and U_5 as a result of the reverse osmosis concentration of maple sap. However, the thermal evaporation process resulted in decrease of major unknown phenolics U_1, U_2, U_3, U_4, and U_5 (data not shown).

Producers Effect. The results (Figure 7D) indicate that the concentrations of phenolic compounds were significantly different among the three producers and that there were, in decreasing order LL > ML > AT.

Analyses of the interaction between phenolic compound and producer sources of variations (Table 4) show a highly significant effect. These results indicate that the proportion of each phenolic compound is different as related to the producer. ANOVA for producers ML, AT, and LL were performed separately and the results (data not shown) demonstrate that there were significant differences between phenolic compound concentra-

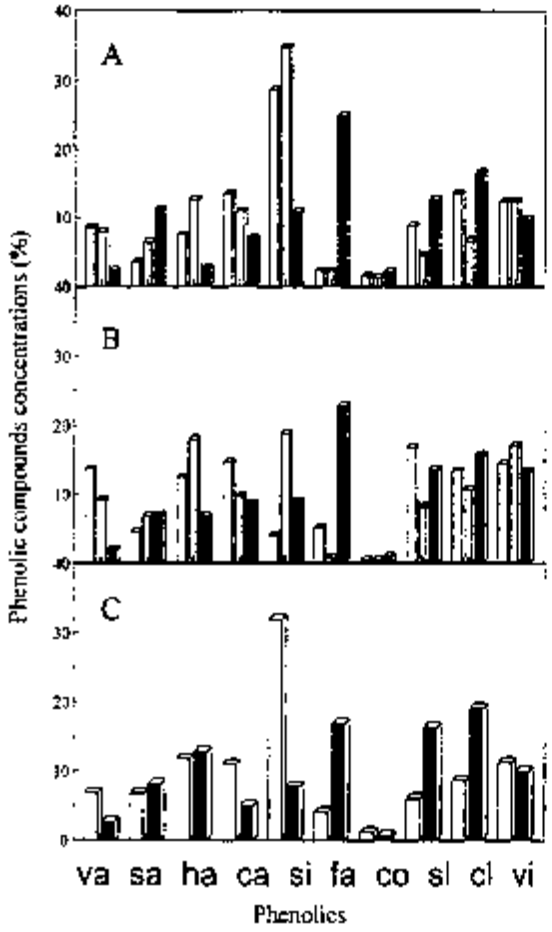

Figure 9. Distribution of phenolic compounds expressed (see Figure 7) as percentage of total phenolics present in maple products from the producers (A) ML, (B) AT, and (C) LL. The bars are differentiated as follows: open, sap; dotted, concentrate; and filled, syrup.

tions for each ANOVA. Corresponding mean plots are reported in Figure 10. The results (Figure 10, parts A–C) show the presence of three similar homogeneous groups for producers ML and LL (group 1, sinapic acid; group 2, vanillic, homovanillic, *p*-coumaric, syringic, and ferulic acids, coniferal, syringal, and vanillin; group 3, coniferol), whereas the proportion of each phenolic is different for producer AT (group 1, homovanillic, *p*-coumaric, sinapic, and ferulic acids, coniferal, syringal, and vanillin; group 2, vanillic and syringic acids; group 3, coniferol).

Those differences between producers may be related to harvest and processing of maple products as well as climatic and soil conditions.

CONCLUSION

The results gathered in this study demonstrated that the optimization of the HPLC analyses using UV and EC detectors allowed the identification and quantification of phenolic and furfural compounds in maple sap, concentrate, and syrup. The present work indicated that HMF concentrations and phenolic profiles of maple products were significantly different as related to harvest time and technological process used to manufacture maple syrup. Highest contents of phenolic compounds occurred at the beginning and at the end of harvest time for all producers. Variations of the quantitative phenolic profile were also observed between the different producers. An increase in the relative proportion of phenolic acids and a decrease in the relative proportions of aldehyde and alcohol was observed during the reverse osmosis processing of maple sap. The thermal evaporation of maple sap or concentrate resulted in an increase of ferulic acid, HMF, vanillin, and syringal and a concomitant drastic decrease of sinapic acid.

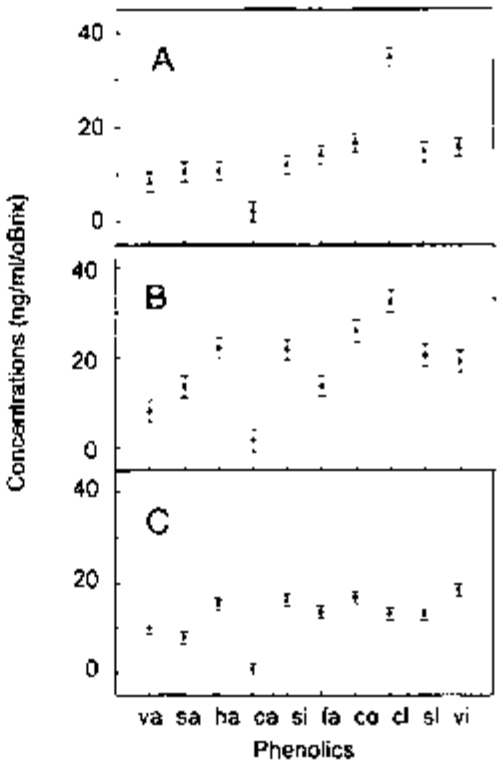

Figure 10. Means plots with 95% confidence for the variable concentration of phenolic compounds (see Figure 7) for the analyses of variance as related to producers (A) ML, (B) LL, and (C) AT.

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Viability of probiotic bacteria in maple sap products under storage and gastrointestinal conditions

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ABSTRACT

This study was undertaken to develop new probiotic products based on liquid maple sap or its concentrate. Sap and concentrate, with or without inulin (2%) were inoculated with *Bifidobacterium lactis* Bb12 and *Lactobacillus rhamnosus* GG valio at initial counts of 10^7–10^8 CFU/ml. Viability was assessed over four weeks of storage at 4 °C and under *in vitro* simulated gastrointestinal conditions using dynamic gastrointestinal model known as TIM-1. Viability was maintained throughout the storage period at the same order of 10^7 to 10^8 CFU/ml. Inulin significantly enhanced the survivability during passage through the gastrointestinal tract simulator. The developed products could be an excellent alternative for delivering probiotics, especially for individuals suffering from lactose intolerance to dairy products.

1. Introduction

Maple sap is a watery liquid produced by maple trees (*Acer saccharum* March) during the spring thaw. Canada is the world's largest producer of maple sap and on average accounts for 84% of the world production of maple sap products (Aider and de Halleux, 2008; Lagacé et al., 2004; Yezza et al., 2007). The sap contains about 97% water and 3% solids. The solids are primarily sucrose and much smaller amounts of glucose and fructose plus nitrogenous, organic and phenolic compounds and minerals (Morselli and Whalen, 1996). Traditionally, maple sap is concentrated by evaporation to produce maple syrup. This process is time-consuming and uses large amounts of energy, since 40 l of sap are required to produce one liter of maple syrup. The prolonged heating also breaks down sucrose to inverted sugars, which may participate in the Maillard reaction (Aider et al., 2007). Due to their nutritional value and wide range of applications in the food industry, maple sap products could be used to develop a variety of functional foods (Yezza et al., 2007).

Consumer interest in functional foods containing probiotics and/or prebiotics is increasing due to their potential role in improving human health and preventing disease (Prado et al., 2008). The development and production of functional foods is one of the fastest growing industries worldwide, with sales expected to exceed $167 billion US by 2010 (Just-Food, 2004). To date, the majority of commercially available probiotic products are based on dairy products or ingredients and none, to our knowledge, are based on maple sap or its products. Maple sap and its concentrate represent good candidates for the production of non-dairy probiotic beverages for avoiding allergic reactions and/or lactose intolerance associated with dairy products (Prado et al., 2008).

It has been suggested that adding non-digestible food ingredients known as prebiotics to certain foods may increase the viability of bacteria passing through the gastrointestinal tract and thus exert a beneficial effect on human health (Chow, 2002; Fooks et al., 1999; Iyer and kailasapathy, 2005; Roberfroid, 2000). Many prebiotic oligosaccharides are now available as consumer products, including fructo-oligosaccharides, inulin, galacto-oligosaccharides, lactulose and isomalto-oligosaccharides (Ozer et al., 2005). Prebiotic and probiotic combination, known as symbiotic, have been shown to improve probiotic proliferation in the intestine (Holzapfel and Schillinger, 1998) and modify gut bacterial community structure (Bartosch et al., 2005). Among prebiotics, inulin and oligofructose have been extensively studied (Gibson et al., 2004) and have been used to develop variety of functional dairy products (Aryana et al., 2007; Cardarelli et al., 2008; Paseephol et al., 2008). These prebiotics have been shown to stimulate the growth of *Bifidobacterium* and *Lactobacillus*, two species used extensively as probiotics and incorporated into various fermented products, especially dairy products such as cheese and yoghurt (Aryana et al., 2007; Maragkoudakis et al., 2006; Ong et al., 2006;

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Tharmaraj and Shah, 2004). *Bifidobacterium lactis* Bb12 and *Lactobacillus rhamnosus* GG are among most studied probiotic strains and their health-promoting properties are well documented (Mohan et al., 2006; Roselli et al., 2006; Wang et al., 2004). The aim of the present study was therefore (1) to develop a symbiotic maple sap product containing the probiotic strains *Bifidobacterium lactis* Bb12 and *Lb. rhamnosus* GG in addition to the prebiotic inulin, (2) to study the viability of these organisms in the product over a proposed shelf life of four weeks at 4 °C, (3) to evaluate chemical changes in the product during storage and (4) to evaluate the capacity of the product to deliver viable probiotic organisms into the human gastrointestinal tract in numbers sufficient to confer a health benefit.

2. Methods

2.1. Maple sap

Maple sap and its concentrate were obtained from the Quebec federation of maple syrup producers (Fédération des producteurs acéricoles du Québec, Longueuil, Quebec, Canada). Samples were collected at the beginning of season 2007 (March–April) and kept frozen at −20 °C until use. Upon thawing, sap and concentrate were passed through a 0.8 μm Versapor filter (Pall, NY, USA) using a laboratory-scale microfiltration system (Osmonics, Oakville, ON, Canada) at 10 psi, held at 63 °C for 20 min and then cooled to 4 °C. Additions of maple syrup aroma (MasterTaste, Teterboro, USA) and edible chicory inulin (Frutafit inulin™, Sunsus America, Monmouth, NJ, USA) at 0.02% and 2%, respectively, were done prior to heating. Cooled samples were immediately dispensed into sterilized glass bottles.

The efficacy of the microfiltration/mild heat treatment on the microbiological quality of the sap and its concentrate was determined by taking aliquots of each product just before and after treatment. Total aerobes, psychotrophes and yeasts and molds were enumerated using specific Petri-films (3 M Inc., London, ON, Canada) incubated aerobically at 30 °C for 48 h, 7 °C for 10 days and at room temperature for five days, respectively. *Pseudomonas* was counted by the drop-plate method described by Herigstad et al. (2001) using *Pseudomonas*-selective medium (Oxoid, Nepean, ON, Canada) supplemented with cetrimide, fucidin and cephaloridine and incubating at 30 °C for 48 h (Lagacé et al., 2006).

2.2. Bacterial strains and probiotic formulation

Bifidobacterium lactis Bb12 (*B. lactis* Bb12) and *Lb. rhamnosus* GG valio (*Lb. rhamnosus* GG) were obtained from Chr. Hansen Ltd. (Barrie, ON, Canada). *B. lactis* Bb12 was reactivated in lactobacilli MRS broth (EMD Chemicals Inc., Gibbstown, NJ, USA) supplemented with 0.05% (w/v) L-cysteine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA) and incubated anaerobically at 37 °C for 24 h in jars using an atmosphere generation system (Oxoid AnaeroGen™, Oxoid Ltd., Basingstoke, Hampshire, England). *Lb. rhamnosus* GG was reactivated in lactobacilli MRS broth and incubated aerobically at 37 °C for 24 h. Both strains were maintained as 20% glycerol stock at −80 °C and sub-cultured at least three times at 24-h intervals before use in experiments.

For probiotic formulation, cells from 24-h MRS cultures were harvested by centrifugation at 8250*g* for 10 min (RC5C Sorvall). After washing with sterilized 0.85% (w/v) sodium chloride solution, cell pellets were resuspended in a small volume of pasteurized maple sap or its concentrate to obtain 10^7–10^8 CFU/ml of each organism. Three maple sap and/or its concentrate were designed; the first one is supplemented only by both probiotic strains, while the second is supplemented by probiotic strains with 0.02% natural maple syrup aroma, and the third contained probiotic strains, maple syrup aroma and 2% of edible chicory inulin prior to heat treatment. Samples were stored at 4 °C for four weeks and samples were taken weekly for chemical and microbiological analyses.

2.3. Total solids and pH measurements

Since over 98% of the dry matter in maple sap is sucrose, Brix measurements could be used to estimate sugar or total solids contents, one degree Brix being equivalent to 1% sucrose (Aider and de Halleux, 2008). This measurement was done at room temperature 25 °C ± 1 using a digital refractometer (Reichert, Depew, NY, USA). The pH was determined using a pH meter.

2.4. Microbiological analysis

Viable counts of *B. lactis* and *Lb. rhamnosus* in maple sap were determined using the drop-plate method described by Herigstad et al. (2001). Samples were serially diluted (1/10) in sterile 0.1% (w/v) peptone water (Difco laboratories, Detroit, MI, USA). For *Lb. rhamnosus,* 20 μl of each dilution were plated in duplicate on lactobacilli MRS agar (EMD Chemicals Inc., Gibbstown, NJ, USA) supplemented with 1 mg/l vancomycin (Sigma-Aldrich Canada Ltd, Oakville, ON, Canada) and incubated at 43 °C for 48 h (Tharmaraj and Shah, 2003). For *B. lactis*, 20 μl of each dilution were plated in duplicate on Beerens agar and incubated anaerobically at 37 °C for 3 days (Beerens, 1990).

2.5. Probiotic delivery capacity

2.5.1. TIM-1 dynamic model

The dynamic gastrointestinal model TIM-1 (TNO Nutrition and Food Research Institute, Zeist, Netherlands) described previously by Minekus et al. (1995) is presented in Fig. 1. The model consists of four compartments connected in series to simulate the stomach, duodenum, jejunum and ileum, separated by valve segments under computer control. Each compartment is composed of two glass

Fig. 1. The multi-compartmental dynamic TIM-1 model of the gastrointestinal system. Vessels (A–D) constitute the gastric, duodenal, jejunal and ileal compartments, respectively. Modules (E) are semi-permeable hollow-fiber membrane dialysis units. (F) Peristaltic valves, (G) ileo-caecal valves, (H) pH electrodes, (I) temperature sensor, (J) stomach secretion inlets, (K) duodenal secretions inlet, (L) and (M) bicarbonate secretion inlet, and (N) volume detecting sensors. Adapted from Kheadr et al. (in press).

jackets in series, in which a flexible tubular membrane is installed. The space between the membrane and the glass jacket is filled with warm water, which maintains the temperature of the chyme in each compartment at 37 °C. The contractions of the space inside the flexible membranes are achieved by varying the water pressure on the two jackets using computer-controlled pumps. This imitates the peristaltic movements of the stomach and small intestine and thus ensures mixing of chyme in each compartment. The contraction frequencies were five and six times per minute for gastric and duodenal compartments, respectively, and seven times per minute for the jejunal and ileal compartments. Two independent sensors connected to the stomach and small intestine compartments ensure temperature control. Hollow-fiber modules connected to the jejunal and ileal compartments provide dialysis of the contents thereof against small intestinal electrolyte solution. The pH in the gastric and small intestine compartments is monitored with four electrodes connected to the computer.

2.5.2. Model disinfection and preparation

Prior to each experiment, the TIM-1 was disinfected for 60 min with 10% (v/v) commercial bleach solution (Lavo Inc., Montreal, PQ, Canada) followed by successive rinsing with sterilized de-mineralized water. To verify complete elimination of hypochlorite, the pH of rinsing water from different sites was measured (pH 6.0 is considered satisfactory). Total bacterial count, yeasts, molds and coliforms in the water at the end of the rinsing protocol were enumerated using specific Petri-films (3 M Inc.) in order to confirm the effectiveness of disinfection procedure. Samples of 0.1 ml of rinsing water were spread onto Beerens agar and MRS-vancomycin agar and incubated anaerobically at 37 °C for 72 h or aerobically at 43 °C for 48 h, respectively, to detect any organism capable of growing on these selective media.

2.5.3. Digestion and sampling

In vitro digestions of probiotic suspensions were done using freshly prepared (i.e. filtered, heated and cooled to 4 °C) maple sap or concentrate. Briefly, 300 g of suspension were introduced into the stomach compartment. Digestions were done according to a fast transit protocol, with delivery half times of 35 and 110 min respectively for the gastric and ileal compartments, based on *in vivo* data on the gastric delivery of liquid milk reported by Marteau et al. (1990, 1991). The gastric pH was initially 4.5 and decreased gradually to 2.4 after 30 min, 1.7 after 60 min and 1.6 after 90 min by injecting 1 M HCl solution and kept at 1.5 from 120 to 300 min. The pH in the duodenal, jejunal and ileal compartments was adjusted to 6.5, 6.8 and 7.2, respectively, by injecting 1 M sodium bicarbonate solution.

Gastric secretions consisted of pepsin (~0.28 mg/ml) from porcine gastric mucosa (EC 3.4.23.1; Sigma-Aldrich Canada Ltd.) and lipase (~0.25 mg/ml) from *Rhizopus oryzae* (EC 3.1.1.3, Amano Pharmaceuticals, Nagoya, Japan), both in an electrolyte solution (NaCl, 3.0 g/l; KCl, 1.1 g/l; $CaCl_2$, 0.15 g/l; $NaHCO_3$, 0.60 g/l) delivered at flow rates of 0.25 and 0.13 ml/min, respectively. Duodenal secretions consisting of 7% pancreatin solution (Pancrex V powder; Paines and Byrne, Greenford, UK) in reverse osmosis-purified water and 4% porcine bile extract (Sigma-Aldrich Canada Ltd.) as well as small intestine electrolyte solution (NaCl, 5.0 g/l; KCl, 0.60 g/l; $CaCl_2$, 0.30 g/l; pH 7.0) were injected respectively at 0.25 ml/min, 0.5 ml/min and 0.25 ml/min and the total injected volumes were logged. According to the manufacturer, 1 gram of Pancrex V powder contains 1400, 25,000 and 30,000 British Pharmacopoeia units of free protease, lipase and amylase, respectively.

Aliquots of 1 ml were taken in duplicate at 0, 20, 40, 60 and 80 min from the gastric compartment to evaluate the impact of this stage of digestion on the viability of both probiotic organisms. The ileal effluent was analyzed at 1 h intervals over the entire 5-h digestion period. Viable *B. lactis* and *Lb. rhamnosus* were determined using the drop-plate method with Beerens and MRS-vancomycin agars as described above. *B. lactis* Bb12 and *Lb. rhamnosus* GG survival was expressed as $\log_{10}$ colony-forming units (CFU), that is, the measured concentration multiplied by the cumulative chyme volume outflow from the gastric or ileal compartment.

2.6. Statistical analysis

Statistical analyses were performed with STATGRAPHICS plus 4.1 (Manugistics, Inc., Rockville, MD). Treatment effects were tested by analysis of variance and Fisher's least-significant differences method (LSD). The level of significance was $P \leqslant 0.05$.

3. Results

3.1. Effect of microfiltering and heating on microbiological quality

In order to determine the effectiveness of microfiltration/mild heat for improving the microbiological quality of maple sap and its concentrate, we analyzed the microbial load before and after treatment. No coliforms or spore-forming bacteria were detected in either the raw maple sap or its concentrate. However, both products contained large numbers of psychrotrophs, total aerobes (10^6–10^7 CFU/ml), *Pseudomonas* sp. (10^5–10^6 CFU/ml) and yeasts and molds (10^4–10^5 CFU/ml). The microfiltration/heat treatment eliminated the vast majority of all of these groups from the maple sap products. Post-treatment viable counts were below the detection limit of the enumeration method (<10 CFU/ml), which indicates that the treatment was effective at improving the microbial quality of both maple sap and its concentrate product (Data not shown).

3.2. Changes in total solids and pH during storage of maple products

The total solids content of raw maple sap and its concentrate varied from 2.5% to 2.7% and 9.6% to 10.8%, respectively (Aider and de Halleux, 2008). Adding inulin increased total solids by almost 2%. In general, total solids contents remained relatively constant over 28 days of storage.

Changes in pH of the maple sap probiotic preparations during 28 days of storage at 4 °C are illustrated in Fig. 2. The initial pH of maple sap and its concentrate was 6.88 ± 0.01 and 7.06 ± 0.06, respectively. The addition of inulin resulted in significant ($P < 0.05$) reduction in pH to 6.62 ± 0.04 and 6.38 ± 0.03 for sap and concentrate, respectively. During storage, inulin-containing

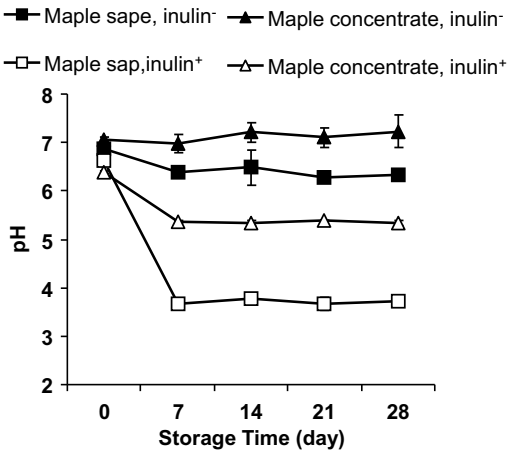

Fig. 2. Changes in the pH of maple sap and its concentrate containing *Bifidobacterium lactis* Bb12 and *Lactobacillus rhamnosus* GG valio both at 10^7–10^8 CFU/ml, with or without added inulin (2%, w/v) over 28 days of storage at 4 °C.

samples had significantly lower pH than their counterparts without inulin. The major shift in pH was observed at day 7, dropping to 3.67 ± 0.03 and 5.36 ± 0.03 in inulin-containing sap and concentrate respectively. However, no further significant reductions were observed between days 7 and 28 of storage. In sap and concentrate without inulin, the pH remained relatively constant during storage and was higher in concentrate than in sap.

3.3. Probiotic viability under storage conditions

In a preliminary trial, we evaluated the capacity of four known probiotic strains to survive in maple sap and its concentrate at 4 °C (data not shown). These were *B. lactis* Bb12, *Lb. rhamnosus* GG valio, *B. longum* ATCC 15707 and *Lb. johnsonii* LA-1. Strains Bb12 and GG valio were selected on the basis of their greater survival under these conditions.

Survival of *B. lactis* and *Lb. rhamnosus* in maple sap and its concentrate at 4 °C is illustrated in Fig. 3A and B, respectively. The average initial counts of viable *B. lactis* Bb12 did not differ significantly among the maple sap products and were approximately 7.8 $\log_{10}$ CFU/ml in each case. Counts remained relatively constant ($P > 0.05$) during the 28 days at 4 °C except for statistically significant decreases by approximately 0.5 $\log_{10}$ CFU/ml for *B. lactis* on day 21 and for *Lb. rhamnosus* GG on day 28, both in sap with inulin. *Lb. rhamnosus* viable counts at day 28 were all around 8 $\log_{10}$ CFU/ml, which was quite similar to the initial counts.

3.4. The viability of probiotics under gastrointestinal conditions

The viability of *B. lactis* Bb12 and *Lb. rhamnosus* GG in maple sap or concentrate under gastrointestinal conditions is expressed relative to the total volume of gastric and ileal effluent at each sample time during *in vitro* digestion in the TIM-1 (Figs. 4 and 5). Over the entire 80-min gastric digestion, maple sap concentrate delivered significantly more viable *B. lactis* Bb12 into the duodenal compartment than sap did (Fig. 4A). The cumulative gastric delivery of viable *B. lactis* Bb12 appeared to be influenced positively by inulin. Based on the initial viable count of 10.2 $\log_{10}$ CFU, the loss in the viability of *B. lactis* Bb12 during the gastric phase was from 0.4 to 1.2 $\log_{10}$ CFU. The total cumulative delivery of viable *B. lactis* Bb12 from the gastric into the duodenal compartment was approximately 9.6, 9.1, 8.9 and 8.8 $\log_{10}$ CFU for concentrate with inulin, concentrate without inulin, sap with inulin and sap without inulin, respectively. The loss of viability at this stage was not significant for concentrate with inulin, but was for the other formulations. In comparison, the cumulative delivery of *B. lactis* Bb12 from the ileal compartment after 5 h of digestion of inulin-free sap was 6.9 $\log_{10}$ CFU, which was significantly lower than 7.7–7.5 $\log_{10}$ CFU determined for the other three samples (Fig. 4B). Thus, major reductions in the viability of *B. lactis* Bb12 occurred during its transit through the intestinal compartment compared to the gastric phase of digestion. This may indicate that intestinal bile salts are more harmful to strain Bb12 than gastric acid. Gastric delivery of viable *B. lactis* Bb12 into the duodenal compartment ranked in the following order: maple concentrate, inulin$^+$ > maple sap, inulin$^+$ > maple concentrate, inulin$^-$ > maple sap, inulin$^-$, respectively. The ranking was the same for delivery into the ileum, albeit with smaller differences.

Similar to *B. lactis* Bb12, the cumulative viable counts of *Lb. rhamnosus* GG delivered from either the gastric or ileal compartments were greater in suspensions containing inulin. The ranking was maple concentrate, inulin$^+$ > maple concentrate, inulin$^-$ > maple sap, inulin$^+$ > maple sap, inulin$^-$. After 80 min of gastric

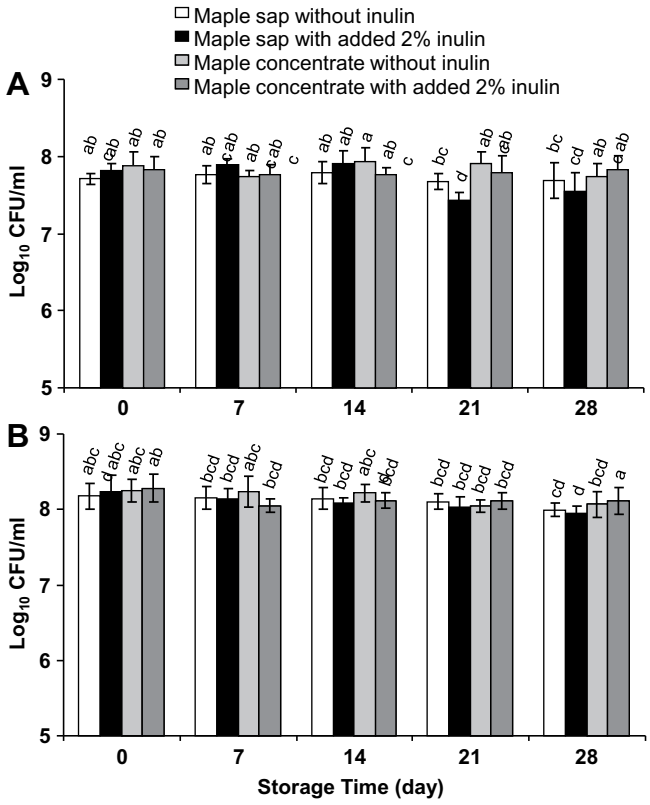

Fig. 3. Survival of *Bifidobacterium lactis* Bb12 (A) and *Lactobacillus rhamnosus* GG valio (B) in probiotic maple sap and its concentrate with or without added inulin (2%, w/v) during 28 days of storage at 4 °C. Means with different letters are significantly different ($P \leqslant 0.05$).

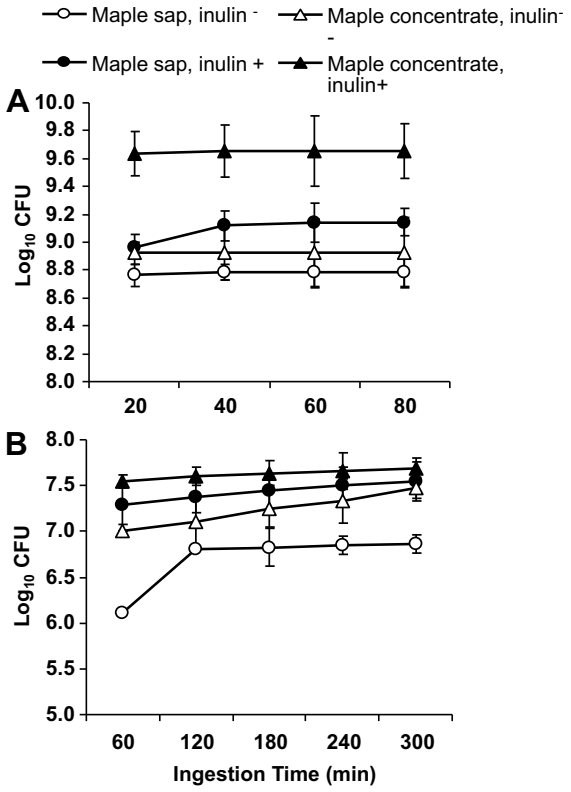

Fig. 4. Cumulative viable counts of *Bifidobacterium lactis* Bb12 suspended in maple sap products with or without added inulin (2%, w/v), delivered from the gastric to the duodenal compartment (A) and from the ileal compartment (B) during *in vitro* digestion in the TIM-1 gastrointestinal model. Means ± SD, two independent repetitions.

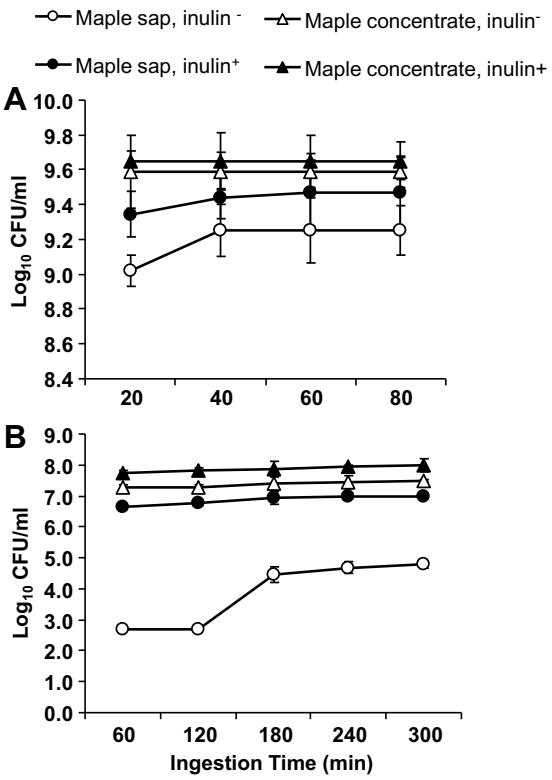

Fig. 5. Cumulative viable counts of *Lactobacillus rhamnosus* GG valio suspended in maple sap products with or without added inulin (2%, w/v), delivered from the gastric to the duodenal compartment (A) and from the ileal compartment (B) during *in vitro* digestion in the TIM-1 gastrointestinal model. Means ± SD, two independent repetitions.

digestion, the total delivery of viable *Lb. rhamnosus* GG into the duodenal compartment was approximately 9.6 $\log_{10}$ CFU for both maple sap concentrates either with or without inulin (Fig. 5A) and 9.45 and 9.25 $\log_{10}$ CFU for maple sap with and without inulin, respectively. Relative to the initial count of 10.6 $\log_{10}$ CFU contained in the 300 g of suspension, the 1.35 $\log_{10}$ CFU reduction in viable *Lb. rhamnosus* GG in maple sap without inulin was the largest loss sustained during gastric digestion. Losses of viable *Lb. rhamnosus* GG ranging from 1.6 to 4.5 $\log_{10}$ CFU occurred during transit through the intestinal compartments (Fig. 5B). The total ileal deliveries of strain GG were 8.0, 7.5, 7.0 and 4.8 $\log_{10}$ CFU after 5 h of digestion of maple concentrate inulin$^+$, concentrate inulin$^-$, sap inulin$^+$ and sap inulin$^-$, respectively.

4. Discussion

The vast majority of the microbial community of maple sap is made up of aerobic, psychotrophic bacteria, particularly *Pseudomonas* sp., as well as yeasts and molds (Lagacé et al., 2004, 2006). The composition of the microbial community is an indicator of microbiological quality, which can significantly affect the chemical and organoleptic characteristics of maple products. The higher the microbiological quality, the better the quality of the maple concentrate or syrup. Several factors have been shown to influence the microbiological quality of maple sap, including temperature, transient time, nutrient availability and the sanitary status and sophistication of the maple sap collection system and its handling (Chapeskie, 2005; Lagacé et al., 2004, 2006). Although conventional evaporation techniques used for the production of maple concentrate and syrup products often improve the microbiological quality of these products, this is at the expense of organoleptic quality and certain valuable nutritional components. For the probiotic preparation, maple sap should be treated in a manner that eliminates its microbial load while protecting its sensitive nutritional components such as sugars and amino acids. A combination of microfiltration and mild heat treatment at 63 °C for 20 min was therefore developed for the present study and found effective for reducing viable bacterial and mold counts in raw maple sap and its concentrate to as low as 10 CFU/ml. In addition to its effectiveness for this purpose, this combination could also decrease the energy required for evaporation and improve the organoleptic and nutritional quality of maple sap products by reducing undesirable effects associated with the prolonged boiling usually done in conventional maple syrup processing.

The pH of raw maple sap and its concentrates was within the range of 6.8–7 and 7–7.5, respectively, as mentioned previously by Dumont et al. (2000). To our knowledge, our study is the first to examine maple sap fortified with inulin, a plant polymer used extensively in dairy product fortification as a prebiotic. In yoghurt and cheese, the addition of up to 3% inulin did not appear to influence titratable acidity and pH (Yasar et al., 2005; Cardarelli et al., 2008). In cheese, inulin is known to increase whey separation and consistency and thus decrease the amount of residual lactose, which could otherwise be further fermented by both dairy starter and probiotic cultures, leading to additional lowering of pH during storage. Contrary to dairy products, maple sap and its concentrate do not contain amphoteric proteins, thus inulin would be expected to behave differently. Generally, chicory inulin has a slight acidic behavior when dissolved in water and could shift the pH to as low as 5.0, depending on its concentration (Franck, 2002). This may explain the instant shift in pH of maple sap and its concentrate upon inulin addition.

Both *B. lactis* Bb12 and *Lb. rhamnosus* GG appeared to have significant potential for survival in maple sap with or without added inulin and high tolerance to refrigerator temperatures. Viable counts as high as 7 $\log_{10}$ CFU/ml over 28 days of storage at 4 °C suggest promise as probiotic candidates for the development of a variety of probiotic products. Although the viability of *B. lactis* and *Lb. rhamnosus* GG decreased in maple sap containing inulin, the counts of >7 $\log_{10}$ CFU/ml after 28 days of cold storage were above the 6 $\log_{10}$ CFU/ml recommended for a probiotic to exert its health benefits (Rasic and Kurmann, 1983).

Unlike other bifidobacteria, *B. lactis* Bb12 in this study appeared to have the ability to survive at 4 °C even at pH as low as 3.7, as determined in maple sap containing inulin since day 7 of storage. In general, the viability of bifidobacteria has been reported significantly reduced in acidic conditions (pH below 5.0) and during storage at 5 °C (Lankaputhra and Shah, 1995). In comparison, the *Lb. rhamnosus* GG strain used in this study is well known for its ability to colonize the intestine and defend against travelers' diarrhea and childhood rotavirus infection (Ouwehand and Salminen 1998; Salminen et al., 1998; Saxelin 2001). Strain GG has also shown the ability to survive in dairy products and to tolerate adverse storage conditions (Alampreese et al., 2005). This is in agreement with its ability to survive in maple sap products under the cold storage conditions in our study.

To our knowledge, the present study is the first to deal with the development of a probiotic product based on maple sap products. Previous attempts have been undertaken to develop probiotic vegetable drinks for vegetarians or consumers who are allergic to dairy products (Yoon et al., 2004, 2005, 2006). In their studies, Yoon et al. used tomato, beet and cabbage juices as raw materials for the production of fermented juices containing either *Lb. acidophilus, Lb. plantarum, Lb. casei* or *Lb. delbrueckii*. Following fermentation at 30 °C for 48–72 h, the juices were stored at 4 °C for four weeks. The resulting lactobacilli counts ranged from 10^6 to 10^8 CFU/ml,

except for *Lb. casei,* which lost viability in fermented cabbage juice after two weeks. The viability of a probiotic organism in such products depends largely on factors such as strain characteristics, relationships between species (when a probiotic mixture is used), pH and/or product acidity, culture conditions and oxygen available in the media (Yoon et al., 2004). Among these factors, strain characteristics are considered the most crucial element in determining the ability of a probiotic organism to survive adverse conditions either during product manipulation and storage or during transit through the gastrointestinal tract.

The tolerance of probiotics to gastrointestinal transit has been evaluated in the past in acidified media (pH 2.0) or in media (usually oxgall) containing 0.3% bile (Lin et al., 2006; Mainville et al., 2005), which does not mimic the sequential stresses to which ingested microorganisms are exposed during their passage *in vivo* (Marteau et al., 1997). Meanwhile, numerous commercial probiotic products have been developed over the past two decades and are available as consumer products but very little is known about their ability to deliver enough viable probiotic to the gastrointestinal tract. We thus sought to determine the capacity of the maple sap products to deliver viable probiotics using the TIM-1 model. This model simulates gastrointestinal stress conditions such as gastric acid, proteolytic enzymes, bile salts and peristaltic movements, which distinguish it from single-compartment *in vitro* models (Marteau et al., 1997; Minekus et al., 1995).

During *in vitro* digestion, *B. lactis* Bb12 and *Lb. rhamnosus* GG showed quite similar tolerance to gastrointestinal conditions. One exceptional tendency, observed for strain GG in maple sap without inulin, was a huge reduction in cumulative viable counts delivered from the ileal compartment, suggesting sensitivity to bile salts. Inulin appeared to improve the tolerance of both strains to gastric fluids and intestinal bile salts. The presence of foodstuffs in general may improve the viability of probiotic bacteria against gastrointestinal conditions by raising gastric pH and increasing tolerance to bile salts (Lin et al., 2006; Madureira et al., 2005).

In a previous study using a simulated gastric juice, it was found that the ability of *Lactobacillus* cultures to tolerate acid varied widely among species and that *Lb. rhamnosus* GG exhibited good survivability (Corcoran et al., 2005). *In vitro* digestion of kefir using a dynamic model simulating the upper gastrointestinal tract (Mainville et al. (2005) revealed that *Lb. rhamnosus* GG survived well at pH 2 for up to 60 min but as little as 0.1% after 90 min. Kheadr et al. (2007) evaluated the ability of 13 bifidobacterial strains to tolerate acid, oxgall and H_2O_2, as well as changes in their antibiogram profile due to these stresses, showed that *B. lactis* Bb12 could be classified among the most tolerant strains, suffering little loss in viability due to oxgall or H_2O_2 and only a slight loss by exposure to pH 2.0 for 60 min.

5. Conclusion

In the present study, a method based on a combination of microfiltration and mild heat treatment has been developed and found adequate for reducing the microbial load of maple sap and increasing its storability at refrigerator temperatures. This method was also found effective in the case of maple products containing inulin. *B. lactis* Bb12 and *Lb. rhamnosus* GG added to maple products remained viable under cold storage conditions for 28 days. The probiotic products developed have thus shown their potential usefulness as vehicles for the delivery of viable *B. lactis* Bb12 and *Lb. rhamnosus* GG in large numbers in the gastrointestinal tract. Further research should be done to validate these *in vitro* results and to broaden the range of bacterial strains and prebiotic agents assayed, both on a pilot scale and *in vivo*.

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Antioxidant Activity, Inhibition of Nitric Oxide Overproduction, and *In Vitro* Antiproliferative Effect of Maple Sap and Syrup from *Acer saccharum*

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ABSTRACT Antioxidant activity, inhibition of nitric oxide (NO) overproduction, and antiproliferative effect of ethyl acetate extracts of maple sap and syrup from 30 producers were evaluated in regard to the period of harvest in three different regions of Québec, Canada. Oxygen radical absorbance capacity (ORAC) values of maple sap and syrup extracts are, respectively, 12 ± 6 and 15 ± 5 μmol of Trolox equivalents (TE)/mg. The antioxidant activity was also confirmed by a cell-based assay. The period of harvest has no statistically significant incidence on the antioxidant activity of both extracts. The antioxidant activity of pure maple syrup was also determined using the ORAC assay. Results indicate that the ORAC value of pure maple syrup (8 ± 2 μmol of TE/mL) is lower than the ORAC value of blueberry juice (24 ± 1 μmol of TE/mL) but comparable to the ORAC values of strawberry (10.7 ± 0.4 μmol of TE/mL) and orange (10.8 ± 0.5 μmol of TE/mL) juices. Maple sap and syrup extracts showed to significantly inhibit lipopolysaccharide-induced NO overproduction in RAW264.7 murine macrophages. Maple syrup extract was significantly more active than maple sap extract, suggesting that the transformation of maple sap into syrup increases NO inhibition activity. The highest NO inhibition induced by the maple syrup extracts was observed at the end of the season. Moreover, darker maple syrup was found to be more active than clear maple syrup, suggesting that some colored oxidized compounds could be responsible in part for the activity. Finally, maple syrup extracts (50% inhibitory concentration = 42 ± 6 μg/mL) and pure maple syrup possess a selective *in vitro* antiproliferative activity against cancer cells.

KEY WORDS: • **Acer saccharum** • ***anti-inflammatory*** • ***antioxidant*** • ***antiproliferative activity*** • ***cancer cells*** • ***nitric oxide inhibition*** • ***phenolic compounds*** • ***sap*** • ***sugar maple*** • ***syrup***

INTRODUCTION

MAPLE SYRUP IS MADE from the sap of *Acer saccharum* (sugar maple). The commercial production of maple syrup is mainly North American, with Québec, Canada, assuming over 80% of the world production.[1] Maple sap is essentially a water-like slightly sweetened solution constituted of sucrose (2–2.5%), organic compounds, and minerals such as potassium, calcium, and magnesium.[2] The main organic compounds identified are amino acids, proteins, and phenolic compounds such as vanillic acid, homovanillic acid, coniferyl alcohol, vanillin, *p*-coumaric acid, syringaldehyde, sinapic acid, and coniferaldehyde.[3] Most phenolic compounds are known to have antioxidant and anti-inflammatory activities.[4] Inflammation is an important factor in the formation (mutagenesis) and development of cancer.[5] Chronic inflammation can predispose an individual to cancer,[6] and, in almost all cases, tumor development induces local or systemic chronic inflammation, thus favoring tumor growth and the formation of metastases.[7] Several works have shown that inflammation leads to an overproduction of nitric oxide (NO) and reactive oxygen species such as superoxide anions ($\bullet O_2^-$).[8] The combination of these two reactants could lead to the formation of peroxynitrite ($ONOO^-$), a powerful mutagenic oxidant.[8,9] Furthermore, NO itself can stimulate tumor growth and metastasis by favoring migration, invasion, and angiogenesis.[10] During the tumor pro-inflammatory process, NO can be generated by inducible NO synthase (iNOS).[10,11] Generally, iNOS is overexpressed in tumor-related activated macrophages and cancer cells.[10,11] Consequently, the use of nutraceutical food having antioxidant and NO inhibition activities could inhibit inflammation and possibly prevent several diseases such as cancer.[12–14] Recently, Thériault *et al.*[15] showed that maple sap and syrup extracts possess interesting antioxidant and antimutagenic activities. However, inhibition of NO overproduction and *in vitro* antiproliferative activity of maple sap and syrup extracts were never studied.

In this study, we measured phenol contents and we assessed the antioxidant activity, inhibition of NO overproduction, and *in vitro* antiproliferative activity against cancer

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cell lines of maple sap and syrup originating from 30 producers in three different regions of the province of Québec with regard to the period of harvest.

MATERIALS AND METHODS

Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) were purchased from Aldrich (Milwaukee, WI, USA), Folin-Ciocalteu and sodium carbonate decahydrate from Fluka (Buchs, Switzerland), fluorescein disodium salt (FL), resazurin sodium salt, lipopolysaccharide (LPS), *N*-1-naphthylethylenediamine dihydrochloride, N_w-Nitro-L-arginine methyl ester hydrochloride, 2′,7′-dichlorofluorescin diacetate (DCFH-DA), *tert*-butyl hydroperoxide, and sulfanilamide were purchased from Sigma (St. Louis, MO, USA).

Harvest of the maple sap

The maple sap samples of 30 producers originating from three regions of the Québec province were harvested in 2007. For region I (Québec region) and II (Beauce), the maple sap samples were harvested as follows: between March 13 and 31 for the beginning of the season; between March 27 and April 14 for the middle of the season; and between April 11 and 23 for the end of the season. For region III (Bas-St-Laurent–Gaspésie), the maple sap samples were harvested as follows: between March 26 and April 14 for the beginning of the season; between April 15 and 22 for the middle of the season; and between April 20 and 30 for the end of the season.

Preparation of ethyl acetate extracts

Sap and syrup samples were frozen upon reception until they were extracted. For sap, a volume of 2.5 L was extracted with ethyl acetate (3×400 mL). For each extraction, a partial emulsion was observed and recovered with the organic phase. The heterogeneous solution was partially evaporated, and then water (150 mL) and ethyl acetate (200 mL) were added until complete solubilization of the residue. After recovery of the organic phase, the aqueous phase, to which NaCl was added, was re-extracted with ethyl acetate (2×200 mL). The final organic phase was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. For syrups, an initial volume of 500 mL was diluted in 1.5 L of distilled water. The solution was extracted with ethyl acetate (3×300 mL). The rest of the extraction procedure performed for the syrup is identical to the procedure previously described for sap.

Preparation of fruit and vegetable juices

Fruits and vegetables were purchased at a local grocery, and their juices were extracted with a juice extractor. The juices were centrifuged, and their supernatant was kept frozen until they were tested without further preparation.

Dosing of total phenol content

The total phenol contents were determined using the Folin-Ciocalteu reagent according to the procedure reported by Singleton and Rossi,[16] with some modifications. In brief, a volume of 50 μL containing increasing concentrations of extract ranging from 0.39 to 50 mg/mL was mixed with 25 μL of 1:1 water-diluted Folin-Ciocalteu reagent in Falcon transparent flat-bottom 96-well plates (BD, Franklin Lakes, NJ, USA). All manipulations were performed in a light-shielded environment. After 5 minutes of reaction, 125 μL of sodium carbonate decahydrate solution (0.27 g/mL) was added to each well. Absorbance was then measured at 758 nm using an automated Varioskan® Ascent plate reader (Thermo, Waltham, MA, USA). The analysis was performed in duplicate, and the results were expressed in tannic acid equivalents.

Oxygen radical absorbance capacity $(ORAC)_{FL}$ assay

The procedure was modified from the method described by Ou *et al.*[17] In brief, the ORAC assay was carried out in Costar black round-bottom 96-well plates (Corning Inc., Corning, NY, USA) on a Fluoroskan Ascent FL™ plate reader (Thermo). Trolox was used as a control standard. The experiment was conducted at 37.5°C and pH 7.4, with a blank sample in parallel. The fluorimeter was programmed to record the fluorescence every 60 seconds after addition of AAPH (375 m*M*). The final results were calculated by comparing the net areas under the FL decay curves between the blank and the samples. ORAC values were expressed in μmol of Trolox equivalents (TE)/mg.

Cell culture

Human skin fibroblasts WS1 (ATCC number CRL-1502) and murine macrophage RAW 264.7 (ATCC number TIB-71) cell line were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were grown in Dulbecco's Minimum Essential Medium supplemented with 10% fetal calf serum (Hyclone, Logan, UT, USA), solution of vitamins (1×), sodium pyruvate (1×), nonessential amino acids (1×), penicillin (100 IU), and streptomycin (100 μg/mL) (Cellgro®, Mediatech, Manassas). Cells were cultured in a humidified atmosphere at 37°C in 5% CO_2.

Antioxidant cell-based assay

Antioxidant activity was evaluated using the DCFH-DA assay as described by Girard-Lalancette *et al.*,[18] with some modifications. In brief, WS1 cells were plated in 96-microwell plates at 10,000 cells per well and incubated for 24 hours at 37°C and 5% CO_2. The cells were washed with 150 μL of Hanks' balanced salt solution at pH 7.4 and incubated for 30 minutes with 100 μL of Hanks' balanced salt solution (pH 7.4) containing 5 μ*M* DCFH-DA (Sigma-Aldrich). The cells were then washed again with 150 μL of Hanks' balanced salt solution. To assess antioxidant activity, the cells were incubated for 1 hour with a growing concentration of extract, and then 100 μL of 200 μ*M* *tert*-

butylhydroperoxide was added. Fluorescence was measured after *tert*-butylhydroperoxide addition and 90 minutes later on an automated plate reader (Fluoroskan Ascent FL) using an excitation wavelength of 485 nm and an emission wavelength of 530 nm. Antioxidant activity is expressed as the concentration of extract inhibiting 50% (IC_{50}) of DCFH oxidation after blank subtraction.

Measurement of NO using the Griess reaction

Exponentially growing cells were plated in 24-well microplates (Falcon, BD) at a density of 2×10^5 cells per well in 400 μL of culture medium and were allowed to adhere overnight. Cells were then left untreated or treated with positive control L-NAME or increasing concentrations of ethyl acetate extracts dissolved in dimethyl sulfoxide: acetone (1:1 vol/vol). Cells were then stimulated with 100 ng/mL LPS and incubated at 37°C in 5% CO_2 for 24 hours. The final concentration of solvent in the culture medium was maintained at 0.25% (vol/vol) to avoid solvent toxicity. After 24 hours, cell-free supernatants were collected, and the NO concentration was immediately determined using the Griess reaction with minor modifications.[19] In brief, 100-μL aliquots of cell supernatants were incubated with 100 μL of a mix of 1% sulfanilamide in 2.5% H_3PO_4 and of 0.1% *N*-1-naphthylethylenediamine dihydrochloride in water at room temperature for 20 minutes. Absorbance at 540 nm was then measured using an automated Varioskan Ascent plate reader, and the presence of nitrite was quantified by comparison with an $NaNO_2$ standard curve.

Determination of maple syrup transmittance

The percentage of light transmission of pure maple syrup (40 mL) having a density of 66.0° Brix was measured at 20°C with a spectrophotometer (Spectronic™ model Genesys™ 20, Thermo) using optical cells with a 8-mm light path at a wavelength of 560 nm. The color values are expressed in percentage of transmittance and were compared to the analytical reagent glycerol (Fisher Scientific, Ottawa, ON, Canada) fixed at 100% transmission.

In vitro *antiproliferative activity*

Exponentially growing cells were plated at a density of 5×10^3 cells per well in Costar 96-well microplates (Corning) in 100 μL of culture medium and were allowed to adhere for 24 hours at 37°C and 5% CO_2 before treatment. Then, 100 μL of increasing concentrations of maple sap and syrup extracts ranging from 0 to 400 μg/mL were added. The final concentration of solvent in the culture medium was maintained at 0.5% (vol/vol) to avoid solvent toxicity. Pure maple syrup, prepared from a mixture from 10 producers, or fruit and vegetable juices were diluted 1:20 in the culture medium. The cells were incubated for 48 hours in the presence or absence of maple sap or syrup extracts, pure maple syrup, or fruit and vegetable juices. The antiproliferative activity was assessed using the resazurin reduction test as described by O'Brien *et al.*[20] Fluorescence was measured on an automated Fluoroskan Ascent FL plate reader using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Antiproliferative activity was expressed as the cell growth-inhibiting IC_{50}.

Statistical analysis

Data were expressed as mean ± SD values from at least 30 producers ($n \geq 30$) for sap and syrups or two determinations in triplicate for fruit and vegetable juices. Comparisons between groups were performed using one-way analysis of variance followed by a Bonferroni *post hoc* test or the Student-Newman-Keuls method. Values of $P \leq .05$ or less were considered as statistically significant. Correlation between phenol contents, ORAC values, NO inhibition, and antiproliferative activities were determined using the Pearson correlation analysis or the Spearman correlation analysis.

RESULTS AND DISCUSSION

The main objectives of this work were to evaluate the *in vitro* antioxidant activity, inhibition of NO overproduction, and antiproliferative activity of ethyl acetate extract of maple sap and syrup from *A. saccharum* and to assess the incidence of the periods of harvest. In 2007, maple sap from 30 producers of three different geographic regions of the province of Québec, including the Québec region, Beauce, and Bas-St-Laurent–Gaspésie, were harvested at three different periods of the season described as the beginning, the middle, and the end of the season. A part of maple sap from each producer for the three periods was transformed into maple syrup. The maple sap and syrup samples were extracted with ethyl acetate to eliminate sugars. Indeed, maple sap and syrup contain relatively a lot of sugar, which can interfere with some bioassays to assess phenol content and nitric oxide. Ethyl acetate was found the best solvent to eliminate sugars in comparison with methylene chloride and hexane (data not shown). In this study, we also investigated *in vitro* antioxidant and antiproliferative activities of pure maple syrup.

Extraction yield and total phenol content of ethyl acetate extracts of maple sap and syrup

For the 30 producers, results show that the extracted quantity ranged from 1 to 28 mg/L of maple sap with a mean of 5 ± 3 mg/L and from 23 to 242 mg/L of maple syrup with a mean of 141 ± 48 mg/L ($n = 90$). The extracted quantities are about 38-fold higher for the maple syrup in comparison with the maple sap. The method of extraction was found reproducible with a coefficient of variation of 4.8% at the 95% confidence interval (data not shown). For ethyl acetate extracts of maple sap and syrup, no significant difference was found between the extraction yields for the three harvest periods ($P > .05$).

The phenol contents of maple sap and syrup extracts were determined using the Folin-Ciocalteu assay.[16] Results, expressed as grams of equivalents of tannic acid per 100 g of

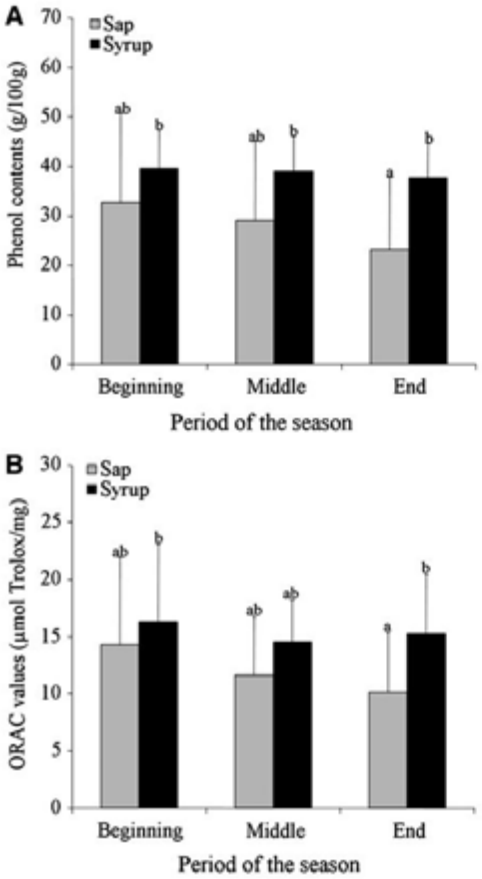

FIG. 1. (**A**) Phenol contents and (**B**) antioxidant activity of ethyl acetate extracts from maple syrup and maple sap using the ORAC assay. Phenol contents are expressed as grams of equivalents of tannic acid per 100 g of extract. Quercetin was used as a positive control with an ORAC value of 22 ± 2 µmol of TE/mg (7.6 ± 0.7 µmol of TE/µmol). All assays were conducted in duplicate. Each value is the mean of 30 different producers for three periods of the season, and the vertical bars represent the SD of each data point. [ab]Values with different letters differ significantly (one-way analysis of variance, $P < .05$).

extract, are presented in Figure 1A. A great variability was observed among the various producers for the maple sap extracts. Indeed, phenolic contents in maple sap extracts ranged from 5 to 75 g/100 g with an average of 28 ± 17 g/100 g. Statistical analysis indicates a significant difference among the 30 producers ($P \leq .001$). As previously reported by Thériault *et al.*,[15] a variability between the three periods of harvest was also observed. The average concentration in phenol tends to decrease during the season from about 29% at the end of the season in comparison with the beginning of the season, but no statistically significant differences were found between three periods of harvest ($P > .05$; $n = 30$). Some parameters could influence phenol contents such as the climatic conditions and the type of soil.[22–24] On the other hand, the phenol contents of maple syrup extracts varied from 21 to 55 g/100 g with a mean of 39 ± 7 g/100 g. In contrast to maple sap extract, no significant difference was found among the producers ($n = 30$; $P = .072$). Moreover, statistical analysis using one-way analysis of variance indicates that the average phenol contents of maple syrup extracts from the periods of harvest are not significantly different ($P > .05$) from each other (Fig. 1A). Most phenolic compounds are known for their antioxidant properties.[4,13,15,25] Therefore, the antioxidant activity of maple sap and syrup was assessed using ORAC and cell-based assays.

In vitro *antioxidant activity of maple sap and syrup using ORAC$_{FL}$ and cell-based assays*

The antioxidant activity of the maple sap and syrup extracts, which are soluble in aqueous solution, was evaluated using the hydrophilic ORAC$_{FL}$ assay developed by Ou *et al.*[17] Quercetin was used as a positive control with an ORAC$_{FL}$ value of 22 ± 2 µmol of TE/mg (7.6 ± 0.7 µmol of TE/µmol). This ORAC value is in good agreement with the literature.[17] For the 30 producers, the mean ORAC value obtained for the extracts of sap is 12 ± 6 µmol of Trolox/mg, whereas for the syrup it is 15 ± 5 µmol of Trolox/mg of extract (Table 1), which corresponds to a portion of maple syrup of 7 mL. These ORAC values are comparable to those of grape seed extract.[17] The antioxidant activity was confirmed with the cell-based assay as described by Girard-Lalancette *et al.*[18] Quercetin was used as a positive control with an IC$_{50}$ of 0.02 ± 0.01 µg/mL. Results show that antioxidant activity of maple sap and maple syrup extracts are similar, with IC$_{50}$ values of 3 ± 1 µg/mL and 6 ± 2 µg/mL, respectively. Figure 1B shows the ORAC values for maple sap and syrup extracts with regard to the periods of the season. As observed for the phenol contents, ORAC values of maple sap extracts tend to decrease about 24% at the end of the season in comparison with the beginning of the season, suggesting that phenolic compounds could be respon-

TABLE 1. *IN VITRO* ANTIOXIDANT ACTIVITY OF MAPLE SAP AND SYRUP FROM *A. SACCHARUM* USING THE ORAC ASSAY

Sample	*ORAC value*	*Reference*
Sap extract	12 ± 6^a	Present study
Syrup extract	15 ± 5^a	Present study
Quercetin	22 ± 2^a	Present study
	24.1 ± 0.7^a	Ou *et al.*[17]
Grape seed extract	11.7 ± 0.9^b	Ou *et al.*[17]
Pure maple syrup	8 ± 2^b	Present study
Blueberry juice	24 ± 1^b	Ou *et al.*[17]
Orange juice	10.8 ± 0.5^b	Prior *et al.*[20]
Red grape juice	19.1 ± 0.6^b	Prior *et al.*[20]
Strawberry juice	10.7 ± 0.4^b	Prior *et al.*[20]

[a]Results expressed as µmol of TE/mg.
[b]Results expressed as µmol of TE/mL.

sible, in part, for the antioxidant activity. A correlation between the phenolic compounds extracted from maple sap and syrup and the antiradical activity was previously reported by Thériault *et al.*[15] Therefore, the coefficient of correlation between the antioxidant activity and the phenol contents was calculated and analyzed using the Pearson correlation method. For maple sap extracts, a weak but significant (0.28; $P < .05$) correlation was found while no correlation was found for maple syrup extracts (0.13; $P = .24$). Therefore, the results of this analysis confirm that phenolic compounds are, in part, responsible for the antioxidant activity of maple sap extract but not for maple syrup extract. Recently, Abou-Zaid *et al.*[26] identified 24 phenolic compounds in soluble ethyl acetate extract from maple syrup, including phenolic acids, cinnamic acid derivatives, and flavanoids. Most of the phenolic compounds identified possess a strong antioxidant activity.[27] The correlations analysis between total phenol contents and antioxidant activity must be taken with caution because the method of dosage of phenols was found to underestimate greatly some flavonoids such as kaempferol 3-*O*-glucoside present in the maple syrup extracts (data not shown). Therefore, it is possible that the variation of some flavonoids influences largely the antioxidant activity of extracts. Moreover, the compounds can act in synergy so a variation of the composition of this complex mixture could also have an important incidence on the antioxidant activity.

On the other hand, the ORAC values of pure maple syrup were also determined from 36 producers and compared with values for various fruit juices in the literature (Table 1). The average ORAC value of pure maple syrup is 8 ± 2 μmol of TE/mL. This result is lower than ORAC values of blueberry (24 ± 1 μmol of TE/mL)[21] and red grape (19.1 ± 0.6 μmol of TE/mL)[21] juices but comparable to ORAC values of strawberry (10.7 ± 0.4 μmol of TE/mL) and orange juices (10.8 ± 0.5 μmol of TE/mL).[21]

Inhibition of NO production by maple sap and syrup extracts

Some phenolic compounds also have an anti-inflammatory activity including inhibition of NO overproduction.[4] The presence of phenolic compounds in maple sap and syrup extracts prompted us to investigate their effect on inhibition of NO overproduction using LPS-stimulated RAW 264.7 macrophages. None of the tested concentrations of extracts inhibited the growth of RAW 264.7 macrophages (data not shown). The stimulation of RAW 264.7 macrophages by LPS induced iNOS and overproduction of NO. L-NAME, an NO synthase inhibitor, prevents the formation of NO in LPS-stimulated RAW 264.7 macrophages and thus was used as a positive control. L-NAME (25 μg/mL) significantly inhibited NO release by 24% in LPS-stimulated RAW 264.7 macrophages (data not shown). A comparable result was obtained with maple sap extracts (Fig. 2A). Indeed, the activity is about 25% NO inhibition per 25 μg/mL of extract, which corresponds to approximately 3.6 mL of pure maple sap. The inhibition of NO production induced by maple syrup extracts is significantly higher in comparison with

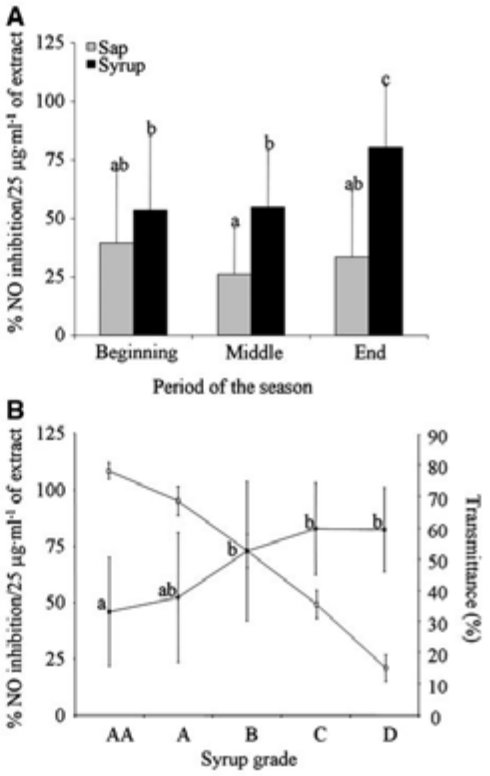

FIG. 2. (**A**)Inhibition of NO production of ethyl acetate from maple sap and maple syrup and (**B**) the relationship with transmittance. L-NAME was used as a positive control with 24% NO inhibition per 25 μg/mL. (A) Anti-inflammatory activity of sap and syrup extracts for three periods of the season. (B) The syrup grade—AA, A, B, C, and D—expressed as percentage of transmittance (□) versus the anti-inflammatory activity (•). All assays were conducted in duplicate. Each value is the mean of 30 different producers for three periods of the season, and the vertical bars represent the SD of each data point. Values with different letters (a–c) differ significantly (one-way analysis of variance, $P < .05$).

maple sap with a mean of about 75% NO inhibition per 25 μg/mL of extract, which corresponds to a portion of 0.18 mL of pure maple syrup. These results show that the constituents of maple syrup extracts strongly inhibit NO production in comparison with positive controls and maple sap extracts. These results show also that transformation of maple sap in syrup improves NO inhibition activity. The heating process to transform maple sap in syrup induces oxidation of the phenolic compounds, suggesting that these could be implied in the activity. The Pearson's correlation analysis between phenol contents and NO inhibition shows a weak correlation for the maple sap extract (0.28; $P < .05$) and no significant correlation for the maple syrup extract (0.20; $P = .06$). As previously discussed, some active flavonoids identified in maple syrup extract are underestimated

such as kaempferol 3-*O*-glucoside, limiting the relationship studied between the total phenol contents and the activity. It is likely that some phenolic compounds are involved in the activity. Indeed, some phenolic compounds identified in the maple syrup extract such as quercetin and kaempferol were found to inhibit NO overproduction.[27,28]

In addition, the incidence of the harvest periods on inhibition of NO production was determined for the maple syrup extracts. Results show that the maple syrup extracts at the end of the season ($75 \pm 25\%$ NO inhibition per 25 μg/mL of extract) have a significantly higher activity in comparison with the beginning ($50 \pm 25\%$ NO inhibition per 25 μg/mL of extract) and the middle of the season ($50 \pm 25\%$ NO inhibition per 25 μg/mL of extract). Interestingly, it is reported that the color of the syrup becomes deeper as the season progresses, suggesting an increase in oxidized phenolic compounds. Indeed, the heating process to transform maple sap in syrup induces oxidation of the phenolic compounds, which contributes to the brown color of the syrup.[26] Therefore, the possible correlation between the NO inhibition of maple syrup extract and the color of syrup was evaluated.

Pure maple syrup is graded by the Canadian Food Inspection Agency based principally on the color and the percentage of light transmission (transmittance) as described in Table 2. Five different grades—AA (extra light), A (light), B (medium), C (amber), and D (dark)—are attributed to maple syrup according to the percentage of transmittance. At the end of the season, a significant ($P < .05$) decrease of the transmittance at $44 \pm 20\%$ was observed in comparison with the beginning and the middle of the season with $69 \pm 9\%$ and $61 \pm 15\%$ transmittance, respectively (data not shown). This result indicates a change in the chemical composition of maple syrup during the season. Figure 2B presents the relationship between the transmittance and inhibition of NO production as a function of the maple syrup grades. Results show that NO inhibition is proportional to the dark color increase of maple syrup and, consequently, is inversely proportional to the percentage of transmittance. Indeed, the inhibition of NO production of maple syrup grade AA (45% NO inhibition per 25 μg/mL) is significantly lower in comparison with grade B (72.5% NO inhibition per 25 μg/mL), C (82.5% NO inhibition per 25 μg/mL), and D (82.5% NO inhibition per 25 μg/mL) maple syrup. These results show that the color of the syrup darkens during the season, whereas NO inhibition of maple syrup extract increases, suggesting a relationship between these two parameters. A correlation analysis using the Pearson method indicates that the percentage of transmittance of maple syrup is negatively correlated (-0.44, $P < .001$) with inhibition of NO production. Therefore, these results suggest that some colored compounds probably related to oxidized phenolic compounds could be involved in NO inhibition of maple syrup extract. However, it is possible that other compounds not detectable by spectrophotometry are also implicated in NO inhibition activity.

Table 2. Maple Syrup Grade According to Color and Transmittance

Grade	*Color class*	*Percentage of light transmission*
AA	Extra light	>75%
A	Light	>60.5% and <75%
B	Medium	>44% and <60.5 %
C	Amber	>27% and <44%
D	Dark	<27%

The criteria are those of the Canadian Food Inspection Agency (http://www.inspection.gc.ca/).[29]

In vitro *antiproliferative activity of maple sap and syrup*

In vitro antiproliferative activity of maple sap and syrup extracts were tested against human lung carcinoma (A549), human colorectal adenocarcinoma (DLD-1), and normal fibroblasts (WS1). Etoposide was used as the positive control. According to the National Cancer Institute, an extract having an IC_{50} less than 100 μg/mL is considered active.[30] Results presented in Figure 3 show that the maple sap extract is inactive against A549, DLD-1, and WS1 cells with an IC_{50} >100 μg/mL. In contrast, the maple syrup extract is moderately active against A549 lung cancer cells (IC_{50} of 42 ± 6 μg/mL) and significantly selective in comparison to DLD-1 (IC_{50} of 72 ± 6 μg/mL) and WS1 (IC_{50} of 84 ± 10 μg/mL) cells. The period of harvest has no significant effect on the antiproliferative activity. However, there is a

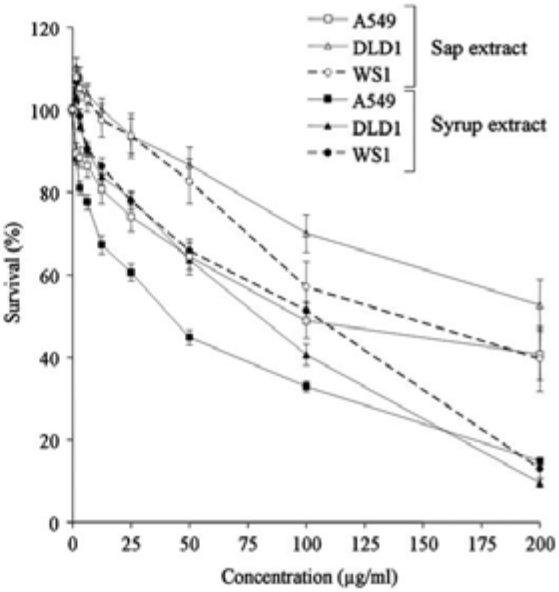

FIG. 3. Anticancer effect of ethyl acetate extracts of maple sap and syrup. Anticancer activity is expressed as percentage of survival. Etoposide was used as a positive control with an IC_{50} of 1.8 ± 0.4 μM, 3.5 ± 0.9 μM, and 6 ± 1 μM for A549, DLD-1, and WS-1 cells, respectively. Each value is the mean of at least 30 different producers, and the vertical bars represent the SD of each data point.

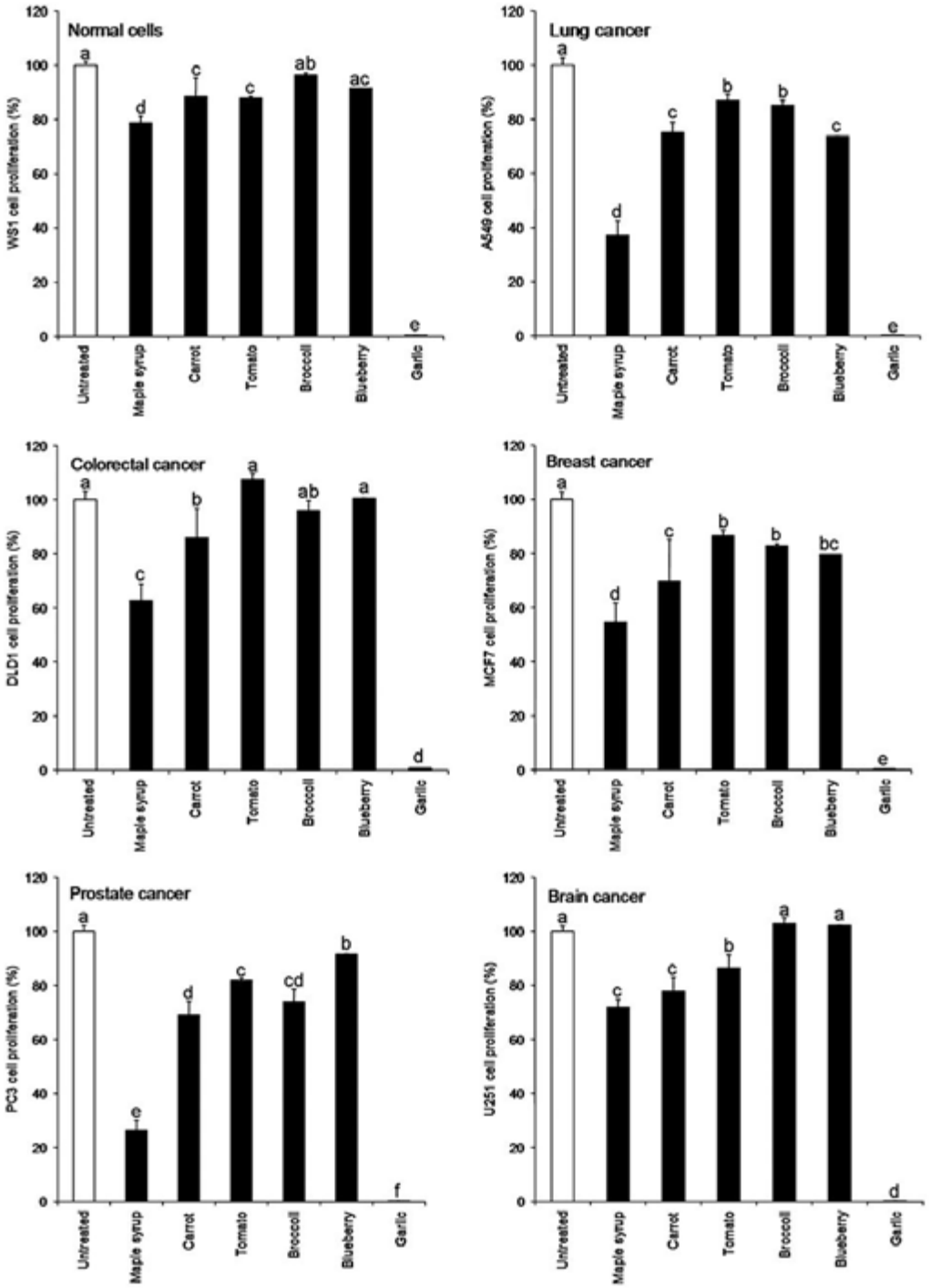

FIG. 4. Anticancer activity of pure maple syrup and fruit or vegetable juices against normal (WS1) and lung cancer (A549), colorectal cancer (DLD-1), breast cancer (MCF-7), prostate cancer (PC-3), and brain cancer (U-251) cells. All cell lines were incubated for 48 hours with or without pure maple syrup and fruit (blueberry) or vegetable (carrot, tomato, broccoli, or garlic) juices diluted 1:20. The vertical bars represent the SD of each data point. Values with different letters (a–f) differ significantly (one-way analysis of variance, $P < .05$).

weak correlation between antiproliferative activity and phenol contents, suggesting that these could be active (0.20; $P = .05$). Altogether, these results prompted us to test antiproliferative activity of pure maple syrup against normal and various cancer cell lines.

The antiproliferative activity of pure maple syrup was evaluated *in vitro* against normal and human tumor cell lines as previously described by Boivin *et al.*[31] The normal (WS1) and cancer cell lines, including lung cancer (A549), colorectal cancer (DLD1), breast cancer (MCF7), prostate cancer (PC3), and brain cancer (U251), were incubated for 48 hours in the absence or in the presence of a 1:20 dilution of maple syrup or the juices of carrot, tomato, broccoli, blueberry, and garlic. Results presented in Figure 4 show that, with the exception of garlic (100% inhibition), pure maple syrup and all tested juices do not, or scarcely, inhibit normal cell growth (4–21% inhibition). As observed by Boivin *et al.*,[31] garlic juice was found very active against all cancer cell lines tested with 100% of inhibition. Interestingly, pure maple syrup selectively inhibits the growth of prostate cancer (74% inhibition) and lung cancer (63% inhibition). Moreover, pure maple syrup possesses a moderate antiproliferative activity against breast cancer and colorectal cancer with 45% and 37% inhibition, respectively. Finally, a weak activity was observed against brain cancer (28% inhibition). In contrast, all the other tested juices do not, or scarcely, inhibit the proliferation of cancer cells with a percentage of inhibition ranging from 0% to 31%. Surprisingly, broccoli juice was inactive against all cancer cell lines, unlike the report of Boivin *et al.*[31] This result could be explained by the bad quality of the cultivar and/or the bad conditions of conservation, suggesting that they are important to have and to maintain a good antiproliferative activity. Altogether, these results show that pure maple syrup possesses an interesting *in vitro* antiproliferative activity mainly against prostate and lung cancer cells.

In conclusion, maple sap and syrup extracts possess *in vitro* antioxidant and NO inhibition activities. The inhibition of NO overproduction induced by maple syrup extract is higher than maple sap extract, suggesting that transformation process improves activity. Interestingly, NO inhibition activity of maple syrup extract is higher at the end of the season, which is correlated with the darkening of the syrup. The maple syrup extract but not maple sap extract was found to be active against lung cancer cells. Finally, pure maple syrup showed inhibition of *in vitro* prostate and lung cancer cell growth.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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Maple Syrup Phytochemicals Include Lignans, Coumarins, a Stilbene, and Other Previously Unreported Antioxidant Phenolic Compounds

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Twenty-three phenolic compounds were isolated from a butanol extract of Canadian maple syrup (MS-BuOH) using chromatographic methods. The compounds were identified from their nuclear magnetic resonance and mass spectral data as 7 lignans [lyoniresinol (**1**), secoisolariciresinol (**2**), dehydroconiferyl alcohol (**3**), 5′-methoxy-dehydroconiferyl alcohol (**4**), erythro-guaiacylglycerol-β-*O*-4′-coniferyl alcohol (**5**), erythro-guaiacylglycerol-β-*O*-4′-dihydroconiferyl alcohol (**6**), and [3-[4-[(6-deoxy-α-L-mannopyranosyl)oxy]-3-methoxyphenyl]methyl]-5-(3,4-dimethoxyphenyl)dihydro-3-hydroxy-4-(hydroxymethyl)-2(3*H*)-furanone (**7**)], 2 coumarins [scopoletin (**8**) and fraxetin (**9**)], a stilbene [(*E*)-3,3′-dimethoxy-4,4′-dihydroxystilbene (**10**)], and 13 phenolic derivatives [2-hydroxy-3′,4′-dihydroxyacetophenone (**11**), 1-(2,3,4-trihydroxy-5-methylphenyl)ethanone (**12**), 2,4,5-trihydroxyacetophenone (**13**), catechaldehyde (**14**), vanillin (**15**), syringaldehyde (**16**), gallic acid (**17**), trimethyl gallic acid methyl ester (**18**), syringic acid (**19**), syringenin (**20**), (*E*)-coniferol (**21**), *C*-veratroylglycol (**22**), and catechol (**23**)]. The antioxidant activities of MS-BuOH (IC_{50} > 1000 μg/mL), pure compounds, vitamin C (IC_{50} = 58 μM), and a synthetic commercial antioxidant, butylated hydroxytoluene (IC_{50} = 2651 μM), were evaluated in the diphenylpicrylhydrazyl (DPPH) radical scavenging assay. Among the isolates, the phenolic derivatives and coumarins showed superior antioxidant activity (IC_{50} < 100 μM) compared to the lignans and stilbene (IC_{50} > 100 μM). Also, this is the first report of 16 of these 23 phenolics, that is, compounds **1**, **2**, **4**−**14**, **18**, **20**, and **22**, in maple syrup.

KEYWORDS: ***Acer saccharum*; sugar maple; maple syrup; butanol extract; phenolics; antioxidant**

INTRODUCTION

Maple syrup is a natural sweetener obtained by concentrating the sap collected from certain maple species including the sugar maple (*Acer saccharum* Marsh.) tree, which is native to North America (*1*, *2*). Maple syrup is primarily produced in northeastern North America, and the vast majority of the world's supply comes from Canada (85%; primarily Quebec), followed by the United States (15%; primarily the New England/New York region) (*2*). Maple syrup is the largest commercially available food product consumed by humans that is derived totally from the sap of deciduous trees.

Maple syrup is produced by thermal evaporation of the colorless watery sap collected from maple trees in late winter to early spring. Because of its high water content, about 40 L of sap is required to produce 1 L of syrup (*1*). During the concentration process of transforming sap to syrup, the characteristic flavor, color, and odor of maple syrup develops. Typically, the color of the syrup becomes darker as the season progresses, and based on Canadian standards, maple syrup is graded as extra light (grade AA), light (grade A), medium/amber (grade B), and dark (grade C) (*2*).

Being a plant-derived natural product, it is not surprising that maple syrup contains phytochemicals (naturally present in the xylem sap), as well as process-derived compounds (formed during thermal evaporation of sap) (*1*−*4*). Apart from sucrose, which is its dominant sugar, maple syrup contains organic acids, amino acids, minerals, and lignin-derived flavor compounds (*1*−*4*). Among the phytochemicals that have been previously reported from maple syrup, the phenolic class predominates. For example, vanillin, syringaldehyde, coniferaldehyde, and cinnamic acid and benzoic acid derivatives, as well as flavonoids (flavanols and flavonols), have been identified in maple syrup extracts (*3*−*6*).

The presence of a diverse range of phenolic subclasses in maple syrup is interesting given that this large class of dietary phytochemicals has attracted significant research attention due to their diverse biological functions and potential positive effects on human health (*6*). Recently, phenolic-enriched extracts of maple syrup were shown to have antioxidant, antimutagenic, and human cancer cell antiproliferative properties (*7*, *8*). Thus, a comprehensive investigation of maple syrup phenolics is necessary to evaluate the biological properties and potential human health benefits of this natural sweetener. Previous phytochemical research has been conducted on maple syrup extracts, namely, ethyl acetate, chloroform, dichloromethane, and diethyl ether extracts (*3*−*6*). Whereas these organic solvents are commonly used for the extraction of phytochemicals from complex food matrices, it is possible that higher polarity solvents, such as *n*-butanol, may contain previously unreported phenolic compounds. However, there are no prior reported studies of compounds found in butanol extracts of maple syrup (MS-BuOH).

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Maple syrup is popularly consumed worldwide and is of significant cultural and economical importance to northeastern North America, particularly in Canada, where it is largely produced. Therefore, increased knowledge of the chemical constituents of Canadian maple syrup would aid in the authentication, characterization, and subsequent detection of intentional adulteration of this premium natural sweetener. Also, characterization of the different chemical subclasses of bioactive phenolics, and ascertaining their levels, would aid in evaluating the potential human health benefits resulting from consumption of Canadian maple syrup. Toward this end, our objectives were (1) to isolate and identify the phytochemicals present in a Canadian MS-BuOH and (2) to evaluate the Canadian MS-BuOH, and its purified constituents, for antioxidant potential in the diphenylpicrylhydrazyl (DPPH) radical scavenging assay.

Here we report the isolation and identification of 23 phenolic compounds, **1**−**23**, from MS-BuOH, among which 16 compounds, namely, **1**, **2**, **4**−**14**, **18**, **20**, and **22**, are being reported from maple syrup for the first time.

MATERIALS AND METHODS

General Experimental Procedures. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained either on a Bruker 400 MHz or on a Varian 500 MHz instrument using deuterated methanol (CD_3OD) as solvent. Electrospray ionization mass spectral (ESIMS) data were acquired on a Q-Star Elite (Applied Biosystems MDS) mass spectrometer equipped with a Turbo Ionspray source and were obtained by direct infusion of pure compounds. Analytical high-performance liquid chromatography (HPLC) was performed on a Hitachi Elite LaChrom system consisting of an L2130 pump, an L-2200 autosampler, and an L-2455 diode array detector, all operated by EZChrom Elite software. Semipreparative scale HPLC was performed on a Beckman-Coulter HPLC system consisting of a Beckman System Gold 126 solvent module pump, a 168 photodiode array (PDA) UV−vis detector, and a 508 autosampler, all operated by 32 Karat 8.0 software. All solvents were of either ACS or HPLC grade and were obtained from Wilkem Scientific (Pawcatuck, RI). Ascorbic acid (vitamin C), butylated hydroxytoluene (BHT), and DPPH reagent were purchased from Sigma-Aldrich (St. Louis, MO).

Maple Syrup Butanol Extract (MS-BuOH). Maple syrup (grade C, 20 L) was provided by the Federation of Maple Syrup Producers of Quebec (Canada). The syrup was kept frozen until extraction, when it was subjected to liquid−liquid partitioning with ethyl acetate (10 L × 3) followed by *n*-butanol (10 L × 3) solvents, to yield ethyl acetate (4.7 g) and *n*-butanol (108 g) extracts, respectively, after solvent removal in vacuo.

Analytical HPLC. All analyses were conducted on a Luna C18 column (250 × 4.6 mm i.d., 5 μM; Phenomenex) with a flow rate at 0.75 mL/min and injection volume of 20 μL. A gradient solvent system consisting of solvent A (0.1% aqueous trifluoroacetic acid) and solvent B (methanol, MeOH) was used as follows: 0−10 min, from 10 to 15% B; 10−20 min, 15% B; 20−40 min, from 15 to 30% B; 40−55 min, from 30 to 35% B; 55−65 min, 35% B; 65−85 min, from 35 to 60% B; 85−90 min, from 60 to 100% B; 90−93 min, 100% B; 93−94 min, from 100 to 10% B; 94−104 min, 10% B. **Figure 1**, panels **A** and **B**, show the HPLC-UV profiles of the butanol extract and all of the isolated phenolics (combined into one solution/injection), respectively. Unfortunately, due to limited sample quantity, we were not able to include compound **13** in the HPLC-UV injection shown in **Figure 1B**.

Isolation of Compounds from the MS-BuOH. The butanol extract (108 g) of Canadian maple syrup was further extracted with methanol (100 mL × 3) to afford methanol-soluble (57 g; dark brown powder) and methanol-insoluble (51 g; off-white powder) fractions. Analytical HPLC-UV analyses of the methanol-soluble extract revealed a number of peaks characteristic of phenolic compounds at 220, 280, and 360 nm (see above for details of methodology; see **Figure 1A** for chromatogram). Therefore, this fraction was selected for further purification by repeated chromatography on a Sephadex LH-20 column (4.5 × 64 cm), eluting with a gradient system of MeOH/H_2O (3:7 v/v to 7:3 v/v to 100:0 v/v), and then with acetone/H_2O (7:3 v/v). On the basis of analytical HPLC-UV profiles, 12 combined fractions, fractions 1−12, were obtained. Fraction 4 (1.5 g) was subjected to column chromatography on a Sephadex LH-20 column (4.5 × 64 cm) using a gradient solvent system of MeOH/H_2O (3:7 v/v to 7:3 v/v) to afford 12 subfractions, fractions 4.1−4.12. These were individually subjected to a series of semipreparative HPLC-UV separations using a Waters Sunfire Prep C_{18} column (250 × 10 mm i.d., 5 μm; flow = 2 mL/min) and elution with a MeOH/H_2O gradient system to yield compounds **1** (4.6 mg), **3** (3.8 mg), **5** (4.0 mg), **6** (41.6 mg), **7** (6.6 mg), **11** (3.5 mg), **15** (0.3 mg), **16** (0.8 mg), **18** (0.2 mg), **20** (1.3 mg), **22** (1.5 mg), and **23** (3.0 mg). Similarly, fraction 5 (0.47 g) was purified by semipreparative HPLC-UV using a Waters XBridge Prep C_{18} column (250×19 mm i.d., 5 μm; flow = 3.5 mL/min) and a gradient solvent system of MeOH/H_2O to afford four subfractions 5.1−5.4. These subfractions were separately subjected to a combination of semipreparative HPLC-UV and/or Sephadex LH-20 column chromatography with gradient solvents systems of MeOH/H_2O to afford compounds **2** (1.9 mg), **4** (1.9 mg), **8** (2.0 mg), **9** (2.3 mg), **14** (2.5 mg), **17** (2.4 mg), **19** (1.8 mg), and **21** (1.3 mg). Similarly, fraction 6 (0.2 g) afforded compounds **12** (1.4 mg) and **13** (1.3 mg), and fraction 11 yielded compound **10** (4.8 mg).

Identification of Compounds. All of the isolated compounds (**Figure 2**) were identified by examination of their ^{1}H and/or ^{13}C NMR and mass spectral data and by comparison of these to published literature reports, when available (**Table 1**). The NMR data for compounds **12** and **13** have not been previously published and are provided here for the first time.

(+)-*Lyoniresinol* (***1***)*:* yellowish amorphous powder; (+) ESIMS, *m*/*z* 443.1719 [M + Na]$^+$, calcd for molecular formula $C_{22}H_{28}O_8$; ^{1}H NMR (CD_3OD, 400 MHz) δ 1.64 (1H, m, H-8), 1.95 (1H, m, H-8′), 2.59 (1H, m, H-7a), 2.72 (1H, m, H-7b), 3.36 (3H, s, 3-OCH_3), 3.51 (2H, m, H-9a, 9a′), 3.61 (2H, m, H-9b, 9b′), 3.75 (6H, s, 3′, 5′-OCH_3), 3.87 (3H, s, 5-OCH_3), 4.31 (1H, d, *J* = 5.6 Hz, H-7′), 6.39 (2H, s, H-2′, 6′), 6.60 (1H, s, H-6); ^{13}C NMR (CD_3OD, 100 MHz) δ 149.09 (C-3′, 5′), 148.77 (C-5), 147.80 (C-3), 139.44 (C-4, 1′), 130.27 (C-1), 135.00 (C-4′), 126.36 (C-2), 107.84 (C-6), 106.91 (C-2′, 6′), 66.87 (C-9), 64.21 (C-9′), 60.26 (3-OCH_3), 56.85 (3′, 5′-OCH_3), 56.69 (5-OCH_3), 49.01 (C-8′), 42.43 (C-7′), 40.98 (C-8), 33.71 (C-7). ^{1}H and ^{13}C NMR data were consistent with the literature (*9*).

Secoisolariciresinol (***2***)*:* yellowish amorphous powder; (+) ESIMS *m*/*z* 385.1447 [M + Na]$^+$, calcd for molecular formula $C_{20}H_{26}O_6$; ^{1}H NMR (CD_3OD, 500 MHz) δ 1.89 (2H, m, H-8, 8′), 2.55 (2H, m, H-7a, 7a′), 2.66 (2H, m, H-7b, 7b′), 3.58 (4H, m, H-9, 9′), 3.74 (6H, s, 3, 3′-OCH_3), 6.55 (2H, d, *J* = 8.0 Hz, H-6, 6′), 6.58 (2H, s, H-2, 2′), 6.66 (2H, s, H-5, 5′); ^{13}C NMR (CD_3OD, 125 MHz) δ 147.38 (C-3, 3′), 144.05 (C-4, 4′), 132.45 (C-1, 1′), 121.28 (C-6, 6′), 114.34 (C-5, 5′), 111.93 (C-2, 2′), 60.69 (C-9, 9′), 54.74 (3, 3′-OCH_3), 42.69 (C-8, 8′), 34.61 (C-7, 7′). ^{1}H and ^{13}C NMR data were consistent with the literature (*10*).

Dehydroconiferyl alcohol (***3***)*:* yellowish amorphous powder; (+) ESIMS *m*/*z* 383.1208 [M + Na]$^+$, calcd for molecular formula $C_{20}H_{24}O_6$; ^{1}H NMR (CD_3OD, 400 MHz) δ 1.81 (2H, m, H-8′), 2.64 (2H, m, H-7′), 3.48 (1H, m, H-8), 3.58 (2H, m, H-9′), 3.70 (1H, m, H-9a), 3.80 (1H, m, H-9b), 3.82 (3H, s, 3-OCH_3), 3.86 (3H, s, 3′-OCH_3), 5.50 (1H, d, *J* = 6.0 Hz, H-7), 6.74 (2H, s, H-4′, 6′), 6.76 (1H, d, *J* = 8.0 Hz, H-5), 6.82 (1H, d, *J* = 8.0 Hz, H-6), 6.96 (1H, s, H-2); ^{13}C NMR (CD_3OD, 100 MHz) δ 149.20 (C-3), 147.61 (C-4, 2′), 145.34 (C-3′), 137.03 (C-5′), 134.92 (C-1), 129.79 (C-1′), 119.81 (C-6), 118.01 (C-6′), 115.97 (C-5), 114.10 (C-4′), 110.56 (C-2), 89.11 (C-7), 65.09 (C-9), 62.35 (C-9′), 56.81 (3-OCH_3), 56.41 (3′-OCH_3), 55.61 (C-8), 35.99 (C-8′), 33.05 (C-7′). ^{1}H and ^{13}C NMR data were consistent with the literature (*11*).

5-methoxydehydroconiferyl alcohol (***4***)*:* yellowish amorphous powder; (+) ESIMS *m*/*z* 413.1464 [M + Na]$^+$, calcd for molecular formula $C_{21}H_{26}O_7$; ^{1}H NMR (CD_3OD, 500 MHz) δ 1.80 (2H, m, H-8′), 2.60 (2H, m, H-7′), 3.47 (1H, m, H-8), 3.58 (2H, m, H-9′), 3.76 (1H, m, H-9a), 3.80 (6H, s, 3,5-OCH_3), 3.84 (1H, m, H-9b), 3.86 (3H, s, 3′-OCH_3), 5.49 (1H, d, *J* = 5.5 Hz, H-7), 6.64 (2H, s, H-2, 6), 6.72 (2H, s, H-4′, 6′); ^{13}C NMR (CD_3OD, 125 MHz) δ 147.91 (C-3, 5), 146.10 (C-2′), 143.80 (C-3′), 135.56 (C-4, 5′), 132.64 (C-1), 128.40 (C-1′), 116.49 (C-6′), 112.71 (C-4′), 102.71 (C-2, 6), 87.68 (C-7), 63.75 (C-9), 60.80 (C-9′), 55.36 (3,5-OCH_3), 55.32 (3′-OCH_3), 54.17 (C-8), 34.39 (C-8′), 31.48 (C-7′). ^{1}H and ^{13}C NMR data were consistent with the literature (*12*).

Erythro-guaiacylglycerol-β-O-4′-coniferyl alcohol (***5***)*:* yellowish amorphous powder; (+) ESIMS *m*/*z* 399.1156 [M + Na]$^+$, calcd for molecular formula $C_{20}H_{24}O_7$; ^{1}H NMR (CD_3OD, 400 MHz) δ 3.81 (6H, s, 3,2′-OCH_3), 3.87 (2H, m, H-9), 4.20 (2H, d, *J* = 5.6 Hz, H-9′), 4.37 (1H, m, H-8), 4.83 (1H, d, *J* = 5.6 Hz, H-7), 6.24 (1H, dd, *J* = 6.0, 16.0 Hz, H-8′), 6.52 (1H,

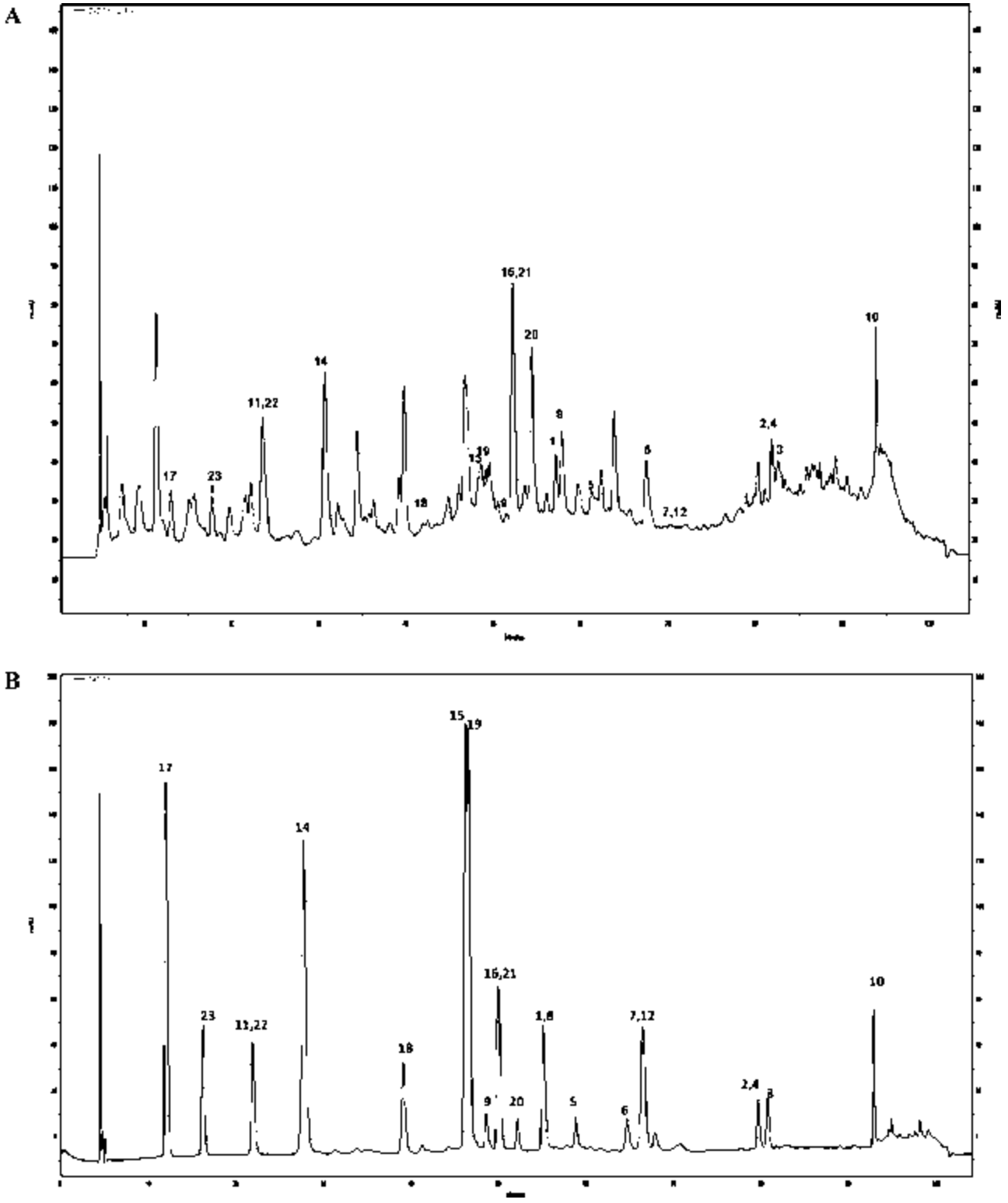

Figure 1. HPLC-UV chromatogram of (**A**) butanol extract of Canadian maple syrup (MS-BuOH) and (**B**) 23 phenolic compounds isolated and identified in MS-BuOH.

d, J = 16.0 Hz, H-7′), 6.73 (1H, d, J = 8.0 Hz, H-5), 6.84 (1H, d, J = 8.0 Hz, H-6), 6.88 (2H, br s, H-5′, 6′), 7.01 (1H, s, H-3′), 7.03 (1H, s, H-2); ^{13}C NMR (CD_3OD, 100 MHz) δ 151.80 (C-2′),149.00 (C-1′), 148.61 (C-3), 147.22 (C-4), 134.18 (C-1), 133.11 (C-4′), 130.81 (C-7′), 128.57 (C-8′), 121.13 (C-6), 120.77 (C-5′), 118.95 (C-6′), 115.74 (C-5), 111.92 (C-2), 110.79 (C-3′), 86.31 (C-8), 74.19 (C-7), 63.90 (C-9′), 62.32 (C-9), 56.58 (3,2′-OCH_3). ^{1}H and ^{13}C NMR data were consistent with the literature (*13*).

Erythro-guaiacylglycerol-β-O-4′-dihydroconiferyl alcohol (***6***)*:* yellowish amorphous powder; (+) ESIMS m/z 401.1602 $[M + Na]^+$, calcd for molecular formula $C_{20}H_{26}O_7$; ^{1}H NMR (CD_3OD, 400 MHz) δ 1.81 (2H, m, H-8′), 2.62 (2H, m, H-7′), 3.47 (1H, m, H-9a′), 3.58 (2H, m, H-9), 3.72 (1H, m, H-9b′), 3.82 (3H, s, 5-OCH_3), 3.85 (3H, s, 2′-OCH_3), 4.21 (1H, m, H-8), 4.90 (1H, m, H-7), 6.71 (1H, d, J = 8.0 Hz, H-5′), 6.77 (1H, d, J = 8.0 Hz, H-3), 6.86 (1H, s, H-3′), 6.88 (1H, d, J = 8.0 Hz, H-2), 6.98 (1H, d, J = 8.0 Hz, H-6′), 7.03 (1H, s, H-6); ^{13}C NMR (CD_3OD, 100 MHz) δ 151.80 (C-2′), 148.95 (C-5), 147.69 (C-4), 147.29 (C-1′), 138.35 (C-4′), 133.88 (C-1), 122.18 (C-5′), 120.93 (C-2), 119.74 (C-6′), 116.01 (C-3), 114.02 (C-3′), 111.82 (C-6), 87.88 (C-8), 74.29 (C-7), 62.36 (C-9), 62.01 (C-9′), 56.65 (2′-OCH_3), 56.48

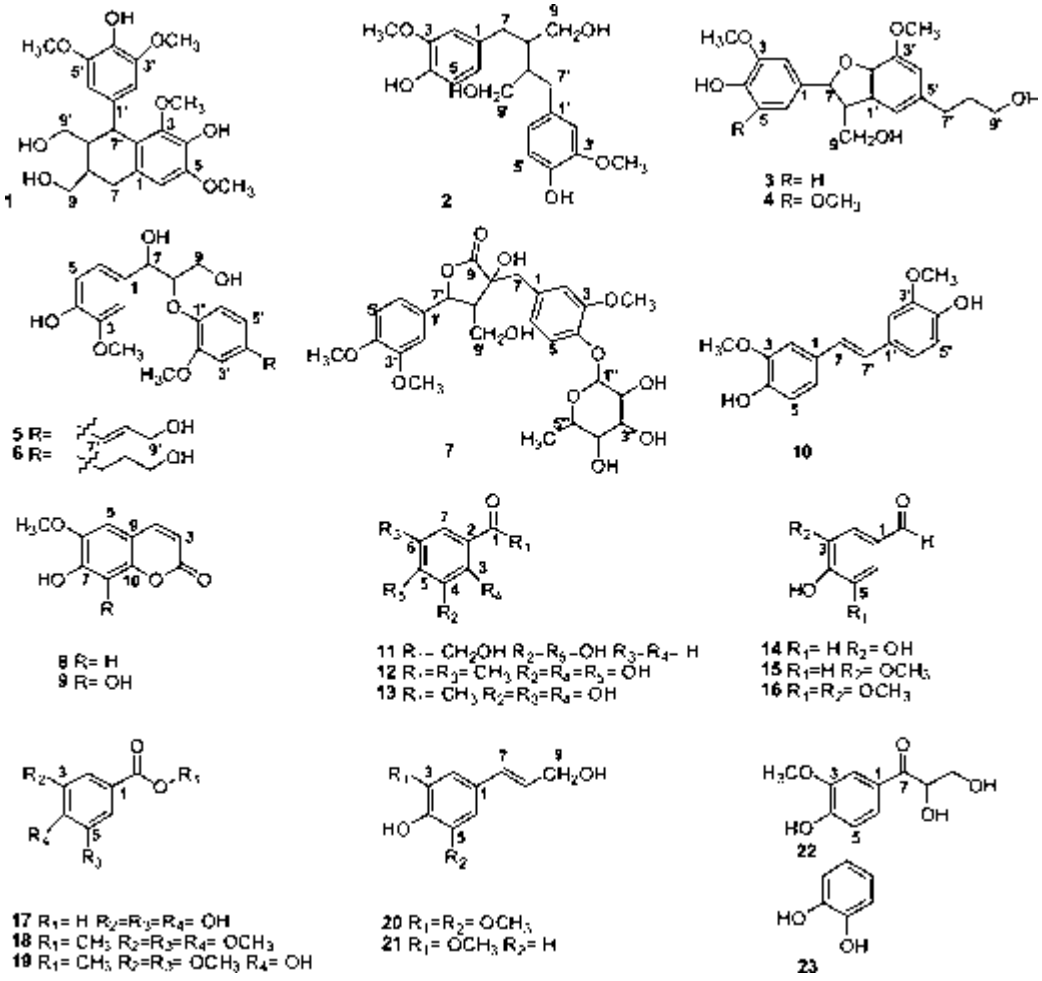

Figure 2. Structures of phenolic compounds (**1**−**23**) isolated and identified from a butanol extract of Canadian maple syrup (MS-BuOH).

(5-OCH_3), 35.71 (C-8′), 32.86 (C-7′). ^{1}H and ^{13}C NMR data were consistent with the literature (*14*).

[*3*-[*4*-[(*6-Deoxy*-α-L-*mannopyranosyl*)*oxy*]-*3-methoxyphenyl*]*methyl*]-*5*-(*3,4-dimethoxyphenyl*)*dihydro-3-hydroxy-4*-(*hydroxymethyl*)-*2*(*3H*)-*furanone* (***7***)*:* yellowish amorphous powder; (+) ESIMS *m*/*z* 573.1913 $[M + Na]^+$, calcd for molecular formula $C_{27}H_{34}O_{12}$; ^{1}H NMR (400 MHz, CD_3OD) δ 1.25 (3H, d, *J* = 6.4 Hz, H-6″), 2.46 (1H, m, H-8′), 3.06 (1H, d, *J* = 13.2 Hz, H-7b), 3.35 (1H, d, *J* = 13.2 Hz, H-7a), 3.5−3.90 (3H, m, H-3″, 4″, 5″), 3.55 (1H, m, 9′b), 3.63 (3H, s, 4′-OCH_3), 3.79 (3H, s, 3′-OCH_3), 3.80 (3H, s, 3-OCH_3), 3.95 (1H, m, 9′a), 4.07 (1H, s, H-2″), 5.10 (1H, d, *J* = 9.2 Hz, H-7′), 5.31 (1H, s, H-1″), 6.37 (1H, s, H-2′), 6.62 (1H, d, *J* = 8.0 Hz, H-6′), 6.85 (1H, d, *J* = 8.0 Hz, H-6), 6.87 (1H, d, *J* = 8.4 Hz, H-5′), 6.97 (1H, s, H-2), 7.05 (1H, d, *J* = 8.4 Hz, H-5); ^{13}C NMR (100 MHz, CD_3OD) δ 179.64 (C-9), 152.11 (C-3), 151.04 (C-3′), 150.74 (C-4′), 146.15 (C-4), 132.66 (C-1), 132.45 (C-1′), 124.54 (C-6), 120.92 (C-6′), 120.11 (C-5), 116.36 (C-2), 112.60 (C-5′), 110.39 (C-2′), 101.82 (C-1″), 82.89 (C-7′), 79.47 (C-8), 73.94 (C-4″), 72.33 (C-3″), 72.25 (C-2″), 71.02 (C-5″), 58.69 (C-9′), 56.75, 56.50 (C3,3′,4′-OCH_3), 51.79 (C-8′), 42.75 (C-7), 18.18 (C-6″). ^{1}H and ^{13}C NMR data were consistent with the literature (*15*).

Scopoletin (***8***)*:* yellowish amorphous powder; (+) ESIMS *m*/*z* 193.0787 $[M + H]^+$, calcd for molecular formula $C_{10}H_8O_4$; ^{1}H NMR (500 MHz, CD_3OD) δ 3.81 (3H, s, 6-OCH_3), 6.10 (1H, d, *J* = 9.4 Hz, H-3), 6.67 (1H, s, H-8), 7.01 (1H, s, H-5), 7.75 (1H, d, *J* = 9.4 Hz, H-4). ^{1}H NMR data were consistent with the literature (*15*).

Fraxetin (***9***)*:* yellowish amorphous powder; (+) ESIMS *m*/*z* 209.0639 $[M + H]^+$, calcd for molecular formula $C_{10}H_8O_5$; ^{1}H NMR (500 MHz, CD_3OD) δ 3.82 (3H, s, 6-OCH_3), 6.22 (1H, d, *J* = 9.4 Hz, H-3), 6.73 (1H, s, H-5), 7.85 (1H, d, *J* = 9.4 Hz, H-4). ^{1}H NMR data were consistent with the literature (*16*).

(*E*)-*3,3′-Dimethoxy-4,4′-dihydroxystilbene* (***10***)*:* yellowish amorphous powder; (+) ESIMS *m*/*z* 294.9650 $[M + Na]^+$, calcd for molecular formula $C_{16}H_{16}O_4$; ^{1}H NMR (400 MHz, CD_3OD) δ 3.83 (6H, s, 3,3′-OCH_3), 6.76 (2H, d, *J* = 8.0 Hz, H-5, 5′), 6.92 (2H, s, H-7, 7′), 6.95 (2H, d, *J* = 8.0 Hz, H-6, 6′), 7.12 (2H, s, H-2, 2′); ^{13}C NMR (CD_3OD, 100 MHz) δ 148.72 (C-3, 3′), 147.35 (C-4, 4′), 131.70 (C-1, 1′), 127.40 (C-7, 7′), 120.94 (C-6, 6′), 116.45 (C-5, 5′), 110.40 (C-2, 2′), 56.53 (3, 3′-OCH_3). ^{1}H and ^{13}C NMR data were consistent with the literature (*17*).

2-Hydroxy-3′,4′-dihydroxyacetophenone (***11***)*:* brown amorphous powder; (+) ESIMS *m*/*z* 191.0227 $[M + Na]^+$, calcd for molecular formula $C_8H_8O_4$; ^{1}H NMR (500 MHz, CD_3OD) δ 4.68 (2H, s, H-8), 6.72 (1H, d, *J* = 8.0 Hz, H-6), 7.27 (1H, d, *J* = 8.0 Hz, H-7), 7.29 (1H, s, H-3). ^{1}H NMR data were consistent with the literature (*18*).

1-(*2,3,4-Trihydroxy-5-methylphenyl*)*ethanone* (***12***)*:* brown amorphous powder; (−) ESIMS *m*/*z* 181.0691 $[M - H]^-$, calcd for molecular formula $C_9H_{10}O_4$; ^{1}H NMR (500 MHz, CD_3OD) δ 2.15 (3H, s, CH_3), 2.51 (3H, s, CH_3CO), 7.08 (1H, s, H-7).

2,4,5-Trihydroxyacetophenone (***13***)*:* brown amorphous powder; (−) ESIMS *m*/*z* 167.0601 $[M - H]^-$; calcd for molecular formula $C_8H_8O_4$; ^{1}H NMR (500 MHz, CD_3OD) δ 2.48 (3H, s, CH_3), 6.28 (1H, s, H-5), 7.16 (1H, s, H-7).

Catechaldehyde (***14***)*:* brown amorphous powder; (−) ESIMS *m*/*z* 137.0341 $[M - H]^-$, calcd for molecular formula $C_7H_6O_3$; ^{1}H NMR (400 MHz, CD_3OD) δ 6.92 (1H, d, *J* = 8.0 Hz, H-5), 7.31 (2H, br s, H-2, 6), 9.70 (1H, s, CHO). ^{1}H NMR data were consistent with the literature (*19*).

Vanillin (***15***)*:* white amorphous powder; (−) ESIMS *m*/*z* 151.0667 $[M - H]^-$, calcd for molecular formula $C_8H_8O_2$; ^{1}H NMR (500 MHz, CD_3OD) δ 6.94 (1H, d, *J* = 8.0 Hz, H-5), 7.43 (1H, d, *J* = 8.0 Hz, H-6), 7.44 (1H, s, H-2), 9.75 (1H, s, CHO). ^{1}H NMR data were consistent with the literature (*20*).

Syringaldehyde (***16***)*:* white amorphous powder; (−) ESIMS *m*/*z* 181.0768 $[M - H]^-$, calcd for molecular formula $C_9H_{10}O_4$; ^{1}H NMR

Table 1. Total Compounds Isolated from a Butanol Extract of Canadian Maple Syrup (MS-BuOH) Showing Those Reported for the First Time from Maple Syrup

compd	identification	references of NMR data
1	lyoniresinol[a]	*9*
2	secoisolariciresinol[a]	*10*
3	dehydroconiferyl alcohol	*11*
4	5′-methoxydehydroconiferyl alcohol[a]	*12*
5	guaiacylglycerol-*β*-*O*-4′-coniferyl alcohol[a]	*13*
6	guaiacylglycerol-*β*-*O*-4′-dihydroconiferyl alcohol[a]	*14*
7	[3-[4-[(6-deoxy-α-L-mannopyranosyl)oxy]-3-methoxyphenyl]methyl]-5-(3,4-dimethoxyphenyl)-dihydro-3-hydroxy-4-(hydroxymethyl)-2(3*H*)-furanone[a]	*15*
8	scopoletin[a]	*15*
9	fraxetin[a]	*16*
10	(*E*)-3,3′-dimethoxy-4,4′-dihydroxystilbene[a]	*17*
11	2-hydroxy-3′,4′-dihydroxyacetophenone[a]	*18*
12	1-(2,3,4-trihydroxy-5-methylphenyl)ethanone[a,b]	
13	2,4,5-trihydroxyacetophenone[a,b]	
14	catechaldehyde[a]	*19*
15	vanillin	*20*
16	syringaldehyde	*20*
17	gallic acid	*21*
18	trimethyl gallic acid methyl ester[a]	*22*
19	syringic acid	*20*
20	syringenin[a]	*20*
21	(*E*)-coniferol	*23*
22	*C*-veratroylglycol[a]	*24*
23	catechol	*25*

[a] First report from maple syrup. [b] NMR data provided for the first time herein.

(500 MHz, CD_3OD) δ 3.86 (6H, s, 3, 5-OCH_3), 7.24 (2H, s, H-2, 6), 9.76 (1H, s, CHO). ^1H NMR data were consistent with the literature (*20*).

Gallic acid (***17***)*:* brown amorphous powder; (−) ESIMS *m*/*z* 169.1226 $[M - H]^-$, calcd for molecular formula $C_7H_6O_5$; ^1H NMR (400 MHz, CD_3OD) δ 7.02 (2H, s, H-2, 6). ^1H NMR data were consistent with the literature (*21*).

Trimethylgallic acid methyl ester (***18***)*:* brown amorphous powder; (+) ESIMS *m*/*z* 249.0735 $[M + Na]^+$, calcd for molecular formula $C_{11}H_{14}O_5$; ^1H NMR (400 MHz, CD_3OD) δ 3.35 (3H, s, $COOCH_3$), 3.92 (9H, s, 3, 4, 5-OCH_3), 7.34 (2H, s, H-2, 6). ^1H NMR data were consistent with the literature (*22*).

Syringic acid (***19***)*:* white amorphous powder; (−) ESIMS *m*/*z* 197.0256 $[M - H]^-$, calcd for molecular formula $C_9H_{10}O_5$; ^1H NMR (400 MHz, CD_3OD) δ 3.90 (6H, s, 3,5-OCH_3), 7.34 (2H, s, H-2, 6). ^1H NMR data were consistent with the literature (*20*).

Syringenin (***20***)*:* brown amorphous powder; (+) ESIMS *m*/*z* 233.0630 $[M + Na]^+$, calcd for molecular formula $C_{11}H_{14}O_4$; ^1H NMR (500 MHz, CD_3OD) δ 3.75 (6H, s, 3,5-OCH_3), 4.10 (2H, d, J = 5.5 Hz, H-9), 6.12 (1H, d, J = 16.0 Hz, H-8), 6.39 (1H, d, J = 16.0 Hz, H-7), 6.60 (2H, s, H-2, 6). ^1H NMR data were consistent with the literature (*20*).

(*E*)-*Coniferol* (***21***)*:* brown amorphous powder; (−) ESIMS *m*/*z* 179.0833 $[M - H]^-$, calcd for molecular formula $C_{10}H_{12}O_3$; ^1H NMR (400 MHz, CD_3OD) δ 3.88 (3H, s, 3-OCH_3), 4.20 (2H, d, J = 5.0 Hz, H-9), 6.20 (1H, d, J = 16.0 Hz, H-8), 6.51 (1H, d, J = 16.0 Hz, H-7), 6.74 (1H, d, J = 8.0 Hz, H-5), 6.86 (1H, d, J = 8.0 hz, H-6), 7.01 (1H, s, H-2). ^1H NMR data were consistent with the literature (*23*).

C-Veratroylglycol (***22***)*:* brown amorphous powder; (+) ESIMS *m*/*z* 235.0582 $[M + Na]^+$, calcd for molecular formula $C_{10}H_{12}O_5$; ^1H NMR (400 MHz, CD_3OD) δ 3.78 (1H, m, H-9a), 3.90 (1H, m, H-9b), 3.93 (3H, s, OCH_3), 5.13 (1H, dd, J = 3.5, 5.5 Hz, H-8), 6.89 (1H, d, J = 8.0 Hz, H-5), 7.60 (1H, d, J = 8.0 Hz, H-6), 7.61 (1H, s, H-2); ^{13}C NMR (100 MHz, CD_3OD) δ 199.52 (C-7), 153.11 (C-4), 150.04 (C-3), 128.14 (C-1), 125.19 (C-6), 116.03 (C-5), 112.51 (C-2), 75.59 (C-8), 66.39 (C-9). ^1H and ^{13}C NMR data were consistent with the literature (*24*).

Catechol (***23***)*:* brown amorphous powder; (−) ESIMS *m*/*z* 109.0448 $[M - H]^-$, calcd for molecular formula $C_6H_6O_2$; ^1H NMR (400 MHz, CD_3OD) δ 6.66 (2H, m, H-2, 5), 6.76 (2H, m, H-3, 4); ^{13}C NMR (100 Hz, CD_3OD) δ 144.67 (C-1,6), 121.04 (C-2,6), 116.52 (C-3,4). ^1H and ^{13}C NMR data were consistent with the literature (*25*).

Table 2. Antioxidant Activities of Pure Compounds Isolated from a Butanol Extract of Canadian Maple Syrup (MS-BuOH) Showing 50% Inhibitory Concentrations (IC_{50}) in the Diphenylpicrylhydrazyl (DPPH) Radical Scavenging Assay[a]

compd	IC_{50} (μM)	compd	IC_{50} (μM)
1	101.5 ± 5.9	**12**	31.3 ± 0.6
2	147.9 ± 3.6	**14**	35.5 ± 3.7
3	1040.9 ± 103	**15**[b]	>2600
4	136.7 ± 3.9	**16**[c]	357.1
5	943.5 ± 21.9	**17**	20.3 ± 0.3
6	1335.9 ± 47.6	**19**	191.85 ± 20.99
7	679.3 ± 45.6	**21**[c]	115
8	68.2 ± 31.2	**22**	641 ± 1 0.6
9	46.5 ± 3.6	**23**	89.5 ± 2.7
10[b]	>2600	vitamin C	58.6 ± 10.7
11	51.8 ± 8.1	BHT	2651.5 ± 285.9

[a] Values are mean ± SD. BHT, a synthetic commercial antioxidant, butylated hydroxytoluene. All compounds were evaluated except **13**, **18**, and **20** (because of limited sample quantity). [b] Stated as >2600 μM when IC_{50} values of sample exceed that of BHT; MS-BuOH and sugar fraction of maple syrup had IC_{50} values >1000 μg/mL. [c] Only tested once because of the quantity.

Antioxidant Assay. The antioxidant potentials of MS-BuOH, the sugar fraction of maple syrup, and the pure compounds were determined on the basis of the ability to scavenge the DPPH radical as previously reported (*26*). The DPPH radical scavenging activity of ascorbic acid (vitamin C) and the synthetic commercial antioxidant, BHT, were also assayed as positive controls (see **Table 2**). The assay was conducted in a 96-well format using serial dilutions of 100 μL aliquots of test compounds (ranging from 2500 to 26 μg/mL), ascorbic acid (1000−10.4 μg/mL), and BHT (250,000−250 μg/mL). Then DPPH (150 μL) was added to each well to give a final DPPH concentration of 137 μM. Absorbance was determined after 30 min at 515 nm, and the scavenging capacity (SC) was calculated as $SC\% = [(A_0 - A_1/A_0)] \times 100$, where A_0 is the absorbance of the reagent blank and A_1 is the absorbance with test samples. The control contained all reagents except the compounds, and all tests were performed in triplicate. IC_{50} values denote the concentration of sample required to scavenge 50% DPPH free radicals.

RESULTS AND DISCUSSION

Isolation and Identification of Compounds in Canadian Maple Syrup Butanol Extract (MS-BuOH). The primary objective of this study was to isolate and identify the phytochemicals present in Canadian maple syrup butanol extract. Because the constituents of ethyl acetate, chloroform, dichloromethane, and diethyl ether extracts of maple syrup have already been reported (*3*−*6*), we focused our isolation and structural elucidation efforts on the butanol extract. We speculated that the butanol extract may contain phenolic compounds not previously identified from the aforementioned organic extracts of maple syrup.

Figure 1A shows the HPLC-UV profile of MS-BuOH, which revealed several peaks at 280 and 360 nm characteristic of phenolic compounds. The extract was subjected to a series of chromatographic isolation procedures to yield 23 (**1**−**23**) phenolics. **Figure 1B** shows the HPLC-UV profile of the purified isolates all combined into a single injection. All of the compounds were identified on the basis of their ^1H and/or ^{13}C NMR and mass spectral data and by correspondence to published literature data when available (**Table 1**). **Figure 2** shows the structures of the compounds grouped into their individual phenolic subclasses for ease of discussion as follows.

Lignans. Seven lignans were isolated from MS-BuOH and identified as lyoniresinol (**1**), secoisolariciresinol (**2**), dehydroconiferyl alcohol (also known as dihydrodehydrodiconiferyl alcohol) (**3**), 5′-methoxydehydroconiferyl alcohol (**4**), erythro-guaiacylglycerol-*β*-*O*-4′-coniferyl alcohol (**5**), erythro-guaiacylglycerol-*β*-*O*-4′-dihydroconiferyl

alcohol (**6**), and [3-[4-[(6-deoxy-α-L-mannopyranosyl)oxy]-3-methoxyphenyl]methyl]-5-(3,4-dimethoxyphenyl)dihydro-3-hydroxy-4-(hydroxymethyl)-2(3*H*)-furanone (**7**).

With the exception of dehydroconiferyl alcohol (**3**), which has been previously reported as a lignin-derived flavor compound in maple syrup (*1*, *2*), this is the first reported occurrence of all of the other lignans in maple syrup. Notably, compound **7** was recently described as a constituent of the hardwood collected from the sugar maple tree, *A. saccharum* (*15*), and thus its occurrence in maple syrup is not surprising. Also, apart from dehydroconiferyl alcohol (**3**), previously found in maple syrup (*1*, *2*), and lyoniresinol (**1**), previously reported from leaves of *Acer truncatum* (*27*), this may be regarded as the first reported occurrence of these lignans in the *Acer* genus.

Lignan-rich foods such as flaxseed, which contains secoisolariciresinol (**2**), have attracted significant research attention for their biological effects (*28*, *29*). Thus, the presence of these compounds in maple syrup is interesting from a human health perspective. However, determination of the levels of these lignans (as well as the other bioactive phenolic subclasses described below) in different grades of maple syrup consumed by humans and whether these compounds achieve physiologically relevant levels after maple syrup consumption would be required to evaluate their impact on human health.

Coumarins. Two coumarins, not previously reported from maple syrup, were isolated from MS-BuOH and identified as scopoletin (**8**) and fraxetin (**9**). Notably, scopoletin (**8**) has recently been identified from the wood of *A. saccharum* (*15*) and has also been reported from the bark of *Acer nikoense* (*30*). From a biosynthetic perspective, it is interesting that coumarinolignans have been previously reported from the heartwood of *A. nikoense* (*31*), which would account for the occurrence of these two individual phenolic subclasses, namely, coumarins and lignans, in maple syrup.

Stilbene. A stilbene was isolated from MS-BuOH and identified as (*E*)-3,3′-dimethoxy-4,4′-dihydroxystilbene (**10**). Whereas stilbene glycosides have been previously reported from the leaves of *Acer mono* (*32*), this is the first reported occurrence of a stilbenoid in maple syrup. Foods containing stilbenes have attracted immense public attention for their potential human health benefits due in large part to emerging research on resveratrol, a stilbene present in red wine, grapes, and berries (*33*).

Phenolic Derivatives. Thirteen phenolic derivatives were found in MS-BuOH including 2-hydroxy-3′,4′-dihydroxyacetophenone (**11**), 1-(2,3,4-trihydroxy-5-methylphenyl)ethanone (**12**), 2,4,5-trihydroxyacetophenone (**13**), catechaldehyde (**14**), vanillin (**15**), syringaldehyde (**16**), gallic acid (**17**), trimethyl gallic acid methyl ester (**18**), syringic acid (**19**), syringenin (**20**), (*E*)-coniferol (**21**), *C*-veratroylglycol (**22**), and catechol (**23**). Whereas several of these compounds have been previously found in maple syrup (*3*, *4*), this is the first report of catechaldehyde (**14**), trimethyl gallic acid methyl ester (**18**), syringenin (**20**), and *C*-veratroylglycol (**22**) in maple syrup.

Other Unidentified Compounds. It is noteworthy that similar to the observations of Abou Zaid et al. (*4*), a number of peaks/compounds in maple syrup remain unidentified (see **Figure 1A**). Despite starting our initial extraction protocol with 20 L of maple syrup, several compounds were unobtainable due to either rapid degradation/decomposition on our columns or low yields.

In addition, we cannot rule out the presence of compounds previously reported in the other organic extracts of maple syrup (*3*−*6*), such as ethyl acetate (MS-EtOAc), being present in the MS-BuOH. Toward this end, we conducted HPLC-UV comparisons of the retention times of authentic phenolic standards of several of these previously reported compounds with the unidentified peaks in **Figure 1A**, along with comparisons of HPLC-UV chromatograms of MS-BuOH and MS-EtOAc (data not shown). However, due to considerable overlapping and coelution of compounds in these HPLC-UV profiles, our results were inconclusive. Our future work will include the isolation and identification of compounds in MS-EtOAc in order to have a comprehensive phytochemical/phenolic characterization of maple syrup.

Finally, we speculate that apart from the "natural products" identified here, there are "un-natural, artifacts or process-derived" compounds present in maple syrup, possibly formed under the conditions of intensive heating involved in transforming sap to syrup. These compounds could potentially be formed in situ as (1) decomposition/degradation products from the natural compounds and (2) due to chemical reactions between native and process-derived compounds. Further research to identify these compounds is warranted because their contribution to the potential health benefits and biological activity of maple syrup may be significant.

Antioxidant Activity. Phenolic compounds identified from maple syrup and maple syrup extracts have been reported to show antioxidant activity (*4*, *7*, *8*). Therefore, MS-BuOH, the sugar fraction of maple syrup, and the pure isolates along with positive controls, vitamin C and the synthetic commercial antioxidant BHT, were evaluated for antioxidant potential in the DPPH assay (**Table 2**). Vitamin C (ascorbic acid) and BHT showed IC_{50} values of 58 μM (ca. 10 μg/mL) and 2651 μM (ca. 583 μg/mL), respectively. Whereas the antioxidant activity of the MS-BuOH (IC_{50} > 1000 μg/mL), the sugar fraction (IC_{50} > 1000 μg/mL), the stilbene (**10**), and vanillin (**15**) all exceeded that of BHT, compounds **11**, **12** and **14** all showed superior antioxidant activity compared to vitamin C. Among the diverse phenolic subclasses of compounds identified in MS-BuOH, the general trend in antioxidant activity was phenolic derivatives, coumarins > stilbene, lignans.

In summary, 23 phenolics (**1**−**23**) with various antioxidant activities were isolated and identified from MS-BuOH. Among the isolates, 16 compounds (**1**, **2**, **4**−**14**, **18**, **20**, and **22**) are being reported from maple syrup for the first time. However, to get a comprehensive phenolic profile and characterization of maple syrup, further isolation work on other extracts (e.g., MS-EtOAc) would be necessary. The results of the current study suggest that the "cocktail" of bioactive phenolics present in Canadian maple syrup may impart potential health benefits to this natural sweetener. However, further research would be required to confirm this.

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Short communication

Quebecol, a novel phenolic compound isolated from Canadian maple syrup

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ABSTRACT

The province of Quebec in Canada leads the world's production of maple syrup, a natural sweetener obtained by thermal evaporation of sap collected from maple (*Acer*) species. As part of our laboratory's detailed chemical investigation of Canadian maple syrup, a novel phenolic compound, 2,3,3-tri-(3-methoxy-4-hydroxyphenyl)-1-propanol, assigned the common name of quebecol, was obtained. Quebecol was isolated using a combination of chromatographic methods and identified by detailed 1D and 2D nuclear magnetic resonance (NMR) and mass spectral (MS) analyses. Liquid chromatography mass spectral (LC-MS) analyses revealed that quebecol is not originally present in maple sap. This observation, as well as the lack of a feasible biosynthetic pathway to explain its origin, suggests that quebecol is formed during the processing and/or extraction of maple syrup. Thus, the identification and biological evaluation of non-natural, process-derived compounds in maple syrup are warranted since such molecules may contribute towards the biological activities reported for this natural sweetener.

1. Introduction

Maple syrup is the largest commercially produced and consumed natural product which is obtained entirely from the sap of deciduous trees. It is produced by thermal evaporation of the colourless watery sap collected from maple (*Acer*) species in the spring months when freeze/thaw cycles cause the sweet sap to rise and flow from taps made in the tree trunk. Maple sap is boiled to concentrate the sugar and approximately 40 L of the sap is required to produce 1 L of the rich 66° Brix syrup (Perkins & van den Berg, 2009). Apart from sucrose which is its predominant sugar, the natural tree sap contains minerals, oligosaccharides, amino acids, polyphenols, and phytohormones (Ball, 2007; Davison & Young, 1973; Perkins & van den Berg, 2009; Potter & Fagerson, 1992). During the intensive heating process required to transform the sap into syrup, a complex cocktail of both native phenolics (originally present in the xylem sap) and derived compounds (formed through chemical reactions during processing) ultimately ends up in maple syrup (Ball, 2007). This is interesting from a human health perspective considering that phenolics have attracted significant research attention for their potential roles in human health promotion and disease prevention (Shahidi & Ho, 2005).

Canada and the United States are the only two countries that commercially produce maple syrup in the world. Canada's production accounts for more than 80% of the world's supply of maple syrup with the province of Quebec leading this

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production with ca. 91% (Ontario Ministry of Agriculture Food & Rural Affairs). Apart from its cultural significance, maple syrup production is thus of great economic importance to the north-eastern region of North America.

Given the worldwide popularity and consumption of this natural sweetener, chemical identification of maple syrup constituents is of great scientific interest. Moreover, published studies have shown that maple syrup extracts have antioxidant, antimutagenic, and human cancer cell antiproliferative properties (Legault, Girard-Lalancette, Grenon, Dussault, & Pichette, 2010; Theirault, Caillet, Kermasha, & Lacroix, 2006). To this end, our laboratory has embarked on a collaborative project to comprehensively identify the chemical constituents in maple syrup from Canada (Li & Seeram, 2010). In that study, we isolated 23 naturally derived phenolics belonging to lignan, coumarin, stilbene, and phenolic acid sub-classes in maple syrup. Here, we report the isolation and structural elucidation of a novel process-derived phenolic compound from the Canadian maple syrup which has been assigned the common name of quebecol.

2. Materials and methods

2.1. General experimental procedures

All 1D and 2D nuclear magnetic resonance (NMR) experiments including correlation spectroscopy (COSY), HSQC (Heteronuclear Single Quantum Coherence) and HMBC (Heteronuclear Multiple Bond Coherence) were acquired on a Varian 500 MHz Biospin instrument using DMSO-d6 as the solvent. Electrospray Ionization Mass Spectral (ESI/MS) data were acquired on a Q-Star Elite (Applied Biosystems MDS) mass spectrometer equipped with a Turbo Ionspray source. Analytical and semi-prep high performance liquid chromatography (HPLC) were performed on a Hitachi Elite LaChrom system consisting of an L2130 pump, L-2200 autosampler, and L-2455 Diode Array Detector all operated by an EZChrom Elite software. Optical rotation was performed on an Auto Pol III Automatic Polarimeter (Rudolph Research, Flanders, NJ, USA) with a sample concentration of 0.5 mg/mL in methanol at 22 °C using a cell with a 1 dm pathway length. The result from the optical rotation experiment was 0 indicating the presence of a racemic mixture. All solvents were either ACS or HPLC grade and were obtained from Wilkem Scientific (Pawcatuck, RI).

2.2. Isolation of quebecol [2,3,3-tri-(3-methoxy-4-hydroxyphenyl)-1-propanol]

A description of the detailed methodologies used for the extraction of maple syrup has been recently reported (Li & Seeram, 2010). Maple syrup (grade C; obtained from Quebec, Canada) is produced by thermal evaporation of sap, without any additives, according to well-established methods in the industry (Perkins & van den Berg, 2009). A 20 L sample of maple syrup was shipped to our laboratory and stored at −20 °C for 1 week prior to the extraction. The entire 20 L maple syrup was subjected to an exhaustive liquid–liquid partitioning with ethyl acetate (10 L × 3; over 6 h) followed by n-butanol (10 L × 3; over 6 h). After the solvent removal *in vacuo* using a rotary evaporator at 37 °C, a portion of the dried n-butanol extract (87 g portion; total = 108 g) was extracted with methanol (100 mL × 3) to afford methanol soluble (36 g) and insoluble (57 g) fractions. The methanol soluble fraction was further purified by repeated Sephadex LH-20 column chromatography followed by C-18 semi-preparative HPLC. First, the extract was chromatographed on 65 × 4 cm Sephadex LH-20 column eluted with a CH_3OH–H_2O gradient system (3:7 to 1:0, v/v) to afford 12 subfractions, A1–A12. Subfraction A4 (1.6 g) was re-chromatographed on a 65 × 4 cm Sephadex LH-20 column eluted with the same gradient system (3:7 to 1:0, v/v) to afford 12 subfractions, B1–B12. Subfraction B5 (137.2 mg) was purified by semi-preparative HPLC using a Waters Sunfire C18 column (250 × 10 mm i.d., 5 μm, flow = 2 ml/min) with a gradient elution system of CH_3OH–H_2O (0.1% trifluoroacetic acid) (1:4, v/v to 1:0, v/v in 60 min) to afford quebecol (0.8 mg; off-white powder).

3. Results and discussion

3.1. Structural elucidation of quebecol

Quebecol (structure shown in Fig. 1A) was isolated as a pale off white amorphous powder from a butanol extract of the maple syrup from Canada. The positive ESI-MS data exhibited a molecular peak at *m/z* 449.1571 $[M+Na]^+$ corresponding to a molecular formula of $C_{24}H_{26}NaO_7$ (calcd. for 449.1576; see online supplementary material). The complete NMR data for quebecol are shown in Table 1. The ^1H NMR spectrum exhibited signals for three sets of an ABX aromatic system as follows: δ_H 6.81 (1H, *J* = 8.0 Hz, H-6), 6.67 (1H, *J* = 8.0 Hz, H-5), 6.98 (1H, s, H-2); δ_H 6.56 (1H, *J* = 8.0 Hz, H-6′), 6.41 (1H, *J* = 8.0 Hz, H-5′), 6.78 (1H, s, H-2′); and δ_H 6.60 (1H, *J* = 8.0 Hz, H-6″), 6.50 (1H, *J* = 8.0 Hz, H-5″), 6.56 (1H, s, H-2″) respectively, suggesting the presence of three aromatic/benzene rings. This was supported by the ^{13}C NMR data and ^1H–1H COSY spectrum analyses (key connectivities are shown in Fig. 1B). In the ^1H NMR spectrum, three individual singlet signals at δ_H 3.76, 3.66 and 3.63, each integrating for three protons were consistent with the presence of three methoxyl (OCH_3) groups. These were corroborated with the accompanying ^{13}C NMR shifts characteristic of methoxyl carbons at 56.14 (C-3), 56.01 (C-3′), and 55.94 (C-3′′), respectively. Additionally, one doublet signal at δ_H 4.02 (1H, *J* = 10.5 Hz, H-7) and two multiplet signals at δ_H 3.41 (1H, m, H-8) and 3.40 (2H, m, H-9) were observed in the ^1H NMR spectrum. This corresponded to a CH–CH–CH_2 substructure which could be deduced from the COSY correlation analyses (Fig. 1B).

All of the proton signals were assigned to their corresponding carbons through direct ^1H–13C correlations in the HSQC spectrum, with the exception of the two singlets at δ_H 8.67 and 8.43 integrating for one and two protons, respectively, which were assigned to the protons of the three aromatic hydroxyl groups. In the HMBC spectrum (Fig. 1B), the correlation signals from δ_H 6.67 (H-5) and 3.76 (3-OCH_3) to C-3 (δ 147.72); δ_H 6.41 (H-5′) and 3.66 (3′-OCH_3) to C-3′ (δ 147.17); and δ_H 6.50 (H-5″) and 3.63 (3″-OCH_3) to C-3″ (δ 147.08), revealed that the three methoxyl groups were substituted on the C-3, 3′ and 3″ positions, respectively. In the same HMBC experiment, correlation signals from δ_H 4.02

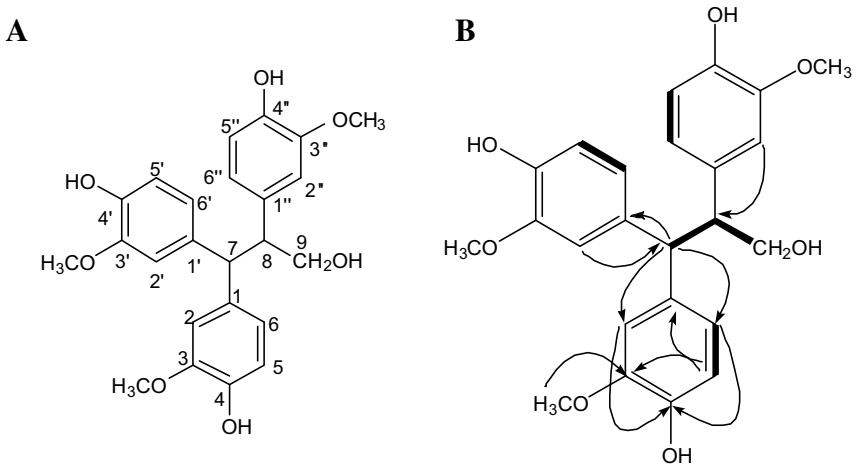

Fig. 1 – Structure of quebecol (1A) and its key COSY (thick lines) and selected key HMBC (arrows) correlations (1B).

Table 1 – ^{1}H and ^{13}C NMR data (in DMSO-d6, 500 and 125 MHz, respectively) of quebecol.

No.	δ_C	δ_H[a]	No.	δ_C	δ_H[a]
1	136.70	–	1′	136.26	–
2	112.56	6.98 (s)	2′	113.15	6.78 (s)
3	147.72	–	3′	147.17	–
4	144.92	–	4′	144.26	–
5	115.72	6.67 (d, 8.0)	5′	115.23	6.41 (d, 8.0)
6	120.33	6.81 (d, 8.0)	6′	121.04	6.56 (d, 8.0)
7	52.67	4.02 (d, 10.5)	1″	134.65	–
8	51.42	3.41 (m)	2″	113.90	6.78 (s)
9	64.92	3.40 (m)	3″	147.08	–
3-OCH_3	56.14	3.76 (s)	4″	144.48	–
3′-OCH_3	56.01	3.66 (s)	5″	115.09	6.50 (d, 8.0)
3″-OCH_3	55.94	3.63 (s)	6″	121.77	6.60 (d, 8.0)
4-OH	–	8.64 (s)	4″-OH	–	8.43 (s)
4′-OH	–	8.43 (s)			

[a] Multiplicity, J_{HH} in Hz.

(H-7) to C-2 (δ 112.56), C-6 (δ 120.33) and C-1′ (δ 136.26), and from δ_H 6.78 (H-2″) to C-8 (δ 51.42) suggested that the three aromatic/benzene rings were attached to the CH–CH–CH_2OH substructure on the C-7, C-7, and C-8 positions, respectively. Thus, consideration of the NMR and ESI/MS spectral data led to the proposed structure of 2,3,3-tri-(3-methoxy-4-hydroxyphenyl)-1-propanol that was assigned the common name of quebecol.

3.2. *Origin of quebecol*

Maple syrup is produced under intensive heating conditions required to transform sap to syrup. Thus, it is not surprising that maple syrup contains naturally occurring phenolics (present in the xylem sap) as well as non-natural, artefacts or process-derived compounds (formed by chemical reactions during its production). Our recent research identified 23 naturally occurring phenolics in maple syrup including coniferol and syringenin, which bear structural similarities to quebecol (Li & Seeram, 2010). Notably, these natural products have well accepted biosynthetic pathways, namely of shikimate origin, and are the precursors of lignans and lignins which are integral components of woody plants (Mann, 1978). On the contrary, the substitution pattern of the aromatic/benzene rings on the 'coniferol moiety' in quebecol (Fig. 1A) and the lack of a feasible biosynthetic pathway to explain its origin suggest that it is of a non-natural origin. Further examination of the maple sap using LC-MS analyses (results not shown) failed to identify quebecol therein, confirming that it is formed during the syrup and/or extract preparation. Also, the result from the optical rotation experiment (i.e. 0) revealed that quebecol occurs as a racemate mixture. Given that quebecol has a stereocentre at position C-8, if it indeed was a natural product, the occurrence of a specific stereoisomer would have been expected. While the exact mechanism/s for quebecol's formation remain/s elusive, we speculate that this compound may be produced *in situ* by chemical reactions between native and/or process-derived phenolic compounds during the maple syrup production and/or extraction. However, further research would be required to confirm this.

4. Conclusion

In summary, a novel phenolic compound, named quebecol, was isolated from Canadian maple syrup. Our finding of a non-natural phenolic compound in maple syrup is interesting considering that such molecules may contribute significantly towards the reported biological activities of maple syrup. Unfortunately, we did not obtain sufficient quantity of the pure isolated compound to conduct biological testing in the current study. Thus, further studies to evaluate the levels and presence of this compound in commercial maple products as well as other grades of maple syrup are warranted. Based on its structural similarities to natural antioxidant phenolics previously identified in maple syrup (Li & Seeram, 2010), it is possible that quebecol may also possess similar biological properties.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jff.2011.02.004.

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Antioxidant, antiradical and antimutagenic activities of phenolic compounds present in maple products

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Abstract

The phenolic compounds in maple sap and syrup were extracted at different periods of the season and were separated to collect the glycosylated compounds and the aglycone compounds. The antioxidant and antiradical activities of each phenolic compound were studied using the thiobarbituric acid reactive substances (TBARS) assay and the *N*,*N*-diethyl-*p*-phenylenediamine (DPD) decoloration test to measure the free radical scavenging. The results showed that in general the phenolic compounds had a good antioxidant and antiradical properties. The glycosylated compounds from maple sap and maple syrup showed a better activity than the aglycones. The antimutagenic effects of each phenolic compounds from maple sap and syrup were also investigated as the inhibition of SOS induction by chemical agents in *Salmonella typhimurium* TA1535/pSK1002 containing the fusion gene umuC-lacZ. Induction of the SOS gene (*umu*C) expression was assayed by measuring accumulated β-galactosidase activity using a modified Umu test. The antimutagenic properties were studied per se and after metabolisation by S9 fraction. The results showed that an optimum of antimutagenic properties of the glycosylated metabolites phenolic compounds from sap and syrup was observed at 75% of the season for the sap and at 25% of the season for the syrup. A higher antimutagenic activity was observed at 25% and 100% of the season for aglycones present in syrup and at 75% of the season for aglycones present in sap.

Keywords: Antioxidant activity; Antiradical activity; Phenolic compounds; Maple sap; Maple syrup; Antimutagenic activity; Umu test

1. Introduction

Oxidative stress by free radicals is an important event in the cell that can cause aging and human degenerative diseases including, cancer, heart diseases, multiple sclerosis, Parkinson's disease, autoimmune disease and senile dementia. Stresses, physical damage, viral infection, cytotoxic or carcinogenic compounds as a consequence of chemical or biological aggression may cause peroxidation of polyunsaturated fatty acids of cell membranes and liberation of toxic substances such as free radicals. Studies concerning the relationship between the morbidity due to cancer and heart diseases and the consumption of fruits and vegetables indicated that polyphenols present in large amount in fruits and vegetables have a significant impact on the morbidity decrease from these diseases (Heim, Tagliaferro, & Bobilya, 2002; Hertog, Hollman, & Van de Putte, 1993; Rice-Evans, 2001). Attention has hence been focused in recent years on antioxidant products from natural sources isolated from plant products. Polyphenolic compounds are found mainly in fruits and vegetables as secondary plant metabolites. Many polyphenols such as kaempferol, quercetin, luteolin,

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myricetin and catechin express strong antioxidative, antiiflamatory, antiallergic and antineoplasic properties (Balasinska & Troszynska, 1998). The high antioxidant activity of plant phenolic compounds attractive to the food industry, prompting their use as replacements for synthetic antioxidants and also as nutraceuticals, playing a role in preventing many diseases.

The discovery and exploration of chemical compounds with antimutagenic and anticarcinogenic potency is at the present time of great importance because of the undesirable consequences of an increased rate of mutations and the related possible risks of cancer in humans (Kaur & Saini, 2000). Human epidemiology has indicated that cancer risk may be modified by changes in dietary habits or dietary components. Humans ingest large numbers of naturally occurring antimutagens and anticarcinogens, like the phytochemicals, in our food. These antimutagens and anticarcinogens may inhibit one or more stages of the carcinogenic process and prevent or delay the formation of cancer. Thus, studies on antimutagens in food are important to research on the physiological functionality of food components.

Maple syrup is one of the most important plant products in Québec, Canada, and represents 72% of the world production (Dumont, Saucier, Allard, & Arouze, 1993). Maple syrup is the product resulting from thermal processing of the sap from *Acer saccharum*. Maple sap represents a solution in which sucrose is the major component; however other compounds like organic acids, minerals and phenolic compounds have been reported (Kermasha, Goetghebeur, & Dumont, 1995; Kuentz, Simard, Zee, & Desmarais, 1976; Mollica & Morselli, 1984). Phenolic compounds, widely distributed in plants, contribute to the sensory properties associated with food quality such as color, aroma and may have potential health benefits, including reduction of cancer risk (Macheix, Fleuriet, & Billot, 1990). The analysis of phenolic compounds from maple sap and syrup have been conducted and from the many reported studies (Côté, 2003; Deslauriers, 2000) none had investigated the antioxidant, antiradical and antimutagenic activities of these compounds.

The aim of the present study was to evaluate the antioxidant, antiradical and antimutagenic activities of total phenolic compounds and phenolic compound extracts from maple sap and syrup collected at different periods of the season.

2. Materials and methods

2.1. Samples

Maple sap and syrup samples were provided by the Centre de recherche, de développement et de transfert technologique en acériculture (ACER, St-Hyacinthe, Québec, Canada). The samples were collected at different periods of the season 2002; 0%, 25%, 50%, 75% and 100% of the season (0% being the beginning and 100% the end of the season).

2.2. Extraction of phenolic compounds

Extraction of phenolic compounds from maple sap and syrup was achieved according to slight modifications of Kermasha et al. (1995) method. The sap and syrup samples (500 ml) were adjusted to pH 7. Three successive extractions in ethyl acetate (Fisher Scientific, Nepean, ON, Canada) were done. The first extraction was done using 500 ml of ethyl acetate and for the two last ones; a volume of 250 ml was used. A 2 l separating funnel was used for the extraction. During each extraction period, 10 min of agitation were used in order to separate the organic phase from the aqueous phase. The organic phase containing the phenolic compounds was recuperated after each extraction and kept at 4 °C. The three organic phases were pooled and mixed with 100 ml of deionised water in order to eliminate the presence of residual sugars. Anhydrous Na_2SO_4 was used to dry the organic phase and then it was filtered on Whatman no. 1 filter (Fisher Scientific). Complete evaporation of ethyl acetate extract was done using the SpeedVac Automatic evaporation system (Savant System, Holbrook, NY). The dry extract was dissolved in methanol (HPLC grade, Fisher Scientific) and dried under nitrogen in preweighed vials.

2.3. Quantification of phenolic compounds

The HPLC analyses were performed on a ProStar 230 (Varian Canada Inc., Mississauga, ON, Canada), equipped with a ternary pump delivery system, a Rheodyne injection valve (500 μl capacity, Waters Ltd., Dorval, QC, Canada) and a ProStar 330 diode-array UV–Vis detector (Varian); integration and data elaboration were performed using Star Chromatography Workstation software (Varian). A Varian analytic column C_{18}, 5 μm, 7.8 × 300 mm column was used. All solvents were filtered with 0.45 μm Millipore (Millipore Canada Ltd., Etobicoke, ON, Canada) filter disk and degassed with helium. A gradient elution was carried out using the following solvent systems: mobile phase A, double distilled water/acetonitrile (Laboratoires Mat, Beauport, QC, Canada)/formic acid (Fluka, Oakville, ON, Canada), (94/5/1, v/v/v); mobile phase B, double distilled water/acetonitrile/formic acid (69./30/1, v/v/v). The linear gradient elution system was: 100–90% A from 1 to 15 min and 90% A to 100% B from 15 to 50 min, keeping 100% B for 10 min, returning to 100% A followed by equilibration for 10 min before injection. Twenty μl of total phenolic compounds dissolved in methanol were injected after filtration through a

0.45 μm filter disk. The flow rate was 0.7 ml min^{-1} and the detection was performed at 280 nm. Total phenolic compounds concentration expressed as gallic acid equivalent was determined with a standard curve ($r^2 = 0.991$) made by concentrations from 0.0050 to 0.0175% (w/v) of gallic acid solution dissolved in 10% (v/v) methanol. This total phenolics concentration was determined using the summed peak area of the different phenolic extracts, and results were expressed as *g* of gallic acid equivalent (GAE) per 100 g of extract. Chromatograms obtained from maple sap and maple syrup during the season are presented Fig. 1. Some peaks were identified by comparison with standards and the retention times of known maple phenolic compound peaks. The phenolic compound standards were obtained from Sigma–Aldrich (Oakville, ON, Canada).

2.4. Separation of the glycosylated and aglycone compounds

The separation of the glycosylated and aglycone compounds from the maple extracts was conducted by dissolving 5 mg of total phenolic extracts from maple sap and syrup in 1 ml of methanol (Fisher Scientific, Nepean, ON, Canada) in order to apply the sample to a Amberlite XAD-2 resin (Supelco, Oakville, ON, Canada) conditionned in SR 10/50 column (Amersham Biosciences Corp., Baie d'Urfé, QC, Canada) (300 mm × 100 mm i.d.) with methanol 100% (grade HPLC, Fisher Scientific). The glycosylated extract was eluted with a methanol/water (60/40, v/v) solution (198 ml) using a peristaltic pump at flow of 0.5 ml/min. The glycosylated fraction was collected in

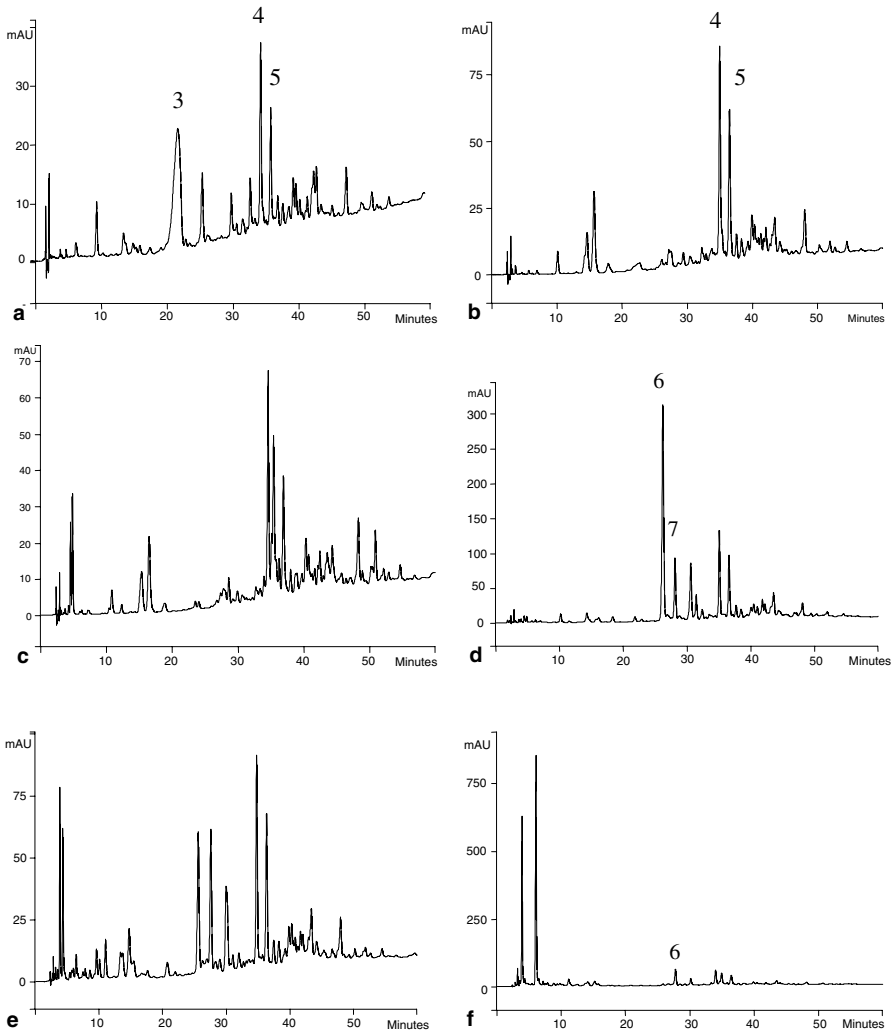

Fig. 1. Chromatograms of Phenolic compounds obtained from maple sap and maple syrup during the season: (a) sap, beginning of the season; (b) sap, mid-season; (c) sap, end of the season; (d) syrup, beginning of the season; (e) syrup, mid-season and (f) syrup, end of the season. (Peak 3) Coniferol; (Peak 4) syringaldehyde; (Peak 5) hydroxycinnamic acid; (Peak 6) Flavonol; (Peak 7) hydrobenzoic acid (products of degradation of phenolic compounds).

a 250 ml amber bottle. The solvent was removed from the fraction using the SpeedVac Automatic evaporation system (System Savant, Holbrook, NY). The sample was dissolved in 2 ml of methanol (Fisher Scientific) and dried under nitrogen in a preweighed vial and stored at −20 °C until analyzed. After the collection of the glycosylated fraction, the aglycone fraction was eluted in the XAD-2 column. This was done using 289 ml of a methanol/acetonitrile (Fisher Scientific) (50/50, v/v) solution. The first 7 ml were used to condition the column. The following 282 ml recuperated in a 300 ml bottle were evaporated with the SpeedVac. The sample was dissolved in 2 ml of methanol and dried under nitrogen in a preweighed vial and stored at −20 °C until analyzed.

2.5. Determination of antioxidant activity (AA)

The determination of the antioxidant activity of phenolic compounds from maple sap and syrup collected at different period of the season was done using a microtechnique based on the non-enzymatic peroxidation of rat liver microsomes method (Esterbauer, Cheeseman, Dianzani, Poli, & Slater, 1982) modified by Lessard (1995) where artificial membranes were used instead of rat liver microsomes, in order to obtain a more stable and reproducible system. This test measures by spectrophotometry the TBARS (thiobarbituric reactive substances) concentration producted during the peroxidation of liposomes exposed to iron ions in 20 mM phosphate buffer solution in presence of ascorbate. The antioxidant activity is equivalent to the lipid peroxidation inhibition capacity.

2.5.1. Liposomes preparation

Liposomes were formed by an injection method, as described by Batzri and Korn (1973). Linoleic acid (Sigma-Aldrich, Oakville, ON, Canada) was dissolved in 95% ethanol. The mixture was injected into phosphate buffer (20 mM, pH 7.4) in a proportion of 1:9 (v/v), using an hypodermic syringe fitted with a fine needle (G26).

2.5.2. Control solution preparation

Positive controls were ascorbic acid (vitamin C) (Laboratoires Mat, QC, Canada) for the hydrophilic compounds and α-tocopherol (vitamin E) (Sigma–Aldrich) for the lipophilic products. Solutions of each compouds were prepared at 1.25 mg/ml. Dilutions at 313 and 79 μg/ml were made. Negative controls were the solvents used meaning distilled water for the hydrophilic compounds and ethanol for the lipophilics.

2.5.3. Microplate preparation

Twenty-five ml of samples (1.25 mg/ml), positive and negative controls were added to a microplate (96 wells). The reaction mixture containing 4 ml of liposomes solution, 2.25 ml of phosphate buffer (20 mM, pH 7,4) and 0.25 ml of ascorbate solution (3.1 mg/ml) was prepared. Sixty-five μl of reaction mixture was added to a microplate using a multichannel pipette. Finally, 10 μl of $FeCl_3$ (Sigma–Aldrich) solution (4.3 mg/ml) were added to the wells. The microplate was then incubated at 37 °C for 15 min. One hundred fifty ml of a fresh solution of 10% (v/v) SDS (Sigma–Aldrich) and 0.67% (v/v) thiobarbituric acid (Sigma–Aldrich) in a 1:2 ratio was added in the microplate. The colorimetric reaction was produced at 80 °C for 30 min. The TBARS of the controls and samples were evaluated at 540 nm with a Microplate Autoreader (model EL 309, Biotek Instruments, Winooski, VT). The reaction was calibrated using the positive control whose the antioxidant activity was 100%. The antioxidant activity (AA) was calculated using the following equation:

$$\mathrm{AA}\ (\%) = [\mathrm{OD}_{(\text{negative control})} - \mathrm{OD}_{(\text{sample})}/\mathrm{OD}_{(\text{negative control})} - \mathrm{OD}_{(\text{positive control})}] \times 100.$$

2.6. Determination of Antiradical activity

Free radical scavenging capacities of phenolic compounds from maple sap and syrup collected at different period of the season were evaluated following a procedure of DPD (*N*,*N*-diethyl-*p*-phenylenediamine) colorimetric method as described by others (Le Tien, Vachon, Mateescu, & Lacroix, 2001; Oussalah, Caillet, Salmiéri, Saucier, & Lacroix, 2004). Two hundred ml of sample from a methanolic extract (1.25 mg/ml) were added in a cell containing 3 ml of 0.15 M NaCl and submitted to electrolysis for 1 min (continuous current, 400 V, 10 mA) using a power supply (Bio-Rad, model 1000/500, Mississauga, ON, Canada). After electrolysis, an aliquot of 200 μl was added to 2 ml of DPD solution (25 mg/ml). The generated oxidative species (superoxide anion (O_2^-), singlet oxygen ($^1O_2$) and OH radicals) and their by-products (hydrogen peroxide (H_2O_2) and hypochlorite ion (OCl^-)) react instantly with DPD, producing a red coloration that can be measured at 515 nm using a DMS 100S spectrophotometer (Varian Canada Inc., Mississauga, ON, Canada). The antiradical activity is equivalent to the capacity of phenolic compounds to inhibit the accumulation of oxidative species (able to oxidize DPD) and consequently the red coloration at 515 nm. The reaction was calibrated using the non- electrolyzed NaCl solution (no oxidative species, ascribed to 100% scavenging) and the electrolyzed NaCl solution (0% scavenging, in the absence of any antioxydants). The scavenging percentage was calculated according to the following equation:

$$\text{Scavenging}\ (\%) = 100 - [(\mathrm{OD}_{\text{sample}}/\mathrm{OD}_{\text{control}}) \times 100],$$

where $OD_{control}$ represents the OD of electrolyzed solution in the absence of phenolic extract and OD_{sample}, the OD of electrolyzed solution with phenolic extract. The OD is directly related to the degree of oxidation of DPD reagent by the oxidative species, meaning that a sample able to reduce completely the level of reactive oxidative species will have a 100% scavenging capacity.

2.7. Determination of antimutagenic activity

The antimutagenicity of phenolic compounds from maple sap and syrup collected at different period of the season was investigated against potassium dichromate and quercetin metabolites in presence of S9 fraction using a modified Umu test. The antimutagenicity per se and of the metabolites was conducted on total phenolic compound extracts from maple sap and syrup samples at 0%, 25%, 50%, 75% and 100% of the season. On the other hand, the glycosylated and aglycone phenolic compounds were analyzed for their antimutagenicity only at 0% and 100% of the season since the middle of the season was not significantly different from those two periods. The Umu test used in this experiment detects the induction of the SOS response following treatment of *Salmonella typhimurium* strain TA1535 with test compounds. This strain carries the plasmid pSK1002 in which the umuC′ gene fused inflame to the lacZ′ gene. The SOS-inducing potency of test compounds would therefore be estimated by the measurement of the induction level of umu operon in terms of intracellular β-galactosidase activity. The SOS response appears after DNA damage or interference with DNA replication (Miyazawa, Sakano, Nakamura, & Kosaka, 2001).

2.7.1. Bacterial strain

Salmonella typhimurium strain TA1535/pSK1002 containing the fusion gene umuC′–′lacZ that produces a hybrid protein with β-galactosidase activity and whose expression is controlled by the umu regulatory region (Oda, Nakamura, Oki, Kato, & Shinagawa, 1985) was purchased from DSMZ (Braunschweig, Germany).

2.7.2. Chemicals

Magnesium chloride ($MgCl_2$) and potassium chloride (KCl) were purchased from Fisher Scientific (Nepean, ON, Canada). SDS, mercaptoethanol, Potassium dichromate, quercetin, the enzyme substrate *o*-nitrophenyl-β-D-galactopyranoside (ONPG), glucose-6-phosphate and β-nicotinamide adenine (β-NAD) were purchased from Sigma–Aldrich (Oakville, ON, Canada). Sodium phosphate (NaH_2PO_4) and disodium phosphate (Na_2HPO_4) were purchased from laboratoires Mat (Beauport, QC, Canada). S9 fraction prepared from livers of male Wistar rats pretreated with Aroclor 1254 was purchased from Invitro Technologies (Baltimore, MD).

2.7.3. Activation mixture

The activation mixture containing 1 ml of S9 fraction, 10 ml of sterile phosphate buffer (0.2 M, pH 7.4), 0.4 ml of 0.4 M $MgCl_2$ + 1.65 M KCl sterile solution, 7.7 ml of sterile distillated water, 0.1 ml of 1 M glucose-6-phosphate sterile and 0.8 ml of 1 M sterile β-nicotinamide adenine (β-NAD) was prepared. This solution must be kept at 4 °C and the S9 fraction and NAD are added last.

2.7.4. Umu test

TGA medium containing 1% Bacto tryptone, 0.5% NaCl, 0.2% glucose and 20 μg/ml ampicilin was inoculated with 1 ml of the tester strain *Salmonella typhimurium* TA1535/pSK1002 and was incubated at 37 °C under moderate agitation during 16 h. The culture was then diluted 10 times with TGA medium and incubated for 2 h or until the bacterial density reached OD_{600} of 0.25–0.3 at 37 °C, resulting in log-phase cells. One ml of the log-phase culture was further added to a test tube containing the test mixture or 1 ml of TGA medium for the control. Two hundred and eight μl of phosphate buffer (0.1 M) for the antimutagenicity per se or 208 μl of the S9 mixture was added for the antimutagenicity evaluation of the phenolic compound metabolites. Forty-two μl of the test phenolic compound solution (1.25 mg/ml) or the solvent (methanol 10% (v/v) for the glycosylated and 100% (v/v) for the aglycone) in which was dissolved the compound (control) was added to each tube. Finally, 42 μl of potassium dichromate (84 μg/ml) or quercetin (313 μg/ml) in presence of the S9 fraction was added to the test tube. The metabolite compounds of quercetin in presence of S9 fraction or the potassium dichromate per se are mutagenic and are used as control. The test mixture and control in test tubes were incubated for 2 h at 37 °C under moderate agitation. At the end of incubation, the cell density from each tube was measured at 600 nm using a spectrophotometer (Unicam model UV4, Cambridge, UK). The β-galactosidase activity was also assayed according to others (Whong, Wen, Stewart, & Ong, 1986). In order to evaluate the β-galactosidase activity, 50 μl of treated cells and 100 μl of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) solution (4 mg/ml in 0.1 M phosphate buffer, pH 7.0) were added to a test tube containing 450 μl of B buffer prepared according to others (Whong et al., 1986) with 16.1 g of Na_2HPO_4, 5.5 g of NaH_2PO_4, 0.75 g of KCl, 0.25 g of $MgSO_4$–7 H_2O, 1 g of SDS, 2.7 ml of β-mercaptoethanol and 1 l of distilled water at pH 7. Finally, the tubes were incubated at 28 °C during 25 min. The enzymatic reaction was stopped by adding 400 μl of 1 M sodium carbonate (Na_2CO_3). The $OD_{420\ nm}$ and $OD_{550\ nm}$ were determined with a spectrophotometer (Unicam, UV4 model, Cambridge, UK). Inhibition of SOS response or antimutagenicity was calculated as follows:

Antimutagenicity (%)
= (β-gal Unit Control-β-gal Unit sample/unit β-gal Control) × 100

β-galactosidase activity was presented as units according to the following formula: Unit = $1000 \times (OD_{420\,nm} - 1.75\ OD_{550\,nm})/(T \times V \times OD_{600\,nm})$ where T represented the time of reaction (min) and V the volume of cells (ml). Enzyme units with a one time dose response were considered as positive results (Miller, 1972).

2.8. Statistical analysis

This experiment was done in replicate and three samples of each replicate were analyzed. Data were analyzed using SPSS for Windows. Analyses of variance were performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range test ($p \leqslant 0.05$).

3. Results and discussion

3.1. Antioxidant and antiradical properties

3.1.1. Total phenolic extracts

The capacity of lipid peroxidation inhibition in relation to the total phenolic content of each extract during the season is illustrated in Fig. 2. The antioxidant properties of phenolic compounds present in maple sap showed antioxidant properties from 84.86% to 96.67% from the beginning of the season to 25% of the season (Fig. 2a). Then a decrease from 96.67% to 42.57% in their antioxidant activity was observed. A maximum of antioxidant activity was observed at the quarter of the season. The concentration of phenolic compounds in the extracts showed an gallic acid equivalent quantity of 16.51–8.51 g/100 g from the beginning of the season to 75% of the season. A value of 24.69 g GEA/100 g was observed at the end of the season. The antioxidant properties of phenolic compounds extracted from maple syrup showed a value of 83.78% (Fig. 2b). Optimal antioxidant properties were observed at 50% of the season representing a value of 95.58% and then a significant ($p \leqslant 0.05$) decrease was observed at 75% of the season and a stable value of 76.02% was observed until the end of the season. The content of the phenolic compounds showed a value of 63.81 g GEA/100 g at the beginning of the season. A significant decrease ($p \leqslant 0.05$) to a value of 17.81 g GEA/100 g was observed until 75% of the season. At the end of the season, a significant increase ($p \leqslant 0.05$) occurred and a value of 59.41 g GEA/100 g was observed. These results showed that there was no correlation ($r^2 < 0.300$) between the

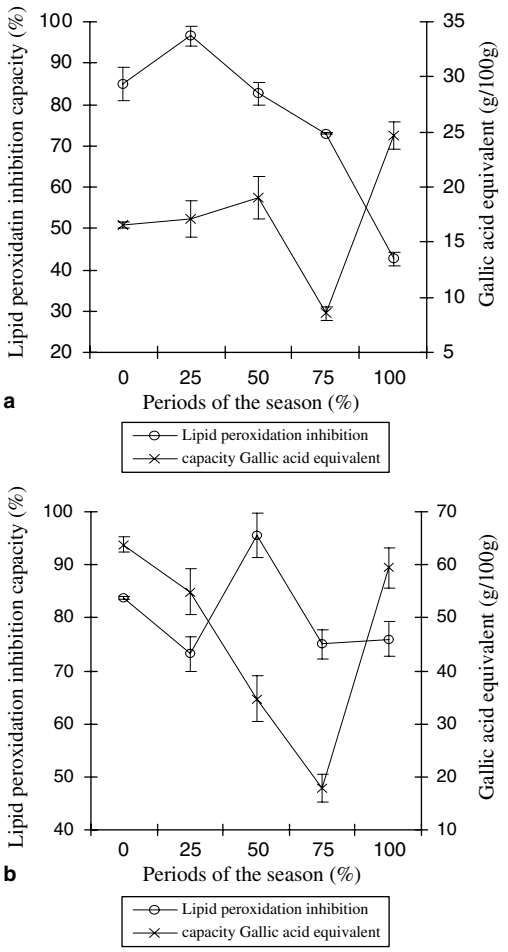

Fig. 2. Lipid peroxidation inhibition capacity and the content of total phenolic compounds in maple sap (a) and maple syrup (b) during the season.

antioxidant activity of maple sap and syrup extracts and the concentration of total phenolic compounds.

The free radical scavenging activity and the content of total phenolic compounds of each extract during the season are presented in Fig. 3. The results showed that the free radical scavenging activity of maple sap extract varies proportionally ($r^2 = 0.947$) with the total phenolic content (Fig. 3a). At the beginning of the season, the extract had a free radical scavenging capacity of 82.95% and it increased at 97.75% at the quarter of the season to remain stable until the end of the mid-season. Then at the three quarter of the season, a significant decrease ($p \leqslant 0.05$) occurred with a free radical scavenging capacity of 67.09%. Finally, at the end of the season, the free radical scavenging capacity increased significantly ($p \leqslant 0.05$) at a value of 75.24%. The free radical scavenging capacity profile of maple sap was different from the one of maple syrup (Fig. 3b). A proportional relation ($r^2 = 0.859$) with the concentration of total phenolic compounds was also observed. At the beginning of the season, the extract had a free radical scavenging capacity of 84.93%. A decrease at

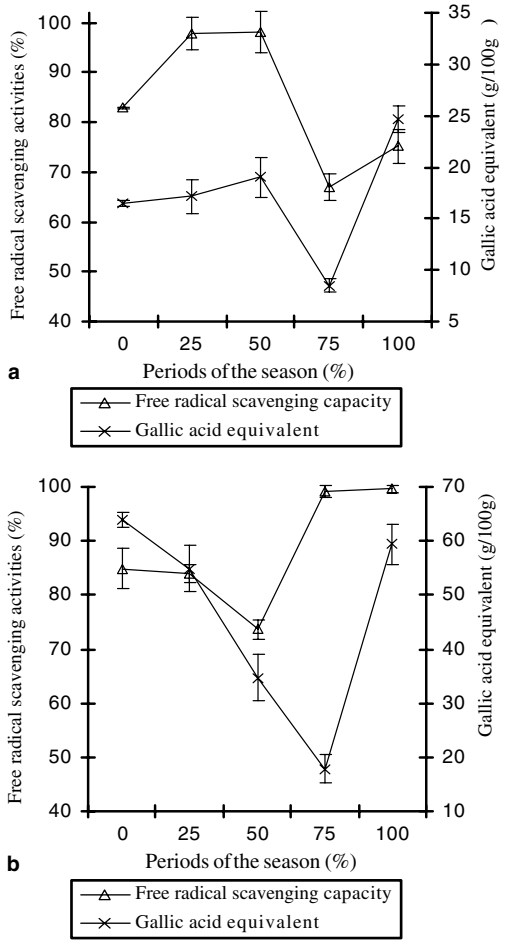

Fig. 3. Free radical scavenging capacities and the content of total phenolic compounds in maple sap (a) and maple syrup (b) during the season.

mid- season (73.62%) and increase were observed at the end of the season (99.63%).

3.1.2. Glycosylated and aglycone compounds

The antioxidant and antiradical properties of the glycosylated and aglycones compounds in maple products extracts was analyzed. The capacity of the glycosylated and aglycone compounds extracted from maple sap to inhibit lipid peroxidation is represented in Table 1. Antioxidant activity values of maple sap extract were found to be superior to 100% regardless of the time of the season, meaning a higher antioxidant activity of the extract than the control. An optimal antioxidant capacity was observed respectively at 25% and 75% of the season with values of 122.46% and 120.63%. A significant decrease ($p \leqslant 0.05$) occurred at the end of the season with a value of 103.69%. The glycosylated compounds from maple syrup, showed also a value superior to 100% until 50% of the season with respective values of 107.89% and 123.14% at 0% and 25% of the season. From 50% to the end of the season, a constant significant decrease ($p \leqslant 0.05$) was observed and a value of the antioxidant

Table 1
Lipid peroxidation inhibition capacity of the glycosylated and aglycone phenolic compounds from maple sap and syrup extracts during the season

Periods of the season (%)	Lipid peroxidation inhibition capacity[a] (%)	
	Sap	Syrup
Glycosylated compounds		
0	113.24 ± 2.66 b	107.89 ± 2.85c
25	122.46 ± 2.03d	123.14 ± 1.93d
50	116.86 ± 2.46bc	100.94 ± 5.31c
75	120.63 ± 2.87cd	70.25 ± 4.09b
100	103.69 ± 1.02a	16.89 ± 4.12a
Aglycone compounds		
0	67.48 ± 3.65b	99.35 ± 1.52b
25	82.73 ± 0.32c	95.41 ± 0.44b
50	96.78 ± 1.45d	127.44 ± 2.67c
75	40.13 ± 0.71a	164.51 ± 3.43d
100	84.67 ± 4.09c	89.75 ± 1.70ab

[a] Means within a column for each type of compound that have different letters are significantly different ($p \leqslant 0.05$). The reaction was calibrated using the ascorbic acid or α-tocopherol (control) whose the lipid peroxidation inhibition capacity was 100%. A lipid peroxidation inhibition capacity value superior to 100% means that maple extract was more antioxidant than control.

activity of 70.25% and 16.89% was respectively observed at 75% and 100% of the season. The results showed that the aglycone compounds showed weaker antioxidant activities. A significant increase ($p \leqslant 0.05$) of the lipid peroxidation inhibition capacity occurred from the beginning to the mid-season. For the aglycone compounds from the sap, a respective value of 67.48%, 82.73% and 96.78% was reached at 0%, 25% and 50% of the season. A significant decrease ($p \leqslant 0.05$) happened at 75% of the season giving a weak value of 40.13% to finally increase at 84.67% at the end of the season. The lipid peroxidation inhibition seems better for the aglycone compounds from the syrup than from the sap. Indeed, the value is similar and respectively of 99.35% and 95.41% at 0% and 25% of the season. The results were superior to 100% at 50% and 75% of the season (127.44% and 164.51%) and decreased significantly ($p \leqslant 0.05$) at the end of the season to a value of 89.75%.

The results of the free radical scavenging activity for the glycosylated and aglycone compounds extracted from maple sap and syrup are presented in Table 2. There is no significant difference ($p > 0.05$) for glycosylated extract present in maple sap at 0% and 50% of the season and the free radical scavenging capacity was, respectively, of 95.42% and 97.75%. At 75% of the season, a significant decrease ($p \leqslant 0.05$) occurred and a value of 69.50% was observed. At the end of the season, a significant increase ($p \leqslant 0.05$) allowed to reach a value of 87.78%. The antiradical properties of the glycosylated compounds from maple syrup extracts was superior to

Table 2
Free radical scavenging capacity of the glycosylated and aglycone phenolic compounds from maple sap and syrup extracts during the season

Periods of the season (%)	Free radical scavenging capacity[a] (%)	
	Sap	Syrup
Glycosylated compounds		
0	95.42 ± 3.59d	94.77 ± 0.4b
25	77.98 ± 1.97b	96.84 ± 0.5c
50	97.75 ± 0.82d	94.72 ± 0.3b
75	69.50 ± 1.13a	90.51 ± 0.61a
100	87.78 ± 1.71c	98.05 ± 2.81d
Aglycone compounds		
0	48.19 ± 1.58b	39.86 ± 1.54a
25	48.28 ± 6.60b	47.24 ± 0.39b
50	89.09 ± 7.58c	79.57 ± 2.94d
75	46.33 ± 0.55b	42.44 ± 3.13ab
100	33.21 ± 4.22a	61.32 ± 2.29c

[a] Means within a column for each type of compound that have different letters are significantly different ($p \leqslant 0.05$).

90% regardless of the period evaluated. At 0%, 25% and 50% of the season, the free radical scavenging acti- vity was 94.77%, 96.84% and 94.72%, respectively. The best antiradical capacity was reached at the end of the season with a value of 98.05%. The aglycone compounds from maple sap showed a weak antiradical activity (inferior to 50%) excepted at mid-season where a significant higher value ($p \leqslant 0.05$) of 89.09% was observed. The aglycone compounds present in maple syrup offered a significant increase ($p \leqslant 0.05$) until mid-season with a value of 39.86%, 47.24% and 79.57%, respectively. A significant decreased ($p \leqslant 0.05$) free radical scavenging capacity occurred at 75% of the season with a weak value of 42.44%. Finally, an increase at the end of the season showed a free radical scavenging capacity of 61.32%.

The results of antioxidant activity of the total phenolic compounds present in the extracts showed some differences. In general, the literature reports that there is a relation between the content of phenolic compounds and the antioxidant property. However, these results showed that other factors should be considered. The nature and the structure of the compounds is indeed very important (Heim et al., 2002). The antioxidant activity of each compound is also important. However a combination of different phenolic compounds structures was observed in maple extracts and some compounds are more active than others. Furthermore, as reported by Shahidi (2000), the efficacy of natural antioxidant contained in bulk oils, emulsions and composite food might be greater than that of individual phenolic compounds. The technological process to manufacture maple syrup can also influence the antioxidant activity since it influences the phenolic content (Kermasha et al., 1995). In addition to the phenolic compounds, Maillard reaction products (MRPs) were found in maple syrup (Akochi, Alli, Kermasha, Yaylayan, & Dumont, 1994). Since these compounds could have been extracted in the organic phase with the phenolic compounds they can add to the antioxidant capacity of the extracts. Indeed, it has been reported that some MRPs found in food processed by heat treatments showed antioxidant properties (Anese, Manzocco, Nicoli, & Lerici, 1999).

The antiradical activities varies also according to the nature of the compounds. Indeed, the glycosylated moety influence the antioxidant properties (Heim et al., 2002). In general, the glycosylated compounds have a weaker antiradical activity than their aglycone equivalent (Rice-Evans, Miller, & Paganga, 1996). However, the present study showed that the glycoside compounds of the maple sap and syrup phenolic extracts had a better antiradical activity than the aglycone compounds. The spatial arrangement of substituents is a greater determinant of antiradical activity than the flavan backbone (Rice-Evans et al., 1996). Consistent with most polyphenolic antioxidants, both the configuration and total number of hydroxyl groups substantially influence several mechanisms of antiradical activity (Burda & Oleszek, 2001; Cao, Sofic, & Prior, 1997). Free radical scavenging capacity is primarily attributed to the high reactivities of hydroxyl substituents. The B-ring hydroxyl configuration is the most significant determinant of scavenging of reactive oxygen species (Burda & Oleszek, 2001). Hydroxyl groups on the B-ring donate hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals, stabilizing them and giving rise to a relatively stable flavonoid radical. Among structurally homologous flavones and flavanones, peroxyl and hydroxyl scavenging increases linearly and curvilinearly, respectively, according to the total number of OH groups (Cao et al., 1997). The differences in antiradical activity between polyhydroxylated and polymethoxylated flavonoids are most likely due to differences in both hydrophobicity and molecular planarity (Heim et al., 2002).

3.2. Antimutagenicity properties

3.2.1. Total phenolic extracts

Results of the antimutagen properties per se of total phenolic compounds extracted from maple sap and syrup are shown in Table 3. The β-galactosidase units, represent the specific enzymatic activity of the β-galactosidase produced by *Salmonella typhimurium* TA1535/pSK1002 due to induction of the SOS response in presence of mutagen agents (McDaniels, Reyes, Wymer, Rankin, & Stelma, 1990). The SOS-inducing potency of test compounds is estimated by the measurement of the induction of the level of umu operon in terms of intracellular β-galactosidase activity (Ong, Stewart, Wen, & Whong, 1987). The percentage of antimutagenicity is calculated to illustrate the inhibition of the SOS response. The results of the β-galactosidase activity at

Table 3
Antimutagenicity per se of total phenol extracts from maple sap and syrup during the season

Samples[a] (%)	β-Galactosidase units[c]		% Antimutagenicity[d]	
	Sap	Syrup	Sap	Syrup
Control[b]	442.31 ± 17.69c	402.31 ± 25.90a	N/A	N/A
0	426.79 ± 12.69bc	396.02 ± 9.51a	3.51	1.56
25	436.68 ± 16.98bc	405.31 ± 26.46a	1.27	−0.75
50	408.24 ± 6.79ab	488.63 ± 27.01b	7.70	−21.45
75	508.98 ± 26.28d	524.43 ± 14.11b	−15.07	−30.35
100	387.25 ± 8.24a	506.74 ± 11.13b	12.45	−25.96

[a] Samples are phenolic extracts collected during the season 0 being the beginning and 100% the end of the season.
[b] Control is the mixture containing only the mutagen agent and the cells.
[c] β-Galactosidase units represents the enzymatic specific activity. Means in the same column bearing the same letter are not significantly different ($P > 0.05$).
[d] Percentage of antimutagenicity is the percentage of inhibition of the SOS response.

0% and 25% of the season are, respectively, 426.79 and 436.68 units for the maple sap extracts and no significant difference ($p > 0.05$) was observed between the control and those samples. At mid-season, a significant decrease occurred ($p \leqslant 0.05$) giving a value of 408.24 units, followed by an increase at 75% of the season to reach 508.98 units. However, a significant decrease ($p \leqslant 0.05$) to a value of 387.35 units was observed at the end of the season. The values of β-galactosidase activity of phenolic extracts from maple syrup showed that at the beginning of the season (0% and 25%) the samples are not significantly different ($p > 0.05$) than the control with respective values of 396.02 and 405.31 units. At mid-season until the end of the season, the values of β-galcatosidase activity increased significantly ($p \leqslant 0.05$) to values of 488.63, 524.43 and 506.74 units at 50%, 75% and 100% of the season, respectively. The percen- tage of antimutagenicity calculated in maple sap was 3.5% at the beginning of the season. At 50% of the season, there is a slight but significant increase ($p \leqslant 0.05$) representing a value of 7.7%. Then at 75% of the season, there is a significant decrease ($p \leqslant 0.05$) of the antimutagenic activity and the obtained value is negative (−15.07%). Finally, there is a significant increase ($p \leqslant 0.05$) of the antimutagenic activity at the end of the season to reach 12.45% of antimutagenicity. The values of percentage of antimutagenicity showed that there is no antimutagenic activity per se for the maple syrup total phenol extracts.

The results for the antimutagenic properties of the metabolites of total phenolic extracts from maple sap and syrup are presented in Table 4. The data of β-galactosidase units obtained for the maple sap showed that only the values obtained for the samples at 0 and 100% of the season are significantly ($p \leqslant 0.05$) different from the control with respective values of 492.56 and 484.45 units compared to 571.16 units for the control. The values at 25%, 50% and 75% of the season were respectively of 569.77, 556.49 and 520.04 units. No significant difference was observed between these values when compared with the control. There is no significant difference ($p > 0.05$) among the values at 75% and 100% of the season. The β-galactosidase activity of the metabolites of maple syrup extracts confirms that there is no significant difference ($p > 0.05$) between samples at 0%, 25%, 50% and 75% of the season and the control. These values are respectively 625.22, 597.03, 614.11 and 652.99 units compared to 621.47 units for the control. A significant decrease ($p \leqslant 0.05$) is visible at 100% of the season where a value of 529.12 units is observed. The antimutagenic activity of the metabolites of the maple sap extracts indicated that at the beginning of the season a value of 13.76% is observed. Then, a significant decrease ($p \leqslant 0.05$) at the quarter of the season occurred to a value of 0.24%. At 50% and 75% of the season the values obtained are respectively of 2.57% and 8.95%. A significant increase at the end of the season allowed reaching a maximum of 15.18%. The total phenolic extracts metabolites from maple syrup showed a weak percentage of antimutagenicity. The percentages of antimutagenicity of the metabolites of phenolic compounds extracted in syrup, were, respectively, of −0.6%, 3.93%, 1.18% and −5.07%, at 0–75% of the season. At the end of the season an improvement was observed to reach a value of 14.86%.

The antimutagenic activity of the metabolites of total phenolic extracts observed showed a low antimutagenic potential but the value obtained was higher than those obtained for the antimutagenicity per se of the same compounds. This means that the compounds are getting their antimutagenic activity after being metabolized in the liver in presence of S9 fraction. The phenolic compounds, especially the flavonoids, possess antioxidant properties, but they are not necessarily able to prevent all kind of mutations that can be induced by this mutagen agent. It has been reported that the phenolic compounds can serve as screens against UV radiation in plants (Escarpa & Gonzalez, 2001). Hence, they can be efficient in preventing mutations in plants but they might need to be metabolized to produce an antimutagenic activity in another organism.

Table 4
Antimutagenicity of total phenolic extracts metabolites from maple sap and syrup during the season

Samples[a] (%)	β-Galactosidase units[c]		% Antimutagenicity[d]	
	Sap	Syrup	Sap	Syrup
Control[b]	571.16 ± 22.93b	621.47 ± 39.70b	N/A	N/A
0	492.56 ± 12.00a	625.22 ± 35.98b	13.76	−0.60
25	569.77 ± 23.92b	597.03 ± 1.65b	0.24	3.93
50	556.49 ± 33.35b	614.11 ± 18.78b	2.57	1.18
75	520.04 ± 33.06ab	652.99 ± 42.52b	8.95	−5.07
100	484.45 ± 24.08a	529.12 ± 40.91a	15.18	14.86

[a] Samples are phenolic extracts collected during the season 0% being the beginning and 100% the end of the season.
[b] Control is the mixture containing only the mutagen agent and the cells.
[c] β-Galactosidase units represents the enzymatic specific activity. Means in the same column bearing the same letter are not significantly different ($P > 0.05$).
[d] Percentage of antimutagenicity is the percentage of inhibition of the SOS response.

Also, the mutagen agent used for the investigation of the antimutagenicity per se is the potassium dichromate, a strong oxidant. The mutations caused by the potassium dichromate can differ in nature from the damage inflicted by UV radiation and then, escape the protection or repair mechanism of bacteria. Furthermore, the phenolic compound extracts were complex mixtures, meaning that different phenomenons such as synergy or co inhibition can interfere with the antimutagenic activity. Kaur and Saini (2000) have associated antimutagenicity with antioxidant properties, due to the capacity of the compounds to inhibit the DNA damage caused by the presence of free radicals. In fact, inhibition of mutagenesis is generally not based on one specific mechanism. Compounds and complex mixtures with antimutagenic activity have different modes of action and act in parallel at different levels. As inhibitors, they may prevent the formation of mutagens. According to others (Krul et al., 2001), as blocking agents, they can prevent the biotransformation of premutagens into reactive metabolites by inhibiting metabolic activation or by scavenging reactive molecules. As suppressing agents they may modulate intracellular processes, which are involved in DNA repair mechanisms.

3.2.2. Glycosylated and aglycone compounds

The antimutagenic properties per se of glycosylated and aglycone phenolic compounds at the beginning and the end of the season are shown in Table 5. The percentages of antimutagenicity show that both phenolic extracts from sap and syrup are inferiors to zero and hence do not present any antimutagenic activity. The results obtained in sap showed a mean value of −15% at the beginning of the season and −13% at the end of the season for both extracts. In the syrup, a value of −56.65% and −31.25% was observed at the beginning of the season for the glycosylated and aglycone extracts, respectively. At the end of the season, the valued obtained for the same compounds were, respectively,

Table 5
Antimutagenicity per se of glycosylated and aglycones phenolic compounds extracted from maple sap and syrup at the beginning and the end of the season

Period of the season[a] (%)	% Antimutagenicity[b]			
	Glycosylated		Aglycones	
	Sap	Syrup	Sap	Syrup
0	−15.82	−56.65	−14.75	−31.25
100	−13.24	−40.46	−12.66	−13.63

[a] Samples are phenolic extracts collected at different periods of the season 0 being the beginning and 100% the end of the season.
[b] Percentage of antimutagenicity is the percentage of inhibition of the SOS response.

−40.46% and −13.63%. Those results could indicate a mutagenic potential, but according to Whong et al. (1986) to be considered a mutagenic positive result with the Umu test the β-galactosidase unit should be one time or more increased over the control, meaning in this case a percentage of −100% or more antimutagenicity.

The antimutagenic activity of the metabolites of glycosylated and aglycone phenolic extracts are illustrated in Table 6. The results showed that the antimutagenic properties of glycosylated compounds in sap were of 3.00% at the beginning of the season and of −18.16% at the end of the season. The values of percentages of

Table 6
Antimutagenicity of glycosylated and aglycone phenolic compounds metabolites extracted from maple sap at the beginning and the end of the season

Period of the season (%)[a]	% Antimutagenicity[b]			
	Glycosylated		Aglycones	
	Sap	Syrup	Sap	Syrup
0	3.00	14.05	−24.81	42.33
100	−18.16	11.81	ND[c]	31.39

[a] Samples are phenolic extracts collected at different periods of the season 0% being the beginning and 100% the end of the season.
[b] Percentage of antimutagenicity is the percentage of inhibition of the SOS response.
[c] Not determined.

antimutagenicity obtained for the glycosylated compounds present in syrup were 14.05% and 11.81% at 0% and 100% of the season respectively. The results for the aglycone compounds present in maple syrup showed the highest values. At the beginning of the season, a percentage of antimutagenicity of 42.33% was obtained. Then at the end of the season, the percentage was 31.39%.

The results obtained for the metabolites of aglycone present in maple syrup showed a higher antimutagenic activity than those from maple sap. These results could be explained by the creation of new chemical compounds produced during the processing of maple sap to syrup. For example, Maillard's reactions produced during the heating can produce new compounds with antimutagenic activity. The antimutagenic activity of glycosylated compound is weak and similar when present in both sap and syrup. These results can be explained by the fact that the glycosylated compounds are more stable and less active than the aglycones. As for the total phenolic extracts there is no antimutagenicity per se for the glycosylated and aglycone compounds from maple sap and syrup.

4. Conclusion

The results observed in this study showed that the phenolic compounds present in maple sap and syrup have antioxidant and antiradical activities. The present work indicated that a variation of the antioxidant activity of the phenolic compounds in maple sap and syrup is observed throughout the season. Also, the nature of the compounds, glycosylated and aglycone, also influenced the lipid peroxidation inhibition and the free radical scavenging capacities. The results obtained in this study have also illustrated the antimutagenic potential of phenolic compounds present in maple sap and syrup, except for the per se glycosylated and aglycone compounds. The aglycone compounds present in sap and syrup are efficient to prevent the mutations in cells. However, the efficienty level in both products is related to the season period.

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Current Research

Total Antioxidant Content of Alternatives to Refined Sugar

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ABSTRACT

Background Oxidative damage is implicated in the etiology of cancer, cardiovascular disease, and other degenerative disorders. Recent nutritional research has focused on the antioxidant potential of foods, while current dietary recommendations are to increase the intake of antioxidant-rich foods rather than supplement specific nutrients. Many alternatives to refined sugar are available, including raw cane sugar, plant saps/syrups (eg, maple syrup, agave nectar), molasses, honey, and fruit sugars (eg, date sugar). Unrefined sweeteners were hypothesized to contain higher levels of antioxidants, similar to the contrast between whole and refined grain products.

Objective To compare the total antioxidant content of natural sweeteners as alternatives to refined sugar.

Design The ferric-reducing ability of plasma (FRAP) assay was used to estimate total antioxidant capacity. Major brands of 12 types of sweeteners as well as refined white sugar and corn syrup were sampled from retail outlets in the United States.

Results Substantial differences in total antioxidant content of different sweeteners were found. Refined sugar, corn syrup, and agave nectar contained minimal antioxidant activity (<0.01 mmol FRAP/100 g); raw cane sugar had a higher FRAP (0.1 mmol/100 g). Dark and blackstrap molasses had the highest FRAP (4.6 to 4.9 mmol/100 g), while maple syrup, brown sugar, and honey showed intermediate antioxidant capacity (0.2 to 0.7 mmol FRAP/100 g). Based on an average intake of 130 g/day refined sugars and the antioxidant activity measured in typical diets, substituting alternative sweeteners could increase antioxidant intake an average of 2.6 mmol/day, similar to the amount found in a serving of berries or nuts.

Conclusion Many readily available alternatives to refined sugar offer the potential benefit of antioxidant activity.

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Oxidative damage has been implicated in the etiology of cancer, cardiovascular disease, and other degenerative disorders (1-3). Antioxidants are compounds with a reductive-oxidative potential and, therefore, have the ability to scavenge free radicals and other reactive oxygen species. Naturally occurring antioxidants in foods include vitamin E (tocopherols), vitamin C (ascorbic acid), flavonoids, lycopene, phenolic acids, and polyphenols, as well as some food additives (eg, butylated hydroxyanisole and butylated hydroxytoluene). Antioxidants prevent oxidative damage induced by free radicals and reactive oxygen species generated in vivo as byproducts of metabolism or inflammatory processes by suppressing their formation, acting as scavengers, or acting as their substrate.

The total antioxidant capacity (TAC) of diets has been correlated with increased concentration of specific antioxidants (eg, carotenoids, tocopherols, vitamin C) and foods (eg, coffee, wine, fruits) (4). Serafini and colleagues (5) observed an inverse relationship between dietary TAC and incidence of gastric cancer. Current dietary recommendations are to increase the intake of antioxidant-rich foods rather than supplement specific nutrients (6). While the interaction of specific antioxidants and other food nutrients as related to physiological effects remain to be completely determined, TAC is generally considered a valuable parameter for identifying potentially rich food sources of biologically active antioxidants that might have beneficial health effects. TAC is assayed by several methods, including ferric-reducing ability of plasma (FRAP) (7), oxygen radical absorbance capacity (ORAC) (8), Trolox-equivalent antioxidant capacity (9), 2,2′-azinobis (3-ethylbenzothiazoline 6-sulfonate) (10), and 2,2-diphenyl-1-picrylhydrazyl (11) radical scavenging assays.

The FRAP assay is a simple, fast, and inexpensive method for quantitative determination of the amounts of antioxidants in samples. The assay has little selectivity and measures most reductants above a certain reduction potential. The FRAP assay does not detect glutathione or protein thiols. This is an advantage over the ORAC and Trolox-equivalent antioxidant capacity assays because these thiols, which are present in high concentrations in animal and plant cells, are mainly degraded in the intestine and poorly absorbed. The original FRAP assay has a limited ability to measure fat-soluble antioxidants (7). Therefore, a modified FRAP assay was developed and reported previously (12) that also measures fat-soluble

antioxidants. On the basis of these and other considerations (13), the FRAP assay was chosen for assessing TAC.

Some recommended dietary changes involve adding or increasing the intake of antioxidant-rich foods, such as berries, dark chocolate, nuts, green tea, and red wine (14-17). Refined sugar and corn syrup are the predominate sweeteners in Western diets. The estimated annual intake of added sugars in the United States (predominately from refined cane and beet sugar, corn syrup, glucose, and dextrose) is 47.5 kg per capita (31 tsp or 130 g per person per day) (18), yet sugar and refined corn syrup are virtually devoid of vitamins, minerals, and phytochemicals. Substitution of whole grains for refined flours and baked goods is recommended because whole grains are richer in antioxidants and nutrients lost in the refining process (19,20). It might be similarly expected that unrefined sweeteners derived from plants would also be richer in antioxidants, but little data exist on the composition of these products. Current attention to reducing refined sugar intake largely translates into replacement by artificial sweeteners (sucralose, aspartame, etc) with the purpose of reducing energy and carbohydrate intake, whereas natural whole-food alternatives represent a way to increase antioxidant and nutrient consumption.

Many alternatives to refined sugar are available, though not widely used. These include plant saps/syrups (eg, maple syrup, agave nectar), syrups made from raw sugar and grains (eg, molasses, barley malt, and brown rice syrup), honey, and fruit sugars (eg, date sugar), as well as raw cane sugar. It was hypothesized that some of these alternatives contain higher levels of antioxidants compared to refined white sugar. Blomhoff and colleagues (12,21,22) recently published values for the TAC of foods using the FRAP method. Results of the analysis of approximately 200 fruits, vegetables, spices and herbs, cereals, supplements, juices and drinks sampled mainly from European countries have been reported (21,22), and a table of the FRAP content of 1,113 US food samples was published recently (12). In the present study, additional results are reported for sweeteners, along with estimates of the impact on total antioxidant intake they could make if used as alternatives to refined sugar.

METHODS

Samples

Samples (Table 1) were procured locally and also through the United States Department of Agriculture's (USDA) National Food and Nutrient Analysis Program (NFNAP) between 2002 and 2006 for the analysis of other nutrients (23). NFNAP is designed to update and improve the food composition data in the USDA's National Nutrient Database for Standard Reference (24). Data for selected artificial sweeteners (eg, aspartame and sucralose) were reported previously (12) and found to contain FRAP <0.05 mmol/100 g; these products were not further considered in this study because the focus was on natural alternatives to refined white sugar that might be utilized by consumers.

The sampling design for NFNAP has been described previously (25). Local samples were procured from major retail outlets and/or health food stores or online distributors and represented major brands available in the US marketplace. Because the purpose of this study was to screen antioxidant content, a full statistical sampling plan was not implemented for all foods, although multiple samples of most products were obtained (see Table 1).

Samples were handled according to standardized, thoroughly documented procedures (26). When composites were prepared, each sample unit was mixed, if necessary, and a representative subsample of no less than 1 cup (240 mL) of liquids and 4 oz (113 g) solids was taken, then combined and stirred thoroughly. Subsamples were dispensed among 30-mL glass jars with Teflon-lined lids (Qorpak, Bridgeville, PA), sealed under nitrogen, and stored at -60 ± 5°C in darkness before analysis. Samples were shipped on dry ice via express air delivery from Blacksburg, VA to Oslo, Norway, received in frozen condition, and stored at -80°C prior to analysis. The range of storage time in Oslo was from 0 to 25 weeks prior to analysis.

Reagents

TPTZ (2,4,6-tri-pyridyl-s-triazine) was obtained from Fluka (Sigma-Aldrich, Deisenhofen, Switzerland), sodium acetate trihydrate and $FeSO_4\times7\ H_2O$ from Riedel-deHaën (Sigma-Aldrich, Germany), acetic acid and hydrochloric acid from Merck (Merck, Darmstadt, Germany), $FeCl_3\times6\ H_2O$ from BDH Laboratory Supplies (Poole, Dorset, UK). MilliQ water (Millipore, Bedford, MA) was used to ensure proper water quality. Methanol of high-performance liquid chromatography–grade was obtained from Merck.

FRAP Analysis

The antioxidant assay of Benzie and Strain (7) was used with minor modifications that allowed quantitation of most water- and fat-soluble antioxidants, as described previously (12). A Technicon RA 1000 system (Technicon Instruments Corporation, Tarrytown, NY) was used for the measurement of absorption changes that appear when the Fe^{3+}-TPTZ2 complex is reduced to the Fe^{2+}-TPTZ2 form in the presence of antioxidants. An intense blue color with absorption maximum at 593 nm develops. Measurements were performed at 600 nm after 4 minutes incubation. An aqueous solution of 500 μmol/L $FeSO_4\times7\ H_2O$ was used for calibration of the instrument. Three analytical portions of each sample were extracted, each extract was analyzed in triplicate, and results are given as reduced TPTZ-Fe^{2+}-complexes in mmol/100 g.

Quality Control

Stability of samples during storage was established in a previous study (12), where it was determined that different homogenized foods could be stored at -80°C for 65 weeks with only negligible changes in antioxidant content. The assay was also fully validated as described in a previous report (12). The within-day repeatability measured as relative standard deviation ranged from 0.4% to 6%. The variation in the values for replicate items obtained from the same source was typically between 3% and 10% relative standard deviation.

Table 1. Antioxidant capacity [ferric-reducing ability of plasma (FRAP)] of sweeteners

Product	NDB number[a]	Brand[b]	Composite type[c]	No. of sample units per composite	FRAP mean mmol/100 g	Standard error[d]	Serving size[e]	FRAP mmol/ serving	Product mean FRAP mmol/100 g	Product mean FRAP mmol/ serving
Honey	19296	Sue Bee	N	10	0.165	0.019	30 mL (1 Tbsp=21 g)	0.035	0.156	0.033
		Sue Bee	N	10	0.139			0.029		
		Store brand	N	10	0.154	0.003		0.032		
		Store brand	N	10	0.159			0.033		
		Multiple brands[f]	N	10	0.135			0.028		
		Dutch Gold	L	1	0.138*			0.029		
		Golden Blossom	L	1	0.193*			0.041		
		FMV	L	1	0.161*			0.034		
Corn syrup, light	19350	Karo	N	12	0.008*		30 mL (1 Tbsp=20 g)	0.002	0.006	0.0012
		Clement Foods Co	P	3	0.005	0.001		0.001		
		Clement Foods Co	P	3						
		Clement Foods Co	P	3						
Molasses, blackstrap	NA	Slow as Molasses	L	1	4.394[z]	0.018	30 mL (1 Tbsp=20 g)	0.879	4.894	0.979
		Plantation	L	1	4.776[y]	0.064		0.955		
		Brer Rabbit	L	1	5.513[x]	0.048		1.103		
Molasses, dark	NA	Grandma's	L	1	4.251	0.090	30 mL (1 Tbsp=20 g)	0.850	4.562	0.912
		Golding Farms	L	1	4.533	0.142		0.907		
		Brer Rabbit	L	1	4.900			0.980		
Maple syrup, 100% pure	19353	Private Selection	L	1	0.412	0.026	30 mL (1 Tbsp=20 g)	0.082	0.412	0.082
		Cary's	L	1	0.371	0.014		0.074		
		Spring Tree	L	1	0.454*			0.091		
Agave nectar, light	NA	Madhava	L	1	0.032	0.024	30 mL (1 Tbsp=21 g)	0.007	0.019	0.004
		Madhava	L	1	0.005	0.003		0.001		
Agave nectar, amber	NA	Madhava	L	1	0.031	0.003	30 mL (1 Tbsp=21 g)	0.006	0.031	0.0065
Agave nectar, raw	NA	Madhava	L	1	0.010	0.002	30 mL (1 Tbsp=21 g)	0.002	0.010	0.0021
Blue agave nectar	NA	Molino Real	L	1	0.034[z]	0.003	30 mL (1 Tbsp=21 g)	0.007	0.089	0.019
		Live Superfoods	L	1	0.143[y]	0.010		0.030		
Brown rice syrup	NA	Lundberg Family Farms	L	1	0.394[z]	0.042	30 mL (1 Tbsp=20 g)	0.079	0.200	0.040
		NOW Foods	L	1	0.006[y]	0.003		0.001		
Brown rice syrup, powdered		Emperor's Kitchen	L	1	1.041*	0.295	1 oz (28.35 g)	0.295	1.041	0.295
Brown rice malt syrup	NA	Sweet Cloud	L	1	0.717*		30 mL (1 Tbsp=20 g)	0.143	0.717	0.143
Barley malt syrup	NA	Eden Organic	L	1	1.008	0.166	30 mL (1 Tbsp=20 g)	0.202	1.565	0.313
	NA	Sweet Cloud	L	1	2.121*			0.424		
Sugar, granulated white	19335	Domino, C&H	R	3	0.009	0.002	1 oz (28.35 g)	0.002	0.009	0.003
		Domino, C&H	R	3	0.017	0.009		0.005		
		Store Brand	R	1	0.004	0.001		0.001		
		Store Brand	R	2	0.009	0.002		0.003		
		Kroger	L	1	0.004*			0.001		
Sugar, light brown	19334[g]	Domino, C&H, Dixie Crystals	N	10	0.385	0.018	1 oz (28.35 g)	0.109	0.361	0.102
		Store brand	N	7	0.337	0.015		0.096		

(continued)

Table 1. Antioxidant capacity [ferric-reducing ability of plasma (FRAP)] of sweeteners (continued)

Product	NDB number[a]	Brand[b]	Composite type[c]	No. of sample units per composite	FRAP mean mmol/100 g	Standard error[d]	Serving size[e]	FRAP mmol/ serving	Product mean FRAP mmol/100 g	Product mean FRAP mmol/ serving
Sugar, dark brown	19334[g]	Store brand	N	6	0.689	0.039	1 oz (28.35 g)	0.195	0.689	0.195
Sugar, turbinado	NA	Sugar in the Raw	R	3	0.079	0.019	1 oz (28.35 g)	0.022	0.126	0.036
		Sugar in the Raw	L	1	0.210*			0.059		
		Hain Pure Foods	R	3	0.090	0.013		0.026		
Sugar, raw cane	NA	Florida Crystals	L	1	0.165	0.017	1 oz (28.35 g)	0.047	0.204	0.058
		Wholesome Sweeteners	L	1	0.120	0.004		0.034		
		Sweet Cloud	L	1	0.327*			0.093		
Date sugar	NA	Bob's Red Mill	L	1	4.586[z]	0.020	1 oz (28.35 g)	1.300	3.273	0.928
		Barry Farm	L	1	2.996[y]	0.053		0.849		
		NOW Foods	L	1	2.237[x]	0.034		0.634		

[a]Entry reference number from US Department of Agriculture (USDA) Nutrient Database for Standard Reference (24). NA=not applicable (food not in database).
[b]Supplier information: Barry Farm (Wapakoneta, OH), Bob's Red Mill (Bob's Red Mill Natural Foods, Milwaukie, OR), Brer Rabbit (B&G Foods, Inc, Roseland, NJ), C&H (C&H Sugar Company, Inc, Crockett, CA), Cary's (Specialty Brands of America, Inc, Westbury, NY), Clements Foods Co (Oklahoma City, OK), Dixie Crystals (Imperial Sugar Company, Sugar Land, TX), Domino (Domino Foods, Inc, Yonkers, NY), Dutch Gold (Dutch Gold Honey, Inc, Lancaster, PA), Eden Organic (Eden Foods, Inc, Clinton, MI), Emperor's Kitchen (Great Eastern Sun, Asheville, NC), Florida Crystals (Florida Crystals Food Corp, West Palm Beach, FL), FMV (Inter-American Products, Inc, Cincinnati, OH), Golden Blossom (John Paton, Inc, Doylestown, PA), Golding Farms (Golding Farms Foods, Inc, Winston-Salem, NC), Grandma's (Mott's, Inc, Stamford, CT), Hain Pure Foods (The Hain Celestial Group, Inc, Boulder, CO), Karo (ACH Food Companies, Inc, Memphis, TN), Kroger (The Kroger Co, Cincinnati, OH), Live Superfoods (Bend, OR), Lundberg Family Farms (Richvale, CA), Madhava (Madhava Honey, Lyons, CO), Molino Real (Dictor S.A. de C.V., Guadalajara, Jalisco, Mexico), NOW Foods (Bloomingdale, IL), Plantation (Allied Old English, Inc., Port Reading, NJ), Private Selection (Inter-American Products, Inc, Cincinnati, OH), Slow as Molasses (Honeytree, Inc, Onsted, MI), Spring Tree (Spring Tree Maple Products, Westbury, NY), Sue Bee (Sue Bee Honey, Sioux City, IA), Sugar in the Raw (Cumberland Packing Corp, Brooklyn, NY), Sweet Cloud (Great Eastern Sun, Asheville, NC), Wholesome Sweeteners (Wholesome Sweeteners, Inc, Sugar Land, TX).
[c]N=national composite of samples; R=regional composite of samples (25); L=sample(s) from a single outlet; P=commodity product provided by directly by producer.
[d]Standard error, based on values from analysis of replicate subsamples.
[e]Based on product label and/or US Department of Agriculture Nutrient Database for Standard Reference (24).
[f]Composite of seven brands (ie, Madhava Mountain, Deep South, Barkmans Busy Bee, Billy Bee, Stollers, Winnie the Pooh, and Beemaid).
[g]"Sugars, brown."
[xyz]Means with different superscript letter (x,y,z), within data for that product, were significantly different for n=3 analytical replicates (α=.05); samples assayed with n=1 are marked with *.

Serving Sizes and Sweetening Equivalents

Weight and serving size of a typically consumed portion of each food was determined from the USDA National Nutrient Database for Standard Reference (24) and/or the product label, generally based on the US Food and Drug Association Nutrition Labeling and Education Act guidelines (27), or actual measurement of average portion weights taken during sample preparation.

Statistical Methods

Means, standard deviations, and standard errors were calculated using Microsoft Excel 2000 (version 9.0, Microsoft Corp, Redmond, WA). Data were subjected to an analysis of variance and Tukey test for multiple comparisons, with $\alpha=0.05$, using SAS (version 8.2 [TS2M0], 2001, SAS Institute, Cary, NC).

RESULTS

Antioxidant Content of Sweeteners

Table 1 summarizes the FRAP content of the individual samples, and the means for each product type. Refined white sugar and corn syrup had FRAP <0.01 mmol/100 g, while raw cane sugar had 0.2 to 0.3 mmol/100 g. Brown sugar was notably higher in antioxidant content relative to refined white sugar, with dark brown averaging nearly twice that of light brown (0.69 vs 0.36 mmol/100 g). Molasses (blackstrap and dark) was richest in antioxidant capacity of all products (4.89 and 4.56 mmol/100 g, respectively), followed by malt syrups (brown rice and barley) with FRAP of about 1 to 1.5 mmol/100 g. Maple syrup had FRAP of 0.41 mmol/100 g), while the antioxidant content of honey was similar to that of raw cane sugar. All types of agave nectar had low FRAP, similar to refined white sugar and corn syrup. In cases where multiple brands of a given sweetener were sampled and analyzed in replicate, there were no large statistically significant differences in antioxidant content except among blackstrap molasses, date sugar, brown rice syrup, and blue agave nectar products (although all samples of the latter contained a relatively low level of antioxidant activity). These differences could be due to processing effects on antioxidant components or inherent variation in the composition of the plant source.

Most refined white sugar worldwide is produced from the sugar cane plant (*Saccharum officinarum*, *S spontaneum*, *S barberi*, *S sinense*, and hybrids thereof), in tropical and subtropical locations, with the remainder coming from sugar beet (28). Sucrose is concentrated in the stalk of the sugar cane, which is harvested for sugar production. The cane is crushed to extract a sucrose-rich juice, then clarified, boiled to a thick syrup, and crystallized to yield raw cane sugar (28), also known as Sucanat, demerara, turbinado, muscavado, or juggeri. Refined sugar results after additional steps are performed to remove color and nonsugar components; and molasses is a byproduct of this process (28). Commercially available "brown sugar" is refined sugar with varying amounts of molasses added (~3.5% and 6.5%, respectively, for light and dark sugars). The relative assayed antioxidant content of molasses, light and dark brown sugar, and raw cane sugar (Table 1 and Figure) are consistent with the high concentration of antioxidants in the molasses syrup vs refined sugar. The higher antioxidant content might be a result of residual components from the sugar cane plant or from byproducts produced during the cooking of the cane juice. Duarte-Almeida and colleagues (29) found a substantial concentration of antioxidant phenolic acids (eg, hydroxycinnamic acids and sinapic acid) and flavonoids (eg, tricin and apigenin) totaling ~160 mg/L.

Maple syrup is produced from the clear sap of the tree, which is boiled and concentrated to yield what is sold as maple syrup. Maple syrup contains approximately 67% solids, mostly sugars, but also minerals and some vitamins, including notable amounts of calcium, potassium, manganese, magnesium, phosphorous, iron, and thiamin (30). Maple syrup also contains phenolic compounds, which also have antioxidant activity (31,32).

Honey is a sugar-rich liquid produced by bees from the nectar of flowers that is partially digested and then regurgitated into the hive and stored in the honeycomb, where evaporation of water concentrates the sugars (33). Honey can be sold raw or refined, with the latter being the case for most commercial retail products in the United States. Honeys assayed in the present study (Table 1) represent major available retail brands in the United States and probably clover as the nectar source because this is the predominant source for honey produced in the United States (34). Data do not reflect the composition of the full range of honeys from different nectar sources or various levels of refining. Phenolic compounds have been widely reported in honey and would be expected to vary with nectar source and level of refining; for example, Baltruăitytë and colleagues (35) found a wide range of antioxidant capacity and phenolic components in honey from different sources, and Blasa and colleagues (36) found considerably greater antioxidant activity, phenolic, and flavonoid levels in raw *Millefiori* vs *Acacia* honey.

Somewhat surprising was the low antioxidant level in agave nectar, which is produced from the sap from hearts of the agave plant, a desert succulent. Agave nectar is the filtered juice expressed from the hearts (piñas) of the plant, which is then heated or enzymatically treated to hydrolyze the complex carbohydrates (mainly fructans) to sugars, then filtered and concentrated to a syrup (37). Although found to be low in antioxidants (similar to refined sugar, Table 1 and Figure), agave nectar is gaining popularity as a healthful alternative sweetener because of its low glycemic index (38,39). The taste and consistency of agave nectar are similar to corn syrup and, because it is unrefined, it might be expected to contain other beneficial non-antioxidant nutrients, trace elements, or phytochemicals.

Date sugar is made by grinding dried dates (the fruit of *Phoenix dactylifera sp.*), which contain 50% to 70% sugar, into a coarse powder (40). Because the product is the whole fruit it possesses the nutrient profile of dates, including considerable amounts of fiber, minerals, and vitamins [see (24), NDB numbers 09087 and 09421]. The antioxidant content of date sugars measured in the present study is consistent with the high FRAP analyzed in dates (0.565 to 0.718 mmol/100 g) (12). Use of date sugar might be limited to specific baking applications, however, because it contains 30% to 50% nonsugar com-

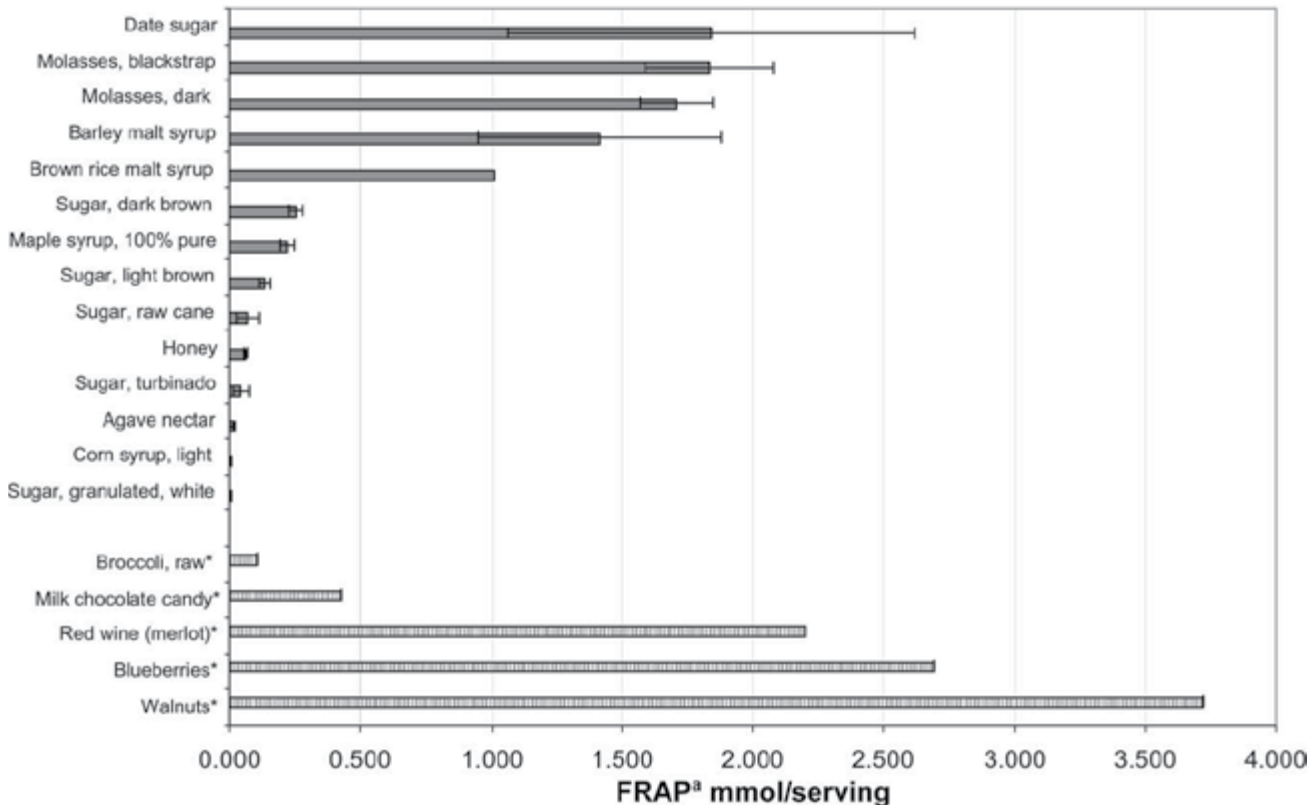

Figure. Estimated antioxidant contribution from sweetener used as a substitute for refined sugar in a standard cake recipe containing 1.5 cups (350 g) granulated sugar and yielding nine servings, compared to 1 serving of selected foods. *Data from Halvorsen and colleagues (12), serving sizes: walnuts, 1 oz. (28.35 g); blueberries, 1 cup (145 g); red wine (merlot), 3.5 oz (103 g); milk chocolate candy, 1 oz (28.35 g); broccoli, raw, 0.5 cup (44 g). Serving sizes for sweeteners: date sugar, dark brown sugar, light brown sugar, raw cane sugar, granulated white sugar, turbinado sugar: 1 oz (28.35 g); blackstrap molasses, dark molasses, barley malt syrup, brown rice malt syrup, maple syrup, agave nectar, corn syrup: 1 Tbsp (30 mL; 20 g); honey: 1 Tbsp (30 mL; 21 g). [a]FRAP=ferric-reducing ability of plasma.

ponents, which will not provide the physical properties needed in many applications, nor does it dissolve in liquids.

DISCUSSION

Potential Impact of Replacing Refined Sugar

Average intake of TAC, determined as FRAP, in a normal adult healthy population in Norway has been reported to be about 15 to 20 mmol (4), with the major contributors to dietary intake being coffee, fruits and berries, tea, wine, and cereals. In an Italian population (41), as measured by three different methods, coffee and tea beverages were the main contributors to TAC intake in women, followed by alcoholic beverages, fruits, and vegetables. In Italian men, the main contributors to TAC intake were alcoholic beverages, followed by coffee and tea, fruits, and vegetables. Similarly, coffee also has been reported to be a major dietary source of TAC in an American diet, followed by tea and other beverages (42).

Estimated average per capita consumption of added sugars in the United States is 130 g/day (18). Table 2 shows the calculated increase in antioxidant content that would result from direct substitution of alternative sweeteners (although not necessarily feasible in all products) for an amount equivalent to the sweetening power of 130 g refined sugar. On average, this amount was 2.6 mmol across all sweeteners (Table 2), ranging from 0.1 to 0.2 mmol (raw cane and turbinado sugars) to 10.7 mmol (blackstrap molasses). Relative to the mean total FRAP content of 17.3 mmol/day and 6.2 mmol/day (in diets including and excluding coffee, respectively), reported by Svilaas and colleagues (4), and of 2.8 mmol/day assayed in an "average American diet (43) (Phillips and colleagues, unpublished data), the contribution of 2.6 mmol/day represents a 15% to 92% increase, respectively. On an absolute basis, the potential increased antioxidant intake from these dietary modifications is similar to the contribution of tea (1.4 mmol/day) and fruit (1.8 mmol/day) in the diets studied by Svilaas and colleagues (4) and to the assayed FRAP content of blueberries (2.7 mmol/1 cup serving), red wine (2.2 mmol/serving) reported for individual foods (12).

Complete substitution of these sweeteners for refined sugar may not be realistic because of changes in product quality, but one possible application is simple replacement of refined sugar in recipes that are routinely prepared. For example, considering a cake prepared with 1.5

Table 2. Calculated increase in antioxidant content resulting from direct substitution of alternative sweeteners for refined white sugar

Product	Sweetening equivalent[ab] to 1 cup sugar (g)	FRAP mmol[c] equivalent to 130 g sugar	Increased mmol/day vs refined sugar
Refined sugar, white granulated	200	0.013	0.0
Raw cane sugar	200	0.186	0.2
Brown sugar, light	220	0.516	0.5
Brown sugar, dark	220	0.986	1.0
Turbinado sugar	200	0.110	0.1
Date sugar	220	4.681	4.7
Honey	339	0.352	0.3
Maple syrup, 100%	322	0.820	0.8
Molasses, dark	337	9.621	9.6
Molasses, blackstrap	337	10.721	10.7
Agave nectar[d]	336	0.085	0.1
Brown rice syrup	337	0.437	0.4
Barley malt syrup	337	2.207	2.2
Average of alternatives to refined white sugar			2.6

[a]Berthold-Bond and Atlas (52).
[b]From nutrition facts panel of product label, or US Department of Agriculture Nutrient Database for Standard Reference (24).
[c]FRAP=ferric-reducing ability of plasma; mean value based on results shown in Table 1.
[d]Mean of all types of agave nectar (Table 1).

cups (350 g) sugar to yield nine servings, the antioxidant content per serving would be increased by ~0.1, 0.25, 1.8, and 1.8 mmol if raw cane sugar, dark brown sugar, dark or blackstrap molasses, or date sugar, respectively, were used instead of refined sugar. These data suggest that the nutrient and antioxidant contribution of alternative sweeteners could be similar to that of whole vs refined flours (19) and foods high in antioxidants (eg, berries, chocolate, and nuts). For example, the FRAP (mmol/100 g) reported previously was 2.15 to 2.33 for blueberries, raspberries, and strawberries; 4.19 for unsweetened chocolate; and 9.67 to 13.12 for pecans and walnuts (12).

There are several examples that antioxidant-rich foods (eg, pomegranates, berries, and nuts) can dampen oxidative stress or reduce risk of developing oxidative stress-related diseases, such as cancer and cardiovascular diseases (44-46). It should be noted that, in general, various methods to estimate total antioxidant capacities/activities give same ranking of the items measured [see references (41,47,48)]. While the absolute values are most often not easily comparable between the methods and in various articles because the assays are performed differently (eg, different incubation time, concentrations), the main result is that the ranking of items is fairly consistent among assays. The dietary antioxidant activity based on measured TAC of foods needs to be correlated with physiological parameters related to oxidative stress in vivo, but the benefit of increasing antioxidant intake is generally recognized (49-51).

CONCLUSIONS

Data provided should be useful to researchers wishing to study the relationship between dietary antioxidants and physiological and/or health effects, as well as to registered dietitians making food-choice recommendations for increasing consumption of antioxidant-rich foods. Use of alternatives to refined sugar can add to the cumulative antioxidant content of the diet by replacing refined sugar. Development of recipes and consumer-friendly methods for replacing refined sugar in baking and cooking could increase antioxidant consumption similar to replacement of refined grains with whole grains.

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The chemical composition of 80 pure maple syrup samples produced in North America

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A total of 80 pure maple syrup samples received from primary producers in Canada and the United States were analyzed for their chemical composition, pH and °Brix. The major carbohydrates found in maple syrup (sucrose, glucose and fructose) were determined employing anion exchange high performance liquid chromatography (HPLC) with pulsed amperometric detection. The sucrose content was found to range from 51.7 to 75.6%; glucose and fructose contents ranged from 0.00 to 9.59% and 0.00 to 3.95%, respectively. The major organic acid present in maple syrup was malic acid. Trace amounts of citric, succinic and fumaric acid were also present. All organic acids were determined by ion exchange HPLC analysis with UV detection at 210 nm. Malic acid levels ranged from 0.1 to 0.7%. Citric, succinic and fumaric acids were found to be present at levels less than 0.06 ppm. Inductively coupled plasma atomic emission spectroscopy was employed for the analysis of potassium, magnesium and calcium, the main minerals found in maple syrup. Potassium was found to be present in the greatest concentration ranging from 1005 to 2990 mg l^{-1}. Magnesium and calcium ranged from 10 to 380 mg/l and 266 to 1702 mg l^{-1}, respectively. The Karl Fischer titration method was employed to determine maple syrup moisture content. The moisture content of maple syrup ranged from 26.5 to 39.4%. The pH and °Brix values for maple syrup ranged from 5.6 to 7.9, and 62.2 to 74.0°, respectively. Copyright © 1996 Canadian Institute of Food Science and Technology. Published by Elsevier Science Ltd

INTRODUCTION

Maple syrup is a product of high commercial value averaging $126 million wholesale per year in North America for 1983–1985 (Lockhart, 1990). This value can be attributed to the limited geographical region of production (it is produced only in North America on a commercial scale), its unique flavor and the high production costs (Stephen, 1981). The unique flavor of maple syrup has made it popular both in the confectionery industry and to consumers. In addition, emphasis on the consumption of natural foods has resulted in the use of maple syrup as an alternative sweetener (Anon, 1984).

Maple syrup is produced from the sap of the maple tree (*Acer Saccharum*). The sap is collected from the trees in early spring when temperatures fluctuate from freezing at night (−5 to −10°C) to thawing during the day (5 to 10°C). The sap itself is a clear water-like substance which tastes only slightly sweet (Willits & Hills, 1976) but which contains all the precursors required for the development of flavor and color which are characteristic of maple syrup (King & Morselli, 1983). Once collected, the sap is concentrated to a Brix value of ~66.5°. This is accomplished by water evaporation or by employing reverse osmosis followed by evaporation; the unique flavor characteristics of maple syrup are developed during this evaporation (~93–110°C for 1.5 h) process (Willits & Hills, 1976). According to trade information, maple syrup consists primarily of sucrose (~65.8%) with small amounts of glucose (0.7%), fructose (0.4%) and trace levels of oligosaccharides, organic acids, minerals, amino acids and vitamins (Morselli, 1975*a*; Anon, 1984).

The purpose of this research was to determine the chemical composition of maple syrup. In particular, the carbohydrate (sucrose, glucose and fructose), organic acid (malic, citric, succinic and fumaric), mineral

(potassium, magnesium and calcium) and moisture content of eighty pure maple syrup samples were determined. The pH and °Brix of these samples were also determined. The chemical composition of maple syrup is of importance because temptation exists to adulterate maple syrup via the addition of inexpensive sweeteners. It is also important for nutritional reasons (Morselli, 1975*b*). If the chemical composition of maple syrup is found to be significantly different from one geographical region to another, it may be possible to detect such adulteration and ensure product purity.

MATERIALS AND METHODS

Materials

In this study, 80 pure maple syrup samples collected during a 3-year production period were analyzed. The geographical regions from which samples were received are shown in Table 1. These seven geographical regions represent the major production areas in North America with the exception of New York. Samples for chemical analysis were prepared upon receipt and were either analyzed immediately or were stored at −20°C until analyzed.

Chemical analysis

Total solids were determined using a Canlab Atago Illuminator refractometer maintained at a temperature of 22°C. All samples were analyzed in duplicate and values reported as °Brix.

Sample moisture content was determined employing a Karl Fischer Automat titrator (Metrohm AG Herisau, Switzerland; Model 633) with a 645 Multi-Dosimat. High performance liquid chromatography (HPLC) grade methanol (Fisher Scientific, Edmonton, AB) was blanked with pyridine-free Karl Fischer reagent (BDH, Edmonton, AB, Comp 5 Aquastar) prior to analysis. The system was standardized by employing 25 μl of distilled, deionized water (Millipore, Milli-Q® Water System, Milford, MA) with the titrator set for a 20 s delay to ensure a stable end point. All samples were analyzed in triplicate at room temperature.

Sample pH was determined in duplicate at room temperature using a Fischer Accumet, Model 620, pH Meter (Fischer Scientific, Edmonton, AB). Calibration was accomplished employing pH 7.0 and 4.0 buffers.

Samples were prepared for carbohydrate analysis by making appropriate dilutions with distilled, deionized water. For sucrose analysis ~0.154 g of maple syrup was accurately weighed and diluted to 1 l. For glucose and fructose analysis ~0.875 g of sample was accurately weighed and brought to a final dilution of 250 ml. All sample solutions were passed through a 0.20 μm syringe filter (Corning Glass Works, Corning, NY) to remove particulates prior to HPLC analysis. Samples were analyzed on a Waters 625 metal free gradient HPLC (Milford, MA) equipped with a Waters 712 Wisp autosampler using a CarboPac PA1 column (250 × 4 mm) coupled with a CarboPac PA1 guard column (50 × 4 mm) (Dionex, Sunnyvale, CA). The mobile phase was 80 mM sodium hydroxide (NaOH; Fischer Scientific, Edmonton, AB) at a flow rate of 1 ml min^{-1} and the sample volume was 100 μl. Detection was achieved employing a Waters Model 464 PAD with dual gold electrode and triple pulsed amperometry at a sensitivity of 50 μA. The potentials and durations of the working electrode were maintained at: E_1 = 0.05 V, t_1 = 0.299 s; E_2 = 0.60 V, t_2 = 0.299 s; E_3 = −0.80 V, t_3 = 0.499 s. Carbohydrates were quantified by comparison to appropriate standards. The concentration of these standards ranged from 6.25 mg l^{-1} to 100 mg l^{-1}. Standards were prepared using D-glucose, D-fructose and sucrose (Aldrich Chemical Company Inc., Milwaukee, WI). Chromatograms were plotted employing Millennium 2010 Chromatography Manager software (Waters Chromatography, Milford, MA). Calibration curves were constructed by plotting peak area versus carbohydrate concentration of the standards. Calibration curves had correlation coefficients of 0.990 or better.

Sample organic acid (malic, citric, succinic and fumaric acid) content was determined by HPLC. Samples were prepared by simple dilution (~0.63 g in 25 ml distilled, deionized water) followed by passage through a 0.20 μm syringe filter to remove particulates. Analysis was accomplished employing a Waters 625 metal free gradient HPLC with a Waters 484 Tunable Absorbance detector set at 210 nm. An anion exchange column (Phenomenex, Torrance, CA; 300 × 7.8 mm; Rezex organic acid) in series with an anion exchange guard column (50 × 7.8 mm; Rezex Organic Guard) was employed for analysis. The mobile phase was 0.005 N H_2SO_4 at a flow rate of 0.5 ml min^{-1}, and the sample volume was 20 μl. Chromatograms were plotted employing a Waters 745B Data Module integrator. The organic acid concentrations were quantified by comparison to standards (62.5 mg l^{-1}, 125 mg l^{-1}, 250 mg l^{-1} and 500 mg l^{-1} for malic, succinic and citric

Table 1. Geographical regions from which samples were received

Country	Region	No. of samples received
Canada	Quebec	27
	Ontario	18
United States	Vermont	14
	Massachusetts	8
	Wisconsin	8
	New Hampshire	4
	Michigan	1

acid). Because fumaric acid had an increased detector response compared with the other organic acids, separate standards with concentrations of 0.625 mg l^{-1}, 1.25 mg l^{-1}, 2.5 mg l^{-1} and 5 mg l^{-1} were employed. Organic acid standards were prepared using DL-malic and fumaric acid (Sigma Chemical Co., St. Louis, MO), succinic acid (Fisher Scientific Co., Edmonton, AB) and citric acid (BDH, Edmonton, AB). Calibration curves were constructed by plotting peak height versus concentration of the standards. Calibration curves had correlation coefficients of 0.990 or better.

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was employed for analysis of magnesium, calcium and potassium in maple syrup (Thermo-Jarrell-Ash Model 16 spectrometer, Waltham, MA). The power employed for calcium, magnesium and potassium analysis was 1350 kW, 1350 kW and 950 kW, respectively. The crossflow nebulizer pressure was 30 psi and the plasma argon flowrates were 12.0 l min^{-1} for the torch gas flow and 1.0 ml min^{-1} for the auxiliary gas flow. Sample uptake rate was 2.6 ml min^{-1}. All samples were diluted 100-fold (~0.25 g in 25 ml HPLC grade water) and aspirated directly into the ICP-AES. The wavelengths at which measurements were taken were 317.9 nm for calcium, 279.1 nm for magnesium and 766.5 nm for potassium. Data was acquired as the average of two 5-s integrations employing a Jarrell Ash PC software system (Waltham, MA). Calcium, magnesium and potassium standards at a concentration of 10 mg l^{-1} were prepared from commercially purchased 1000 mg l^{-1} stock solutions (SCP Science, Montreal, PQ). Blanks (distilled, deionized water), standards and duplicates were run at a 10% frequency. The duplicates had an average percent difference of 0.9% for calcium, 2.6% for magnesium and 2.5% for potassium. Eight samples, randomly chosen, were spiked at a level of 5 mg l^{-1} and analyzed. The average percent recoveries were 102% for calcium, 96% for magnesium and 113% for potassium.

Statistical analysis was performed to study whether maple syrup composition varied significantly with geographical origin. This analysis was accomplished employing ANOVA with a multiple comparison procedure (Sheffe's) on the program StatView 4.01 (Abacus Concepts, Berkeley, CA). A significance level of 5% was employed to identify statistical differences.

RESULTS AND DISCUSSION

Results of all chemical analyses are shown in Table 2. A summary of these results is presented in Table 3. The major carbohydrate found in the 80 maple syrup samples analyzed in this study was sucrose. The concentration of sucrose in the maple syrup samples ranged from 51.7 to 75.6% and had a mean value of 68.0 ± 4.0%. The glucose and fructose concentrations ranged from 0.00 to 9.59% (0.01% limit of detection) and 0.00 to 3.95% (0.01% limit of detection), respectively, with mean concentrations of 0.43 ± 1.11% and 0.30 ± 0.54%, respectively. Mean values of 65.8% sucrose, 0.7% glucose and 0.4% fructose have been reported for samples collected in 1984 in a trade publication (Anon, 1984). In this study, the mean sucrose value obtained for maple syrup was higher, and the glucose and fructose values lower, than those reported in that trade publication. Differences in glucose, fructose and sucrose concentrations may be due to the age of the maple syrup samples analyzed in the 1984 study. Unpublished results from our study have shown that monosaccharide levels increase as storage time increases. Two samples (#18, #22) contained levels of glucose and fructose considerably higher than those found in the remaining 78 samples in this study and in the aforementioned trade publication. These levels cannot be explained by sucrose inversion during storage as these samples were analyzed within 1 month of sap collection and processing. The high levels of glucose and fructose observed in these samples may be due to processing, processing method and/or microbial load (Whalen & Morselli, 1985).

Sample °Brix values ranged from 62.2 to 74.0° and had a mean value of 67.0 ± 1.6°. While °Brix is not a measure of the actual carbohydrate content of a maple syrup sample, the values obtained did correlate quite closely to carbohydrate content. For example, maple syrup sample #2 had a combined carbohydrate (glucose, fructose and sucrose) content of 67.5% which agreed with the Brix value of 67.3°. This is because carbohydrate comprises approximately 99% of the total solids present in a maple syrup (Morselli, 1975*a*). By definition, °Brix is the grams of sucrose per 100 g of sample material, thus, a refractometer can be used to determine the carbohydrate content of the sap and/or finished syrup (Willits & Hills, 1976).

The US legal definition of maple syrup is "syrup that is made by the evaporation of maple sap or by the solution of maple concrete (maple sugar) and that contains not more than 35% water and weighs not less than 11 pounds to the gallon" (Willits & Hills, 1976). Under this definition, 68 samples in the study met this standard.

Sample moisture content had a mean value of 31.7 ± 2.9% and ranged from 26.5 to 39.4%. A value of 33% has been reported for maple syrup (Anon, 1984). The mean value reported for the 80 maple syrup samples in this study was significantly different from that previously reported, with 20% of the samples having a moisture content <29% and 1% having a moisture content >37%. Combining moisture with carbohydrate and °Brix values gave mean values of 100.5% and 98.7% with a range of 92.8–105.0% and 94.1–103.1%, respectively. As the major chemical components of maple syrup are carbohydrate and water the combination of these experimentally determined results should and did approximate 100%.

Table 2. Chemical data for maple syrup[1]

Sample	Origin	G (%)	F (%)	S (%)	°Brix	MC (%)	MA (%)	FA (%)	pH	Ca (mg/l)	Mg (mg/l)	K (mg/l)	CHO + MC (%)	°Brix + MC (%)
1	VT	0.42	0.32	65.1	66.6	31.5	0.38	0.010	6.78	1415	154	1681	97.4	98.1
2	MI	0.43	0.15	66.9	67.3	32.9	0.04	0.003	7.11	NA	NA	NA	100.5	100.2
3	WI	0.02	0.13	68.3	67.7	32.2	0.44	0.005	7.35	880	122	2404	100.7	99.9
4	MA	0.39	0.09	71.5	66.3	32.4	0.32	0.001	6.59	493	10	1820	104.4	98.7
5	MA	0.13	0.08	69.3	66.9	29.0	0.52	0.003	6.98	842	63	2323	98.4	95.9
6	MA	0.95	0.65	71.4	68.7	28.3	0.52	0.005	6.35	961	219	2314	101.3	97.0
7	NH	0.14	0.14	67.9	67.4	32.0	0.28	0.001	6.88	266	56	2261	100.2	99.4
8	NH	0.55	0.33	68.3	74.0	29.1	0.54	0.005	6.97	1161	211	2990	98.3	103.1
9	NH	0.13	0.09	67.7	66.9	28.8	0.48	0.002	6.79	884	105	2739	96.7	95.7
10	VT	0.38	0.25	74.9	68.3	27.0	0.06	0.003	6.36	1074	232	1968	102.5	95.3
11	VT	0.09	0.05	73.4	68.4	28.9	0.52	0.004	6.37	1148	264	2054	102.4	97.3
12	VT	0.22	0.15	72.6	66.7	29.6	0.42	0.003	6.79	793	133	1809	102.5	96.3
13	VT	0.46	0.31	65.6	65.2	30.5	0.42	0.002	7.04	1012	86	1675	96.8	95.7
14	VT	1.08	0.78	65.3	66.1	29.7	0.55	0.006	6.11	1168	204	1932	96.8	95.8
15	VT	0.27	0.22	66.9	66.7	32.4	0.62	0.003	6.39	856	204	1957	99.8	99.1
16	VT	0.77	0.52	69.3	66.2	29.7	0.52	0.004	6.54	838	228	2120	100.2	95.9
17	VT	0.13	0.12	64.3	66.8	33.3	0.36	0.001	6.47	992	96	1594	97.8	100.1
18	NH	2.88	2.23	60.7	62.2	36.2	0.24	0.002	5.91	440	95	1005	100.0	98.4
19	Que	0.41	0.32	68.0	65.9	29.8	0.36	0.004	5.66	482	269	1760	98.5	95.7
20	Que	0.44	0.34	68.6	66.5	29.3	0.40	0.005	6.37	527	177	2134	98.7	95.8
21	Que	0.08	0.04	70.3	66.1	28.9	0.42	0.004	6.83	581	186	1853	99.4	95.0
22	Que	9.59	3.95	51.7	65.6	32.6	0.56	0.008	6.14	806	367	1502	97.8	98.2
23	Que	0.36	0.28	65.3	65.6	33.8	0.33	0.003	6.42	598	164	1373	99.7	99.4
24	Que	0.76	0.54	64.0	65.6	33.2	0.56	0.004	6.85	488	280	2173	98.4	99.8
25	Que	0.02	0.14	69.2	66.0	32.8	0.35	0.005	6.90	446	178	1724	102.2	98.9
26	Que	0.11	0.11	65.7	65.1	32.8	0.63	0.005	6.57	738	380	1950	98.6	97.9
27	Que	0.30	0.26	71.0	69.0	28.3	0.50	0.003	6.19	490	281	1674	99.9	97.3
28	Que	0.59	0.16	73.6	68.7	28.3	0.52	0.003	6.95	724	195	2380	102.6	97.0
29	Que	0.33	0.24	69.9	69.5	28.0	0.58	0.005	6.37	712	236	2540	98.5	97.5
30	Que	1.25	1.08	61.7	67.6	28.8	0.46	0.007	6.37	1707	195	1425	92.8	96.4
31	VT	0.25	0.36	71.3	66.5	32.2	0.40	0.004	6.62	428	187	2113	104.2	98.7
32	VT	0.64	0.46	68.1	68.5	29.5	0.48	0.005	6.36	960	173	2717	98.7	98.0
33	MA	0.61	0.83	67.1	68.2	29.9	0.62	0.005	6.27	983	182	2577	98.4	98.1
34	MA	0.60	0.80	72.8	68.0	30.0	0.60	0.008	6.40	768	175	2463	104.2	98.0
35	MA	0.54	0.89	70.4	68.0	29.7	0.59	0.006	6.55	753	173	2434	101.5	97.7
36	MA	0.46	0.61	70.5	68.6	30.7	0.57	0.006	6.58	NA	NA	NA	102.3	99.3
37	MA	0.15	0.15	65.7	65.5	32.5	0.52	0.005	6.52	NA	NA	NA	98.9	98.0
38	VT	0.12	0.05	73.2	66.4	27.7	0.40	0.002	7.01	619	207	1591	101.0	94.1
39	VT	0.97	1.23	64.0	65.9	35.9	0.50	0.005	6.72	626	223	1987	102.1	101.8
40	VT	0.33	0.18	70.5	66.3	28.5	0.42	0.004	6.57	392	181	1960	99.5	94.8
41	WI	0.52	0.48	71.2	67.7	31.8	0.66	0.006	6.53	1380	188	2415	103.9	99.5

continued

Table 2. continued

42	WI	0.65	0.40	68.5	68.3	32.4	0.44	0.006	7.90	1005	68	2439	102.0	100.7
43	WI	0.18	0.14	70.7	67.7	32.0	0.44	0.002	6.82	609	103	2001	103.0	99.7
44	WI	0.14	0.10	71.1	67.5	32.5	0.36	0.002	6.75	720	55	2200	103.9	100.0
45	WI	0.14	0.09	69.1	65.8	33.0	0.38	0.002	6.33	625	116	1733	102.3	98.8
46	WI	0.34	0.29	70.8	68.7	28.1	0.47	0.002	6.20	NA	NA	NA	99.5	96.8
47	WI	0.19	0.13	69.8	66.5	31.4	0.36	0.004	6.91	457	109	2075	101.6	97.9
48	Ont	0.05	0.02	70.6	67.1	28.1	0.48	0.004	7.85	991	115	1446	98.8	95.2
49	Ont	0.14	0.06	67.2	66.7	28.3	0.43	0.007	7.50	793	160	1660	95.7	95.0
50	Ont	0.13	0.10	67.9	67.7	32.3	0.35	0.002	6.92	530	89	1854	100.5	100.0
51	Ont	0.22	0.16	67.1	66.5	33.2	0.62	0.006	6.89	1172	210	2373	100.7	99.7
52	Ont	0.06	0.05	68.5	68.8	31.3	0.30	0.002	7.13	465	54	1715	99.9	100.1
53	Ont	0.09	0.05	68.9	67.3	32.0	0.48	0.005	7.31	695	165	2304	101.0	99.3
54	Ont	0.08	0.05	71.4	67.6	27.7	0.45	0.005	7.04	614	181	1894	99.3	95.3
55	Ont	0.73	0.95	68.2	66.4	32.8	0.40	0.002	5.70	660	155	1596	102.7	99.2
56	Ont	0.30	0.17	67.6	67.5	31.9	0.47	0.005	6.86	783	157	1605	99.9	99.4
57	Ont	0.17	0.12	70.2	66.8	32.1	0.42	0.004	6.64	1010	138	1667	102.6	98.9
58	Ont	0.07	0.05	68.5	67.5	32.3	0.50	0.005	7.41	755	198	1992	100.9	99.8
59	Ont	0.27	0.20	65.3	65.9	33.2	0.66	0.003	6.61	1395	157	1964	99.0	99.1
60	Ont	0.14	0.10	66.2	66.2	33.1	0.33	0.004	6.80	805	61	2638	99.6	99.3
61	Ont	0.12	0.08	72.4	68.3	31.2	0.45	0.003	7.17	772	187	2046	103.8	99.5
62	Ont	0.14	0.10	75.6	69.1	28.4	0.32	0.002	6.72	556	108	1974	104.3	97.5
63	Ont	0.20	0.14	72.1	63.6	31.4	0.66	0.002	6.76	1336	101	2049	103.9	95.0
64	Ont	0.11	0.08	75.2	69.1	26.5	0.39	0.002	6.96	787	126	1414	101.9	95.6
65	Ont	0.24	0.19	73.5	68.0	27.3	0.45	0.002	6.46	1140	152	1306	101.3	95.3
66	Que	0.02	0.01	66.1	66.1	36.6	0.07	0.002	6.05	950	137	2384	102.8	102.7
67	Que	0.04	0.02	63.8	63.2	39.4	0.48	0.004	5.64	560	206	1728	103.2	102.6
68	Que	0.00	0.00	67.1	66.7	35.7	0.62	0.005	5.94	428	273	2158	102.8	102.4
69	Que	0.00	0.00	69.0	67.2	34.3	0.49	0.002	7.33	748	58	2508	103.3	101.5
70	Que	0.01	0.01	68.9	66.4	36.0	0.62	0.003	6.95	888	155	2112	105.0	102.4
71	Que	0.00	0.00	67.6	66.9	36.0	0.52	0.005	7.74	526	199	2236	103.6	102.9
72	Que	0.44	0.14	57.0	66.7	35.7	0.58	0.012	6.76	636	188	2295	93.3	102.4
73	Que	0.01	0.01	64.1	65.2	36.8	0.50	0.001	6.16	841	112	1924	100.9	102.0
74	Que	0.01	0.01	66.1	67.8	34.2	0.47	0.002	6.43	497	196	2049	100.2	102.0
75	Que	0.00	0.00	65.0	67.0	34.2	0.55	0.004	5.95	476	304	2306	99.1	101.2
76	Que	0.01	0.01	63.7	66.7	35.6	0.46	0.005	6.59	493	233	2090	99.3	102.3
77	Que	0.01	0.03	61.2	63.7	38.6	0.42	0.004	6.10	776	23	1878	99.9	102.3
78	Que	0.00	0.00	62.8	67.3	34.9	0.56	0.003	6.88	584	175	2317	97.6	102.2
79	Que	0.01	0.01	62.0	66.4	35.9	0.56	0.003	6.66	767	216	2207	97.9	102.3
80	Que	0.02	0.01	66.0	67.5	34.9	0.55	0.003	6.53	624	158	2412	100.9	102.4
X[a]		0.43	0.30	68.0	67.0	31.7	0.46	0.004	6.66	775	167	2026	100.5	98.74
SD[b]		1.11	0.54	3.99	1.56	2.89	0.13	0.002	0.46	279	72	375	2.47	2.44

[a]X: mean. [b]SD: standard deviation. NA: not available; VT: Vermont; MI: Michigan; WI: Wisconsin; MA: Massachusetts; NH: New Hampshire; Que: Quebec; Ont: Ontario; G: glucose; F: fructose; S: sucrose, MC: moisture content; MA: malic acid; FA: fumaric acid; Ca: calcium; Mg: magnesium; K: potassium; CHO: carbohydrate.

Sample pH ranged from 5.64 to 7.90 and had a mean value of 6.66. These results agree with those of Robinson *et al.* (1989) who reported the mean pH of 61 pure maple syrup samples to be 6.60. The variation in sample pH can be related to microbial contamination, the removal of organic acids with the niter, or their conversion to flavor compounds during evaporation of the sap (Robinson *et al.*, 1989; Willits & Hills, 1976).

Table 3. Mean and range of chemical composition data for 80 pure maple syrup samples

	Mean	Range
Sucrose (%)	68.0 ± 4.0[a]	51.7 75.6
Glucose (%)	0.43 ± 1.11	0.00–9.60
Fructose (%)	0.30 ± 0.54	0.00–4.00
°Brix	67.0 ± 1.6	62.2 74.0
Moisture content (%)	31.7 ± 2.9	26.5 39.4
pH	6.7 ± 0.5	5.6 7.9
Malic acid (%)	0.47 ± 0.11	0.06 0.66
Fumaric acid (%)	0.004 ± 0.002	0.001 0.012
Calcium (mg/l)	775 ± 279	266 1707
Magnesium(mg/l)	167 ± 72	10–380
Potassium (mg/l)	2026 ± 375	1005 2990

[a]Standard deviation.

The major organic acids present in maple syrup are malic, citric, succinic and fumaric. Of these four organic acids, only malic and fumaric acid were quantitated in this study. This is because the citric and succinic acid levels present in the maple syrup samples approached the detection limits of the HPLC system (< 62.5 mg l $^{-1}$) employed for analysis. Sample concentration of fumaric acid ranged from 0.001 to 0.012% and had a mean value of 0.004 ± 0.002%. The concentration of malic acid ranged from 0.06 to 0.66% with a mean concentration of 0.47 ± 0.11%. Morselli (1975*a*) reported the mean fumaric and malic acid concentrations of maple syrup to be 0.0040% and 0.0938%, respectively. The mean value obtained for malic acid in this study was considerably higher than that reported by Morselli (1975*a*). This may be due to the fact that only Vermont samples were analyzed in her study. Our results showed the Vermont samples to have a mean malic acid concentration of 0.43 ± 0.13% which is only slightly lower than the value reported for all 80 samples; however, only Vermont had a sample with a malic acid concentration as low as 0.06%.

Two maple syrup samples were subjected to scanning ICP AES to identify the major minerals present. Results from these experiments showed that calcium, magnesium and potassium were the major metals present in this natural product. Mineral analysis of the remaining

Table 4. Chemical composition of maple syrup according to geographical origin

	Ontario	Quebec	Vermont	Massachusetts	Wisconsin	New Hampshire
No. of samples	18	26	14	9	8	4
Glucose	0.18[a] ± 0.16[b] (0.05–0.73)[c]	0.52 ± 1.86 (0.00–9.59)	0.48 ± 0.38 (0.09–1.25)	0.50 ± 0.26 (0.13–0.95)	0.27 ± 0.22 (0.02–0.65)	0.93 ± 1.32 (0.13–2.88)
Fructose	0.15 ± 0.21 (0.02 0.95)	0.26 ± 0.77 (0.00 3.95)	0.40 ± 0.37 (0.05 1.23)	0.51 ± 0.33 (0.08–0.89)	0.22 ± 0.51 (0.09 0.48)	0.70 ± 1.03 (0.09 2.23)
Sucrose	69.8 ± 3.0 (65.3–75.6)	65.7 ± 4.5 (51.7–73.6)	68.4 ± 4.2 (61.7–74.9)	69.6 ± 2.3 (65.7–72.8)	69.9 ± 1.2 (68.3–71.2)	66.2 ± 3.7 (60.7–68.3)
CHO = MC	100.9 ± 2.1 (95.7–104.3)	100.2 ± 2.5 (93.3–105.0)	99.7 ± 3.1 (92.8–104.2)	100.9 ± 2.4 (98.4–104.4)	102.1 ± 1.5 (99.5–103.9)	98.8 ± 1.6 (96.7–100.2)
MC	30.7 ± 2.3 (26.5–33.2)	30.7 ± 3.2 (28.0–39.4)	30.4 ± 2.4 (27.3–35.9)	30.2 ± 1.5 (28.3–32.5)	31.6 ± 1.5 (28.1–33.0)	31.6 ± 3.4 (28.8–36.2)
°Brix	67.2 ± 1.3 (63.6–69.1)	66.5 ± 1.4 (63.2–69.5)	66.7 ± 0.9 (65.2–68.4)	67.6 ± 1.1 (65.5–68.7)	67.5 ± 0.9 (65.8–68.7)	67.6 ± 4.9 (62.2–74.0)
°Brix + MC	98.0 ± 2.1 (95.0–100.1)	100.2 ± 2.6 (95.0–102.9)	97.1 ± 2.2 (94.1–101.8)	97.8 ± 1.0 (95.9–99.3)	99.2 ± 1.3 (96.8–100.7)	99.2 ± 3.0 (95.7–103.4)
Malic acid	0.45 ± 0.11 (0.30–0.66)	0.51 ± 0.09 (0.33–0.65)	0.43 ± 0.13 (0.06–0.62)	0.53 ± 0.09 (0.32–0.62)	0.44 ± 0.10 (0.36–0.66)	0.39 ± 0.15 (0.24–0.54)
Fumaric acid	0.004 ± 0.002 (0.002 0.007)	0.004 ± 0.002 (0.001 0.012)	0.004 ± 0.002 (0.001 0.010)	0.005 ± 0.002 (0.001 0.008)	0.004 ± 0.002 (0.002 0.006)	0.003 ± 0.002 (0.001 0.005)
pH	6.96 ± 0.46 (5.70–7.85)	6.50 ± 0.49 (5.64–7.74)	6.58 ± 0.26 (6.11–7.04)	6.51 ± 0.21 (6.27–6.98)	6.85 ± 0.56 (6.20 7.90)	6.88 ± 0.09 (6.79–6.97)
Ca	848 ± 271 (465–1395)	630 ± 149 (428–950)	934 ± 366 (392 1707)	823 ± 173 (493 983)	811 ± 310 (457 1380)	688 ± 409 (266 1161)
Mg	140 ± 45 (54–210)	206 ± 82 (23–380)	185 ± 51 (86 264)	142 ± 75 (10 219)	109 ± 43 (55 188)	117 ± 66 (56–211)
K	1860 ± 351 (1306 2638)	2064 ± 309 (1373 2540)	1840 ± 211 (1425 2120)	2421 ± 295 (1820–2717)	2091 ± 385 (1446 2438)	2249 ± 882 (1005 2990)

[a]Mean. [b]Standard deviation. [c]Range. CHO: carbohydrate; MC: moisture content; Ca: calcium; Mg: magnesium; K: potassium.

pure maple syrup samples was completed by employing ICP-AES. Sample calcium levels were found to be present at a mean concentration of 775 ± 279 mg l^{-1} and ranged from 266 to 1707 mg l^{-1}. The mean magnesium and potassium concentrations were 167 ± 72 mg l^{-1} and 2026 ± 375 mg l^{-1}, respectively, and ranged from 10 to 380 mg l^{-1} for magnesium and 1005 to 2990 mg l^{-1} for potassium. Morselli (1975*a*) reported ranges of 266 to 1862 mg/l for calcium, 7.8 to 239 mg l^{-1} for magnesium and 865 to 2594 mg l^{-1} for potassium. The calcium range reported in this study was slightly tighter and the magnesium and potassium minimum and maximum levels were higher than those reported by Morselli (1975*a*).

As samples were obtained from each of the major maple syrup producing regions in North America, statistical correlation of chemical data and geographical origin was investigated. Chemical data based on geographical origin is presented in Table 4. Unfortunately, no statistical differences were found between geographical regions for carbohydrate, moisture, °Brix, organic acid or pH. However, statistical differences (at a significance level of 5%) were observed for the three minerals analyzed. Calcium content varied significantly between Quebec and Vermont, magnesium content varied significantly between Quebec and Wisconsin and potassium content varied significantly between both Massachusetts and Ontario and Massachusetts and Vermont, but not between Ontario and Vermont. These results suggest that the mineral content of maple syrup may be used to establish the origin of the syrup. This hypothesis is supported by literature. Robinson *et al.* (1989) found statistically significant differences in interprovincial levels for copper in both sap and syrup (27 sap and syrup samples analyzed from Nova Scotia, New Brunswick and Quebec). Robinson *et al.* also reported the site location to have a significant influence on sap composition as they found statistical differences in the amounts of copper, iron, lead and zinc found in sap collected from different sites.

CONCLUSIONS

The results of this study, which may be the first peer-reviewed study of its kind, represent chemical compositional data for 80 pure maple syrup samples obtained from six geographical locations in North America. The chemical results showed some natural variation. This variation may have been due to genetic and metabolic characteristics of the tree, environmental factors (King & Morselli, 1983), tree spacing (Willits & Hills, 1976), the time of sap collection (Laing & Howard, 1990), the time span between collection and processing, microbial load, processing method, the type of equipment employed and the type and the length of maple syrup storage (Whalen & Morselli, 1985). As determined herein, small variations do exist in all parameters analyzed. However, only the mineral content of maple syrup showed any significant differences due to geographical origin.

This lack of chemical difference in these samples may be useful in the detection of adulterated syrup via a matrix approach (i.e. where a number of chemical parameters are measured). Mineral analysis, specifically calcium, magnesium and potassium, may also be useful in determining the geographical origin of the syrup.

ACKNOWLEDGEMENTS

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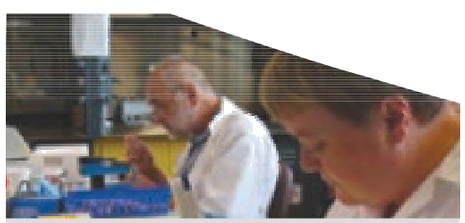
Maple sap and syrup are rich sources of ABA and polyphenols with potential benefits to health

Yves Desjardins, Ph. D., Agr.

IHC-2010, Lisbon, 25 August 2010



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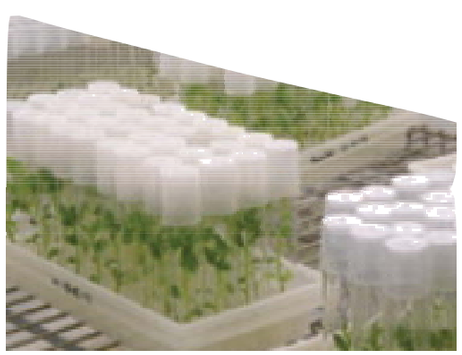
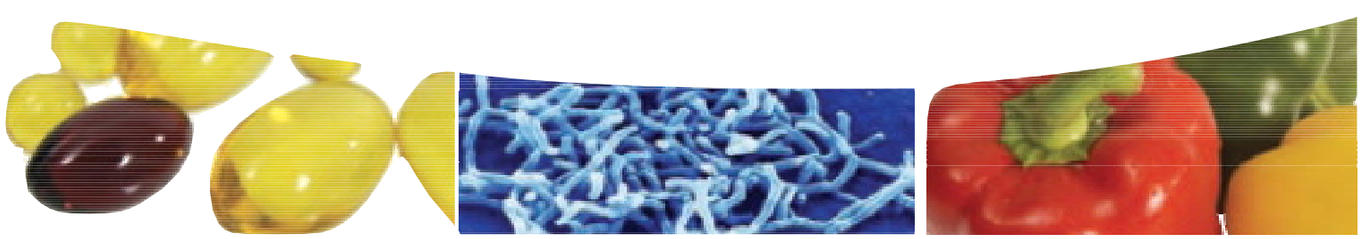





1


2


3


4

Maple sap composition

92-99% water

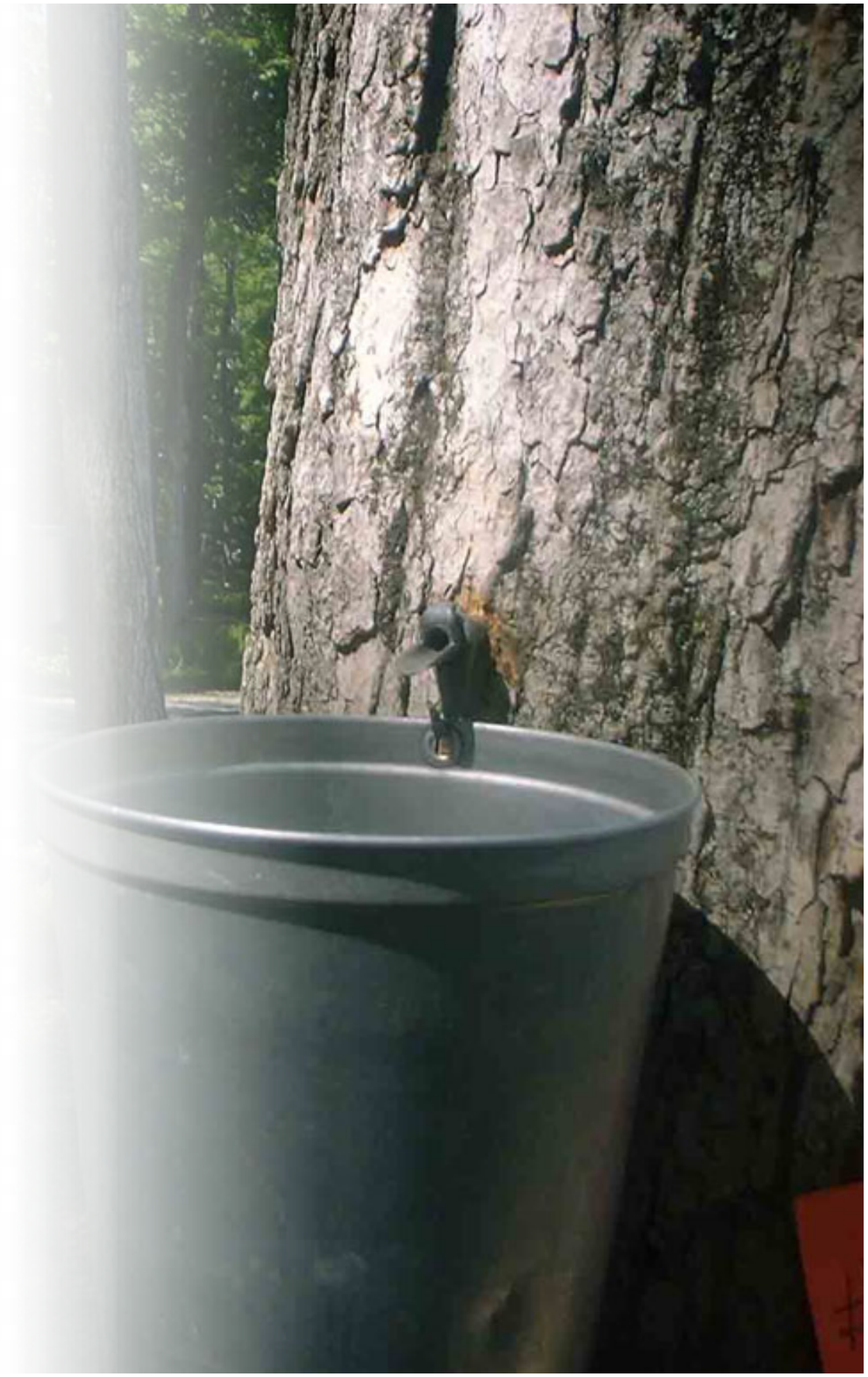
99% sucrose d.w.

0.15 % glucose d.w.

2 % oligosaccharides

Organic acid

Polyphenols



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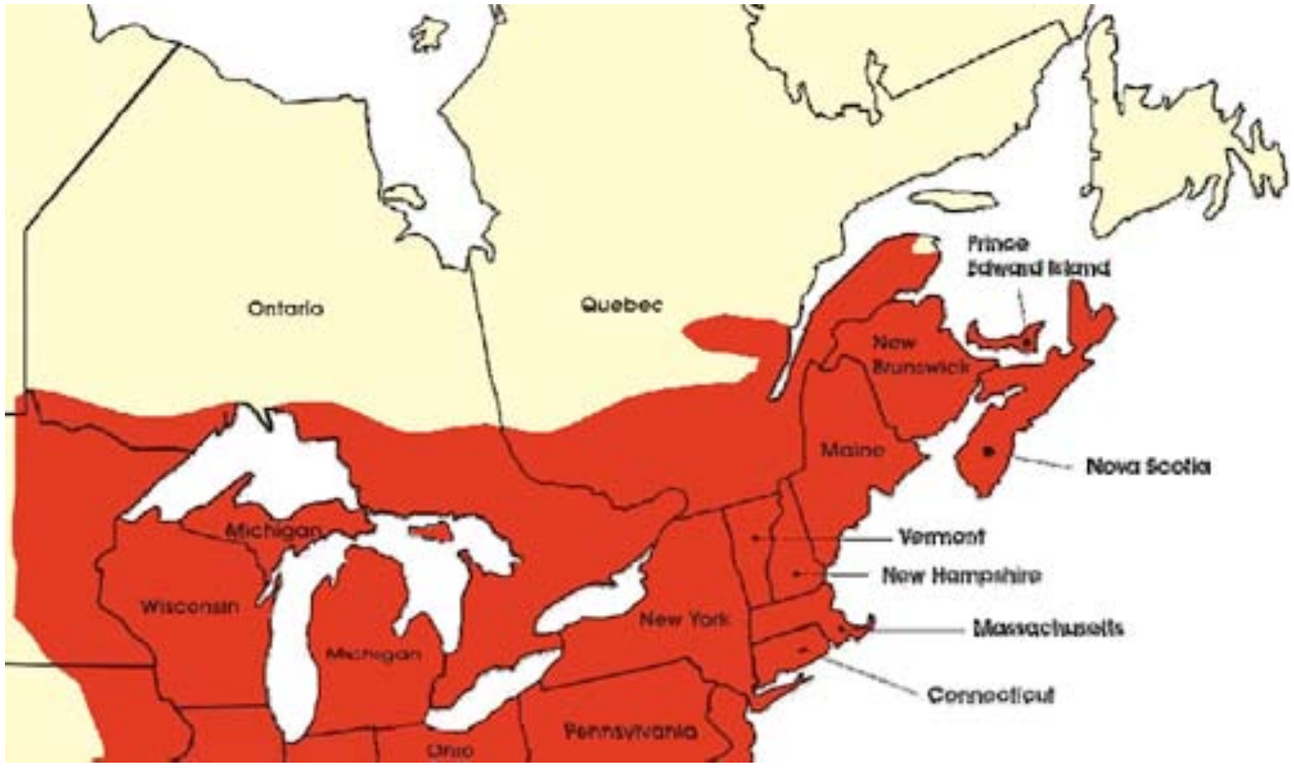
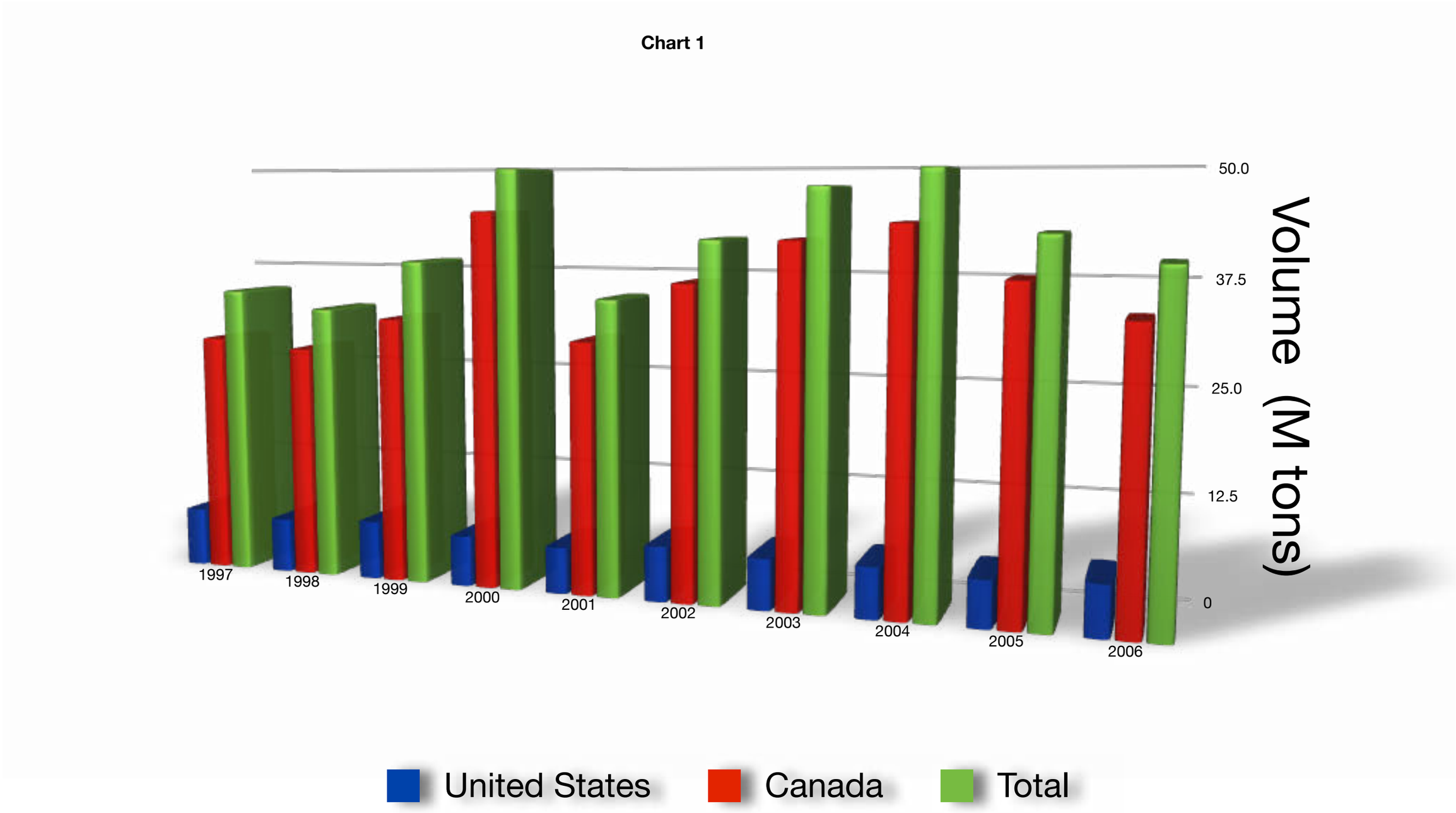
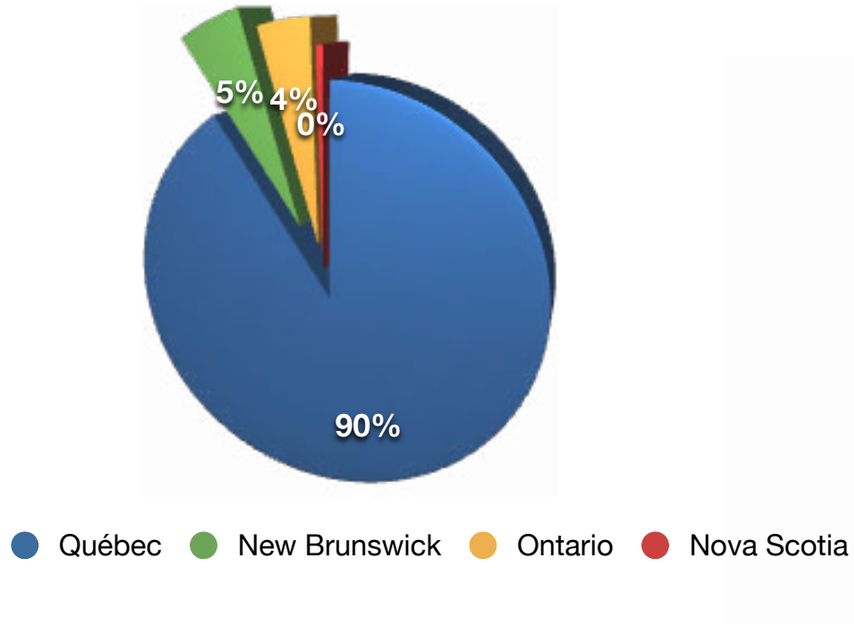


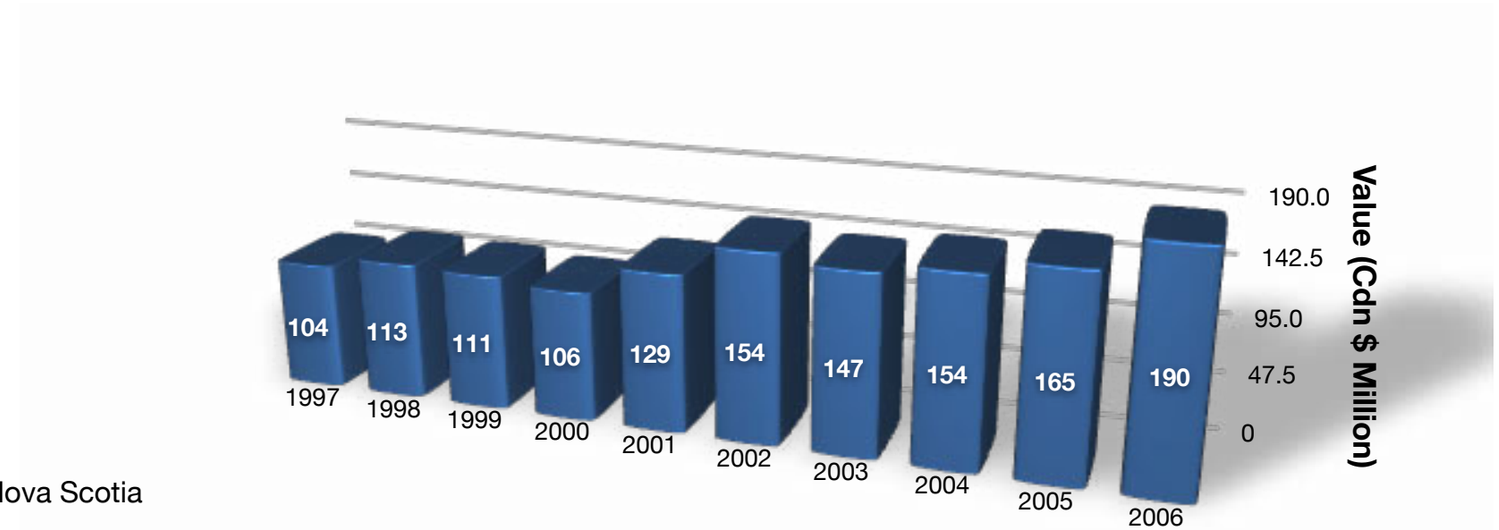
Acer saccharum
Acer saccharinum
Acer rubrum
Betula alleghaniensis

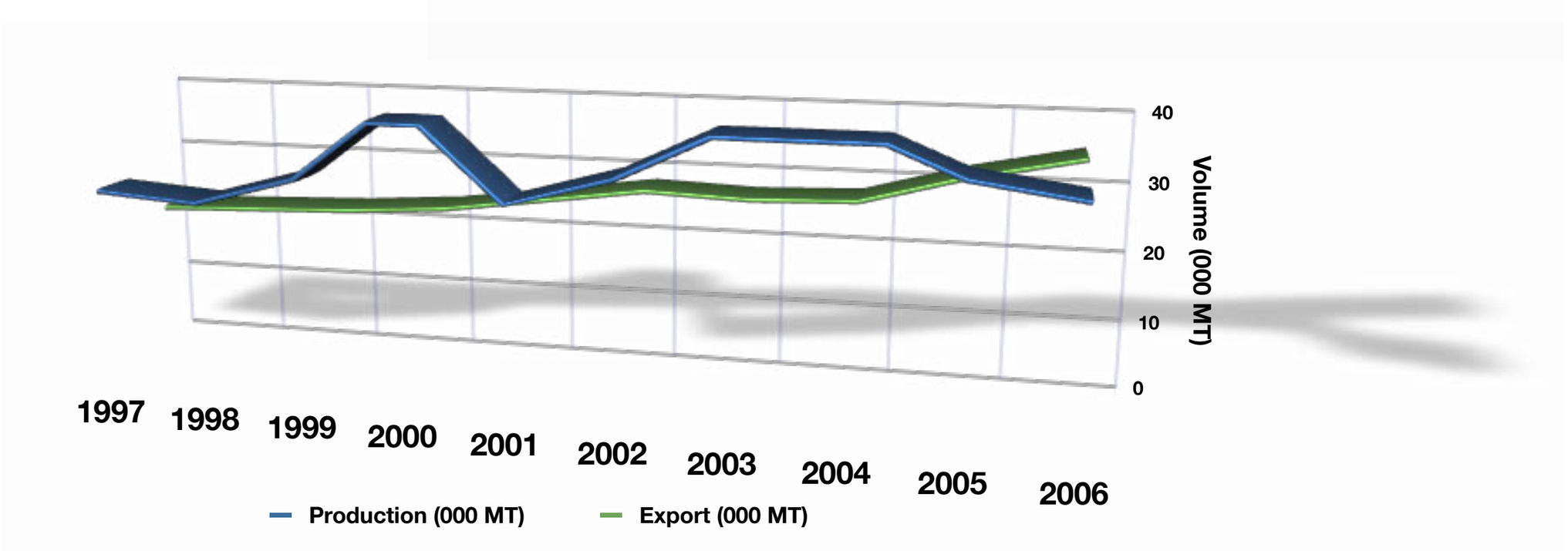
World Production of Maple Syrup



Production Statistics for Maple Products







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Antioxidant, antiradical and antimutagenic activities of phenolic compounds present in maple products

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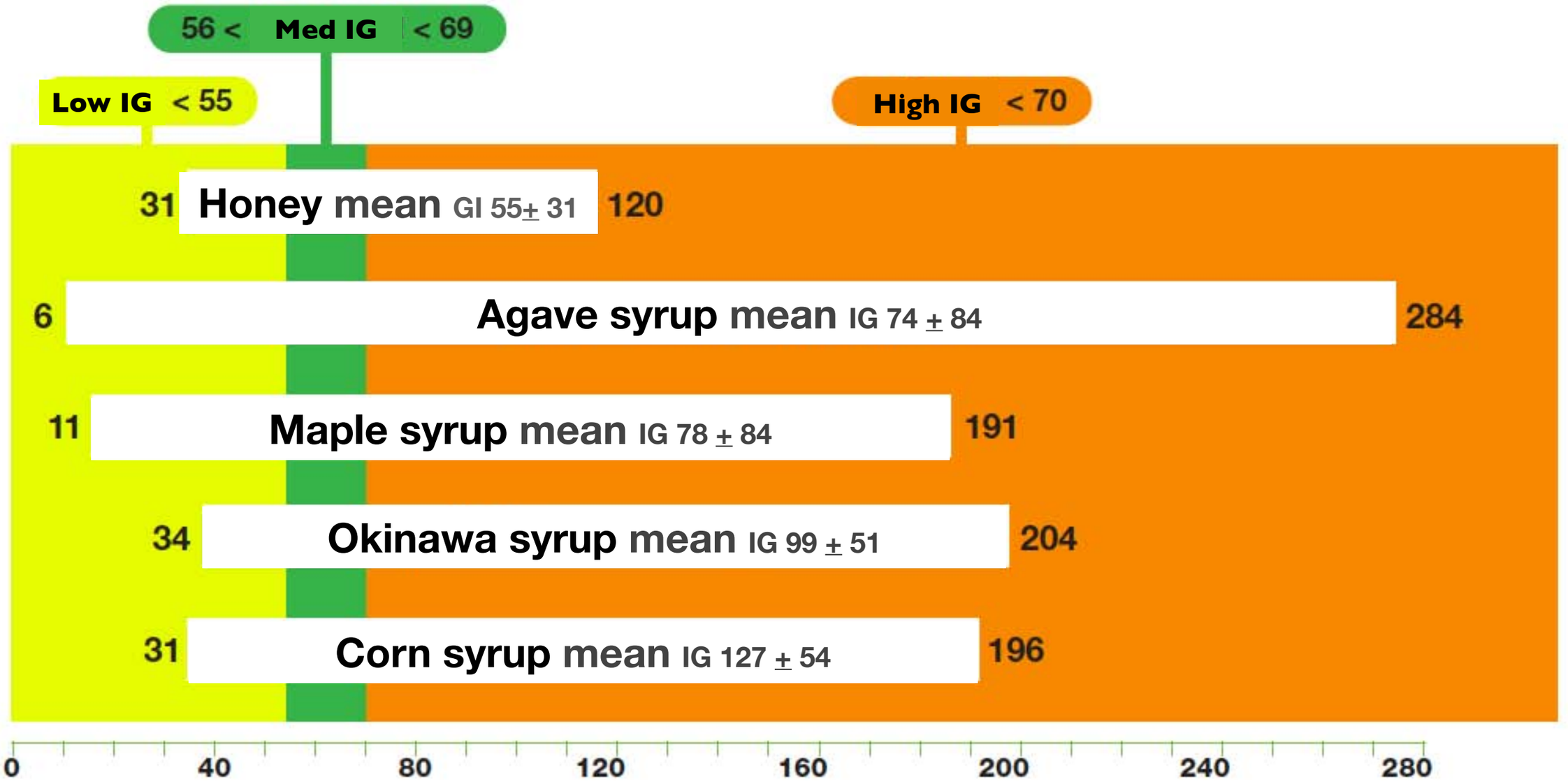
Glycemic index of Canadian Maple syrup



Glycemic index (GI)

Fédération des producteurs acéricoles du Québec

Mandate

- Characterize the physico-chemistry of maple products in order to determine their potential use as a functional food of nutraceutical with health added value

Variable measured during project

Total sugar

Antioxidant potential
(ORAC)

Total phenols

Types of phenols

Organic and amino acids

Acid invertase activity

Minerals

Riboflavin

Triacontanol

Polysaccharides
nature
Molecular mass

Peptides

Growth regulators

Material and Methods

Starting material: lyophilized

Maple Sap

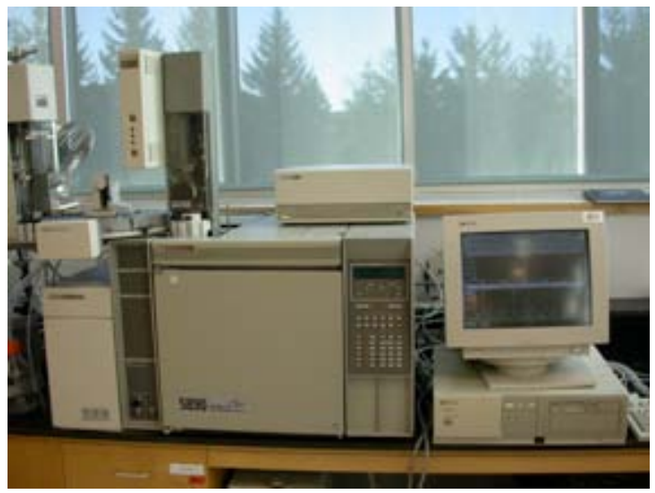
10 % conc.

66 % conc. syrup

Polyphenol analysis

Total phenols :
Folin & Ciocalteu

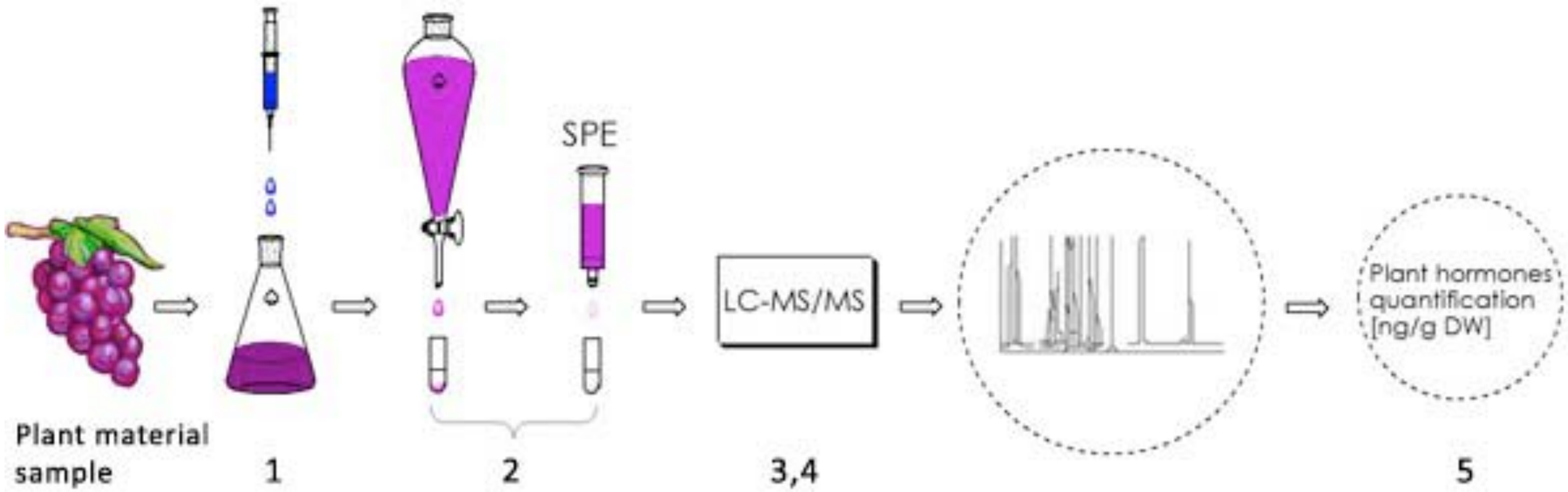
Liquid/liquid extraction with ethyl acetate to remove sugars



Phenols compomposition:
Extraction: Kermasha et al (1995)
Derivatization : Deslauriers (2000)
GC-MS:

GC Agilent 5890 - quadrupole MS 5972
SIM detection
Phenomonex ZB-5 column

ABA analysis



Labelled Isotope spiking

Extraction according to Chiwocha et al. Plant J.2003

Selectivity
Sensitivity
Multiple reaction monitoring

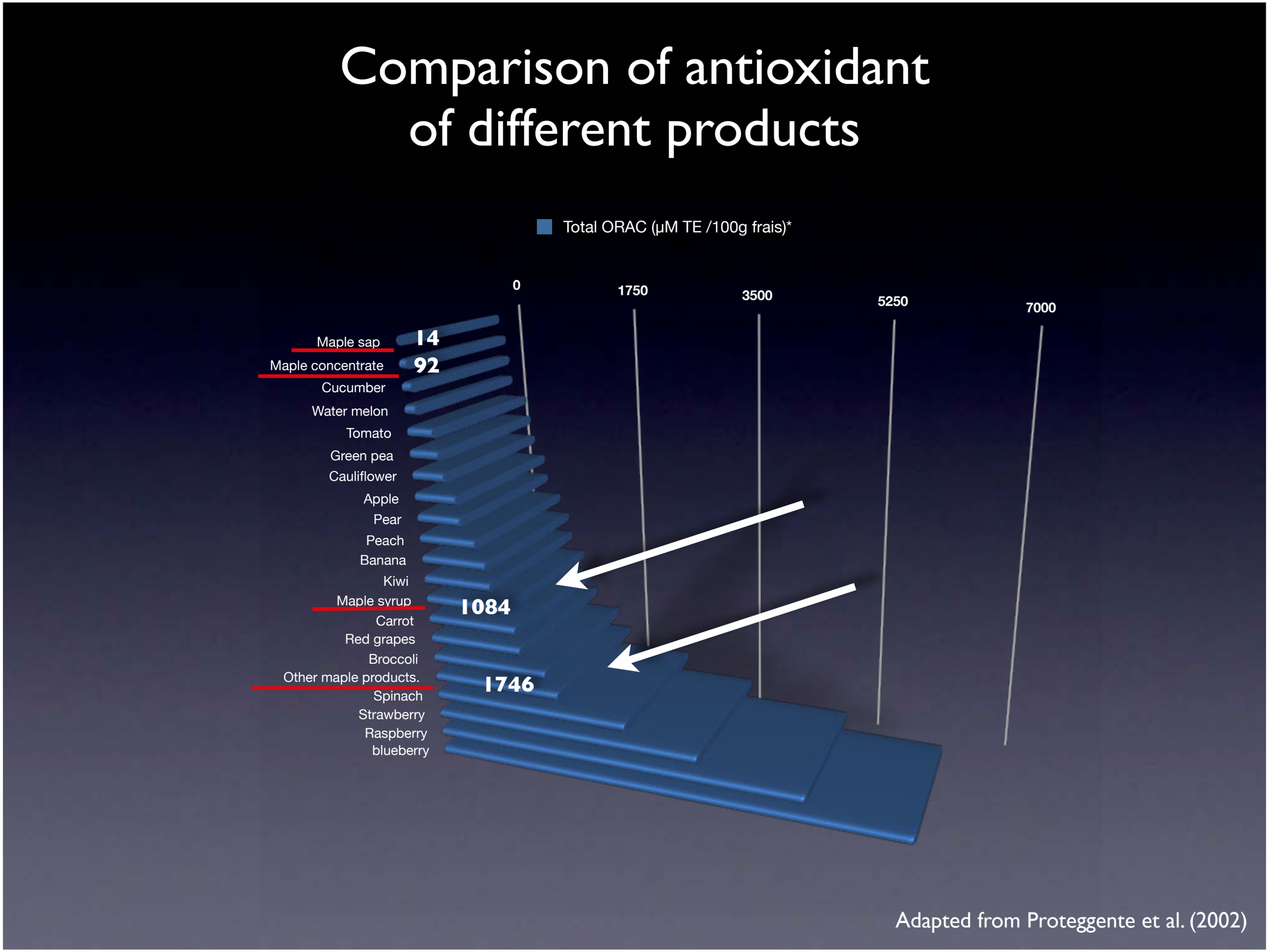
Sugar content of the lyophilized maple products

	% saccharides				
Season	Fruct.	Gluc.	Sacch.	Olig.	Poly.
Early	0	0	99	0	1
Mid	0	0	97	0	3
Late	4	4	87	2	3

ORAC activity

	Total ORAC (µM TE/100g fresh)	Total ORAC (µM TE/60 ml fresh)
Early		
Sap	15	
Concentrate	68	
Syrup	726	549
Mid		
Sap	20	
Concentrate	125	
Syrup	964	768
Late		
Sap	7	
Concentrate	76	
Syrup	805	635

Comparison of antioxidant of different products

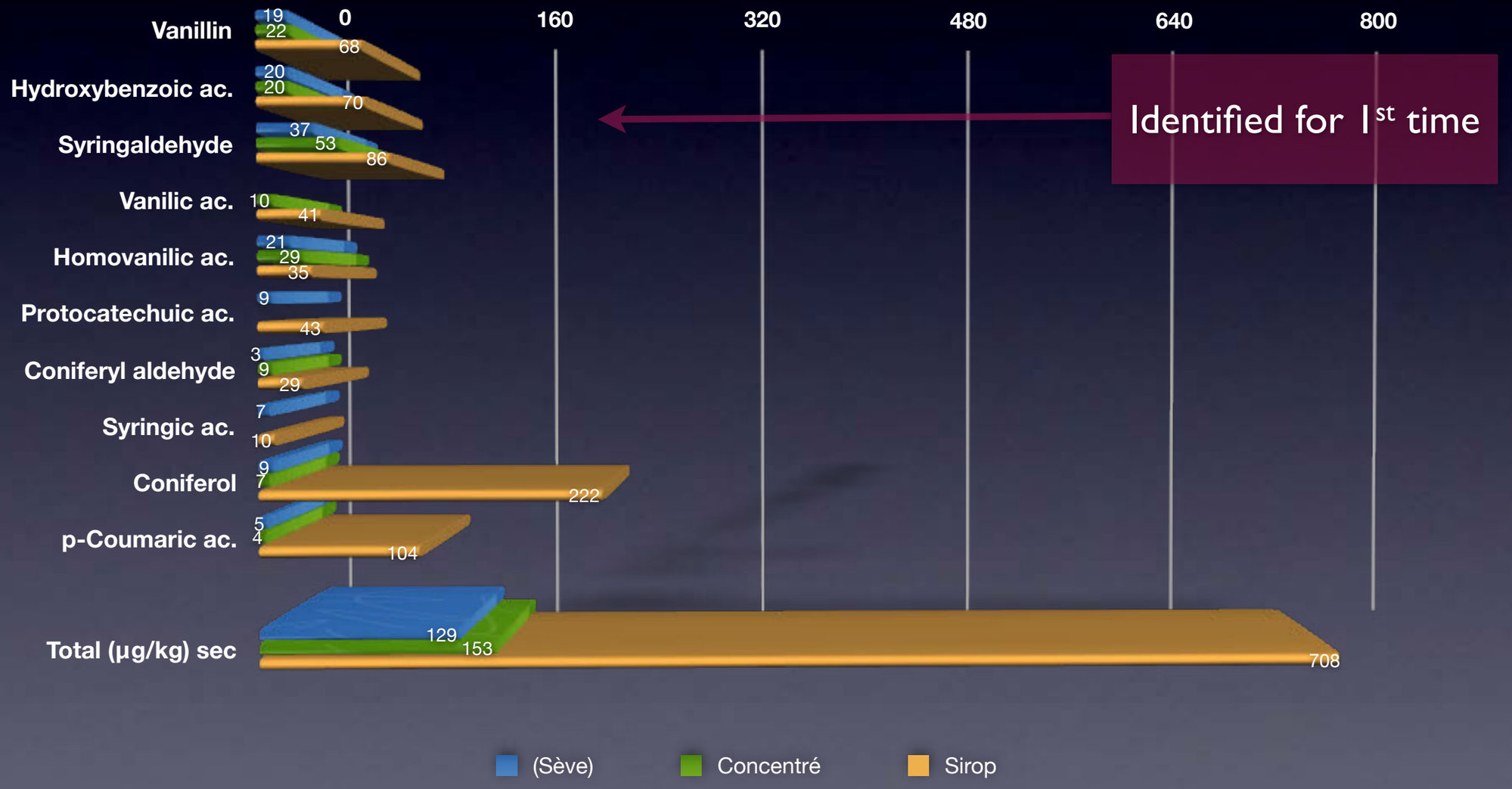


Adapted from Proteggente et al. (2002)

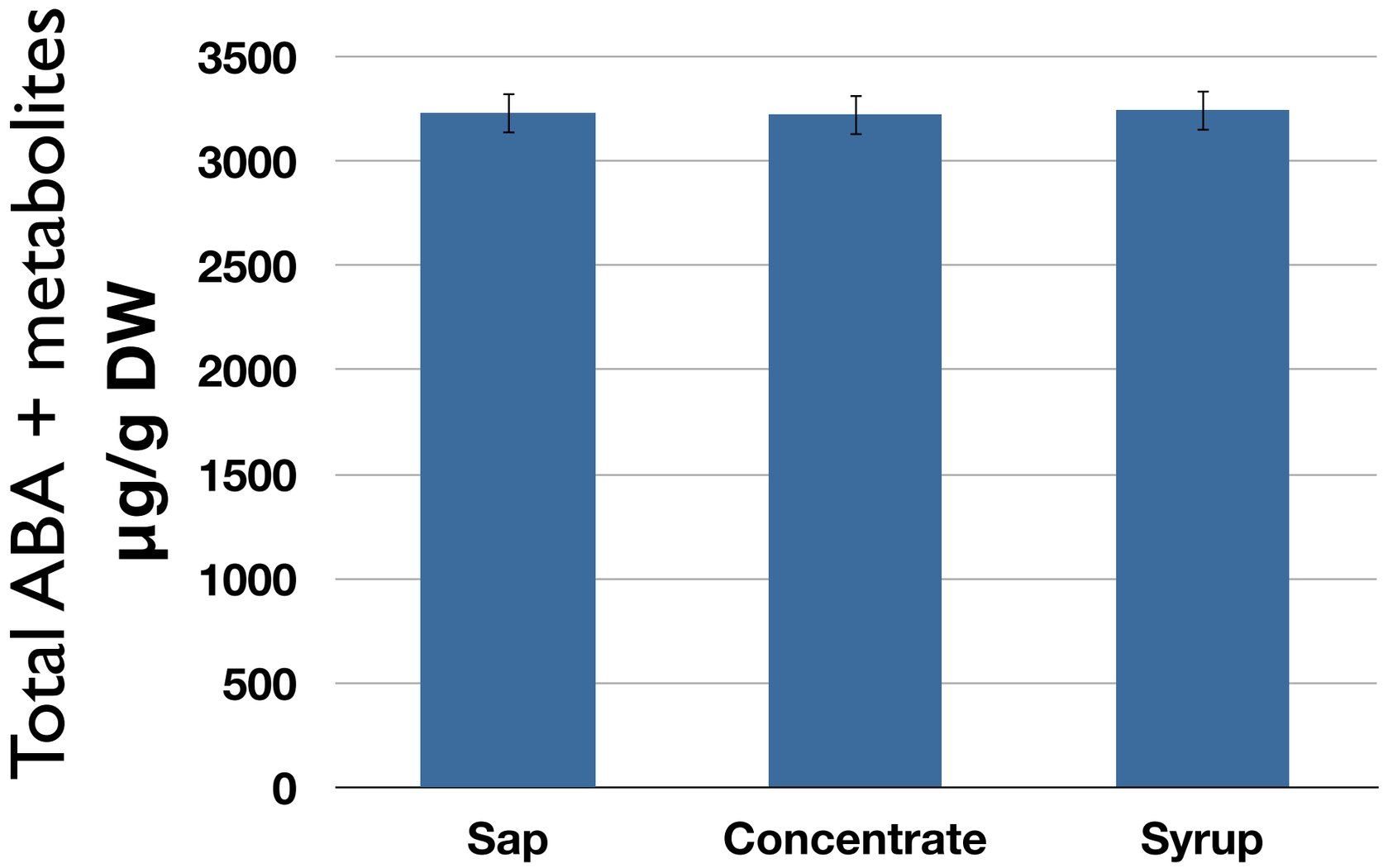
Total phenol concentration
(express in gallic acid eq.)

	Total Phenols (mg/g Dry Weigth)	Total Phenols (mg/g Dry Weigth)
Early		
Sap	0.18	0.24
Concentrate	0.15	0.96
Syrup	0.30	4.92
Mid		
Sap	0.15	0.19
Concentrate	0.14	0.92
Syrup	0.30	5.18
Late		
Sap	0.12	0.20
Concentrate	0.16	1.45
Syrup	0.26	5.14
Honey	0.08	
Sucrose	0.03	

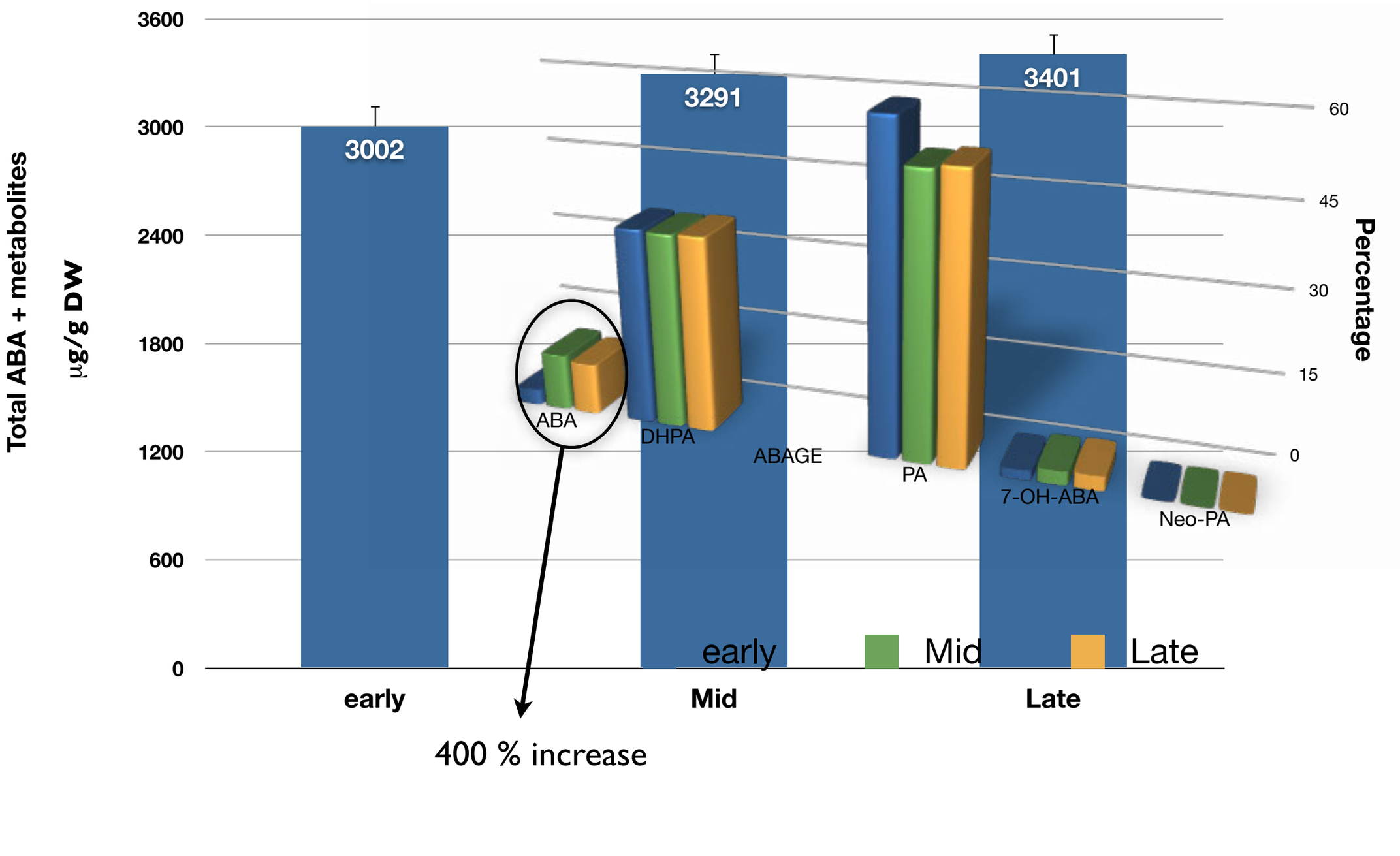
Concentration in phenolic compounds
(µg/kg dry weight)



Total ABA and metabolites concentration in different lyophilized maple products



Percentage change in ABA and metabolites over times



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Results: We found that ABA induced transactivation of PPARγ in 3T3-L1 pre-adipocytes in vitro. Dietary ABA-supplementation for 36 days decreased fasting blood glucose concentrations, ameliorated glucose tolerance, and increased mRNA expression of PPARγ and its responsive genes (i.e., adiponectin, aP2, and CD36) in WAT. We also found that adipocyte hypertrophy, tumor necrosis factor-α (TNF-α) expression, and macrophage infiltration in WAT were significantly attenuated in ABA-fed mice.

Conclusions: These findings suggest that ABA could be used as a nutritional intervention against type II diabetes and obesity-related inflammation.

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In maple products we measure 3000 ng/g DW ABA and its metabolites.

These levels correspond to 3 mg/kg DW.

If we consider that the dry weight matter of maple syrup is is about 20 %, one would find about 0.06 µg d'ABA/g in syrup.

The consumption of 80 g of syrup contains about 5 ppm of ABA and its metabolites to the body, a quantity close to the levels showing anti-diabetes effects in mice.

Conclusions/perspectives

- Maple syrup has an antioxidant activity comparable to that of carrots and kiwis
- The antioxidant activity correlates with the phenolic content
- Presence of ABA may be related to better control of glycemia despite the high sugar content of Maple syrup

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NOTE

Aromatic compounds and their antioxidant activity of *Acer saccharum*

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Abstract A new lignan glycoside, 5-(3″,4″-dimethoxyphenyl)-3-hydroxy-3-(4′-hydroxy-3′-methoxybenzyl)-4-hydroxymethyl-dihydrofuran-2-one 4′-*O*-α-L-rhamnopyranoside (**1**), with seven known compounds, compound 2, koaburside, icariside E_4, cleomiscosin C, cleomiscosin D, scopoletin, and 5′-demethylaquillochin, were isolated from the EtOH extract of the wood of *Acer saccharum* (Aceraceae). Their structures were determined by 1D and 2D nuclear magnetic resonance (NMR) and mass spectroscopy analysis. All of the isolated compounds, **1**–**8**, were tested for their antioxidant activity in superoxide dismutase (SOD)-like assay.

Keywords *Acer saccharum* · Lignan glycoside · Phenylpropanoid · Aromatic compound · Antioxidant activity · SOD · Aceraceae

Introduction

As a part of a research program aimed at the discovery of biologically active compounds from natural sources, we reported previously the isolation, structure elucidation, and the antimicrobial activity of phenylpropanoid, flavonol, and lignan from *Firmiana simplex* [1]. Here, we have paid attention to the chemical study for *Acer saccharum* (sato-ukaede in Japanese, Aceraceae), which is known for fallen arbor leaves belonging to Aceraceae as the tree for which their autochthonism is North America and to obtain maple syrup [2]. The previous chemical studies on this tree led to the isolations of scopoletin [3], urusane and oleanane type triterpenes, and steroids [4].

Results and discussion

The wood of *Acer saccharum* was exhaustively extracted with EtOH at room temperature for 1.5 months. The extract was separated by ordinary-phase silica gel and reverse-phase silica gel to furnish a novel lignan glycoside, compound 1 (**1**), along with seven known compounds, compound 2 (**2**) [5], koaburside (**3**) [6], icariside E_4 (**4**) [5], cleomiscosin C (**5**), cleomiscosin D (**6**) [7], scopoletin (**7**) [3], and 5′-demethylaquillochin (**8**) [8] (Fig. 1). This is the first report of the isolations of **2**–**6** and **8** from *A. saccharum*.

Compound **1** was obtained as a colorless oil, and showed a $[M+H]^+$ peak at *m*/*z* 549.2006 in the high-resolution (HR)-FAB-MS, which corresponded to the molecular formula $C_{27}H_{34}O_{12}$, including eleven unsaturations. The IR spectrum of **1** showed absorptions at 3400, 1760, and 1050 cm^{-1}. The ^{13}C-nuclear magnetic resonance (NMR) distortionless enhancement by polarization transfer (DEPT) and the ^{1}H-NMR spectra were similar to those of olivil [9], except for the presence of one carbonyl carbon (δ 178.8), one methoxy carbon (δ 55.6), and 6-deoxy-hexose moiety. On acid hydrolysis with 2.5% H_2SO_4, **1** liberated L-rhamnose identified by HPLC analysis using an optical rotation detector (see the following section) [10]. The gross structure of **1** was determined by analysis of the 2D NMR data,

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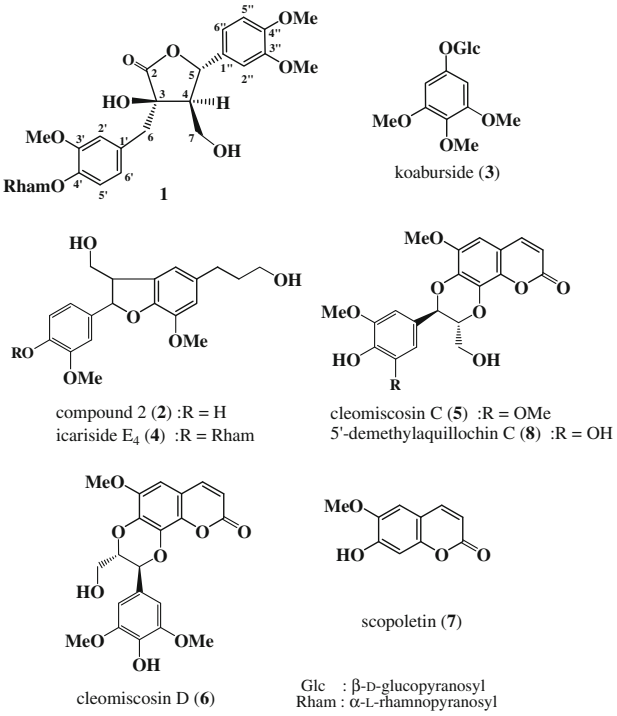

Fig. 1 The chemical structures of compounds **1**–**8**

including heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC), and rotation-frame Overhauser enhancement spectroscopy (ROESY) experiments. Two 1,3,4-trisubstituted aromatic rings, one α-rhamnopyranosyl group, and CH–CH–CH_2 partial structure were deduced from COSY correlations (Fig. 2). In the HMBC data, the connectivity from H_2-6 (δ 3.88, 3.71) to C-2 (δ 178.8), C-3 (δ 81.8), C-4 (δ 52.0), C-1′ (δ 131.8), C-2′ (δ 116.1), C-6′ (δ 124.0), from H-5 (δ 5.61) to C-1″ (δ 131.9), C-2″ (δ 110.1), C-6″ (δ 120.0), from H-6′ (δ 7.30) to C-4′ (δ 145.7), and from H-1‴ (δ 5.99) to C-4′ revealed the presence of γ-butyrolactone, that is, 3-hydroxy-4-hydroxymethyl-dihydrofuran-2-one, 4′-hydroxy-3′-methoxy-benzyl, and 3″,4″-dimethoxy-phenyl, and then they were connected between C-3 and C-6, C-5 and C-1″, α-rhamno-pyranosyl group, and C-4′ positions (Fig. 2, Table 1). The relative configurations of three successive chiral centers at C-3, C-4, and C-5 in **1** were indicated by the following NOE analysis as shown in Fig. 3. The NOEs between H-5 (δ 5.61)/H_2-7 (δ 4.63, 4.14), H-4 (δ 3.06)/H-2′ (δ 7.40), /H-6′ (δ 7.30), /H-2″ (δ 6.80), and /H-6″ (δ 6.90) indicated the β-orientations of HO-3 and H-5, and the α-orientation of H-4. Three methoxy groups at two 1,3,4-trisubstituted aromatic rings could also be confirmed at the C-3′, C-3″, and C-4″ positions from the NOEs between H-2′/OMe (δ 3.65), H-2″/OMe (δ 3.69), and H-5″ (δ 6.82)/OMe (δ 3.62) (Fig. 3). Thus, from the above findings, the structure of **1** was formulated as shown for **1**.

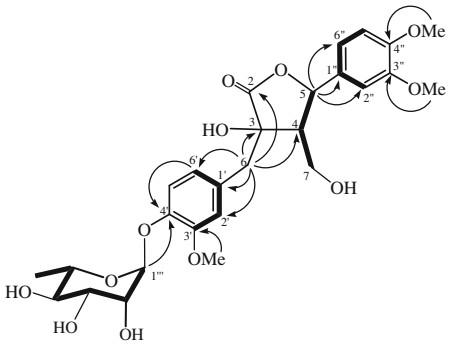

Fig. 2 COSY (*thick lines*) and HMBC (*arrows*) correlations for compound **1**

Table 1 NMR spectral data for compound **1** (in pyridine-d_5, 150 and 600 MHz)

Position	δ_C	δ_H (*J*, Hz)	Position	δ_C	δ_H (*J*, Hz)
2	178.8		1‴	101.8	5.99 (d, 1.3)
3	81.8		2‴	72.1	4.81 (dd, 3.3, 1.3)
4	52.0	3.06 (m)	3‴	72.6	4.72 (dd, 9.3, 3.3)
5	78.8	5.61 (d, 9.3)	4‴	73.8	4.36 (t, 9.3)
6	42.6	3.88 (d, 13.1)	5‴	71.1	4.47 (m)
		3.71 (d, 13.1)	6‴	18.6	1.60 (d, 6.3)
7	57.9	4.63 (dd, 11.0, 7.4)			
		4.14 (dd, 11.0, 4.4)			
1′	131.8				
2′	116.1	7.40 (d, 1.6)			
3′	151.0				
4′	145.7				
5′	119.0	7.38 (d, 8.2)			
6′	124.0	7.30 (d, 8.2, 1.6)			
1″	131.9				
2″	110.1	6.80 (d, 1.9)			
3″	150.2				
4″	150.2				
5″	111.9	6.82 (d, 8.2)			
6″	120.0	6.90 (d, 8.2, 1.9)			
3′-OMe	55.8	3.65s			
3″-OMe	55.8	3.69s			
4″-OMe	55.6	3.62s			

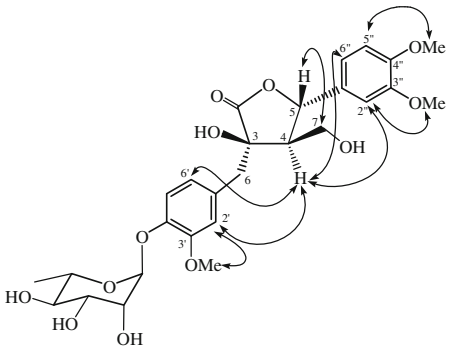

Fig. 3 ROESY correlations for compound **1**

The antioxidant activity of **1**–**7** has been studied with superoxide dismutase (SOD) assay kit. Vitamin C was used as a positive control (IC_{50} 66.2 μM). Among these, compounds **2**, **5**, and **6** exhibited significant SOD-like activity, IC_{50} 3.6, 46.0, and 21.2 μM, respectively.

Experimental

General

Optical rotation was taken on a JASCO DIP-1000 polarimeter. IR spectra were measured on a JASCO FT/IR-5300 instrument. NMR spectra were recorded on a Varian UNITY 600 spectrometer. The chemical shifts are given in δ (ppm) in C_5D_5N solution, using tetramethylsilane (TMS) as an internal standard. NMR experiments included COSY, HMQC, HMBC, and ROESY. Coupling constants (*J* values) are given in Hz. HR-FAB-MS were measured on a JEOL JMS-700 MS station.

Material

A. saccharum was collected at St. Roberts, Quebec, Canada, in May 2003. A voucher specimen (TB 5429) is deposited at the Herbarium of the Department of Pharmacognosy, Tokushima Bunri University, Tokushima, Japan.

Extraction and isolation

The wood of *Acer saccharum* (3.7 kg) was exhaustively extracted with EtOH at room temperature for 1.5 months. The EtOH extract was partitioned between EtOAc and H_2O. The EtOAc soluble-portion (54.0 g) was subjected to silica gel column chromatography with hexane–isopropyl ether–MeOH (10:1:0 → 0:1:10). Fraction 6 (3.14 g) and 7 (2.64 g) were purified by silica gel column chromatography with isopropyl ether–MeOH (20:1) to yield scopoletin (**7**, 157.8 mg) from fraction 6, and compound **2** (14.6 mg) from fraction 7, respectively. Fraction 9 (2.25 g) was subjected to silica gel column chromatography with isopropyl ether–MeOH–H_2O (25:3:0.1) and purified by HPLC (ODS, 40–50% MeOH) to afford cleomiscosin C (**5**, 85 mg) and 5′-demethylaquillochin (**8**, 4.3 mg). Fraction 10 (4.99 g) was also subjected to silica gel column chromatography with isopropyl ether–MeOH–AcOEt–H_2O (6:2:4:1) and purified by HPLC (ODS, 30–60% MeOH) to afford compound **1** (22 mg), koaburside (**3**, 38.7 mg), icariside E_4 (**4**, 138.6 mg), cleomiscosin C (**5**, 37.3 mg), and cleomiscosin D (**6**, 13.4 mg).

Compound 1

Amorphous solid. $[\alpha]_D$ −40.1° (c 1.7, MeOH). FT-IR (dry film) cm^{-1}: 3400 (OH), 1760 (C=O), 1050 (OH). HR-FAB-MS *m/z*: 549.2006 (calculated for $C_{27}H_{34}O_{12}$: 549.1973).

Acid hydrolysis of compound 1

A solution of **1** in 5% H_2SO_4–dioxane (1:1) was heated at 100°C for 2 h. The reaction mixture was diluted with H_2O and then neutralized with Amberlite IRA-35 and evaporated in vacuo to dryness. The identification and the D or L configuration of rhamnose was determined by using RI detection (Shimadzu RID-10A) and chiral detection (Shodex OR-1) by HPLC (Shodex RSpak NH_2P-50 4D, CH_3CN–H_2O–H_3PO_4, 95:5:1, 1 mL/min, 47°C), by comparison with an authentic sugar (10 mmol of L-rham). The sugar portion gave the following peak of L-(+)-Rham at 4.60 min.

Superoxide dismutase-like activity

SOD-like activity was determined according to the method of Ukeda et al. [11] using an SOD Assay Kit-WST (Dojindo Lab., Kumamoto). A test sample was dissolved in DMSO to obtain a final DMSO concentration of 0.8% (v/v).

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