Effect of the new high vacuum technology on the chemical composition of maple sap and syrup

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A B S T R A C T

Background: Techniques used to produce maple syrup have considerably evolved over the last decades making them more efficient and economically profitable. However, these advances must respect composition and quality standards as well as authenticity of maple products. Recently, a new and improved high vacuum technology has been made available to producers to achieve higher sap yields. The aim of the present study was therefore to evaluate the effect of this new system on the yield of sap and on the sap and syrup chemical composition.
Results: Sap yield was monitored during the 2013 and 2014 seasons for high vacuum collection systems (25–28 InHg) and compared to the control systems (20 InHg). Samples of sap and syrup were also collected for chemical analysis. During the 2013 season, a sap volume of 166.19 L/tap was recorded at 25 InHg vacuum level while the control vacuum level permitted to collect 139.47 L/tap, corresponding to a yield increase of 19.2 %. The following season, a yield increase of 38.2 % was measured when control and 28 InHg vacuum levels were compared with 118.06 and 163.13 L/tap, respectively. Results on the pH, color, flavor, minerals, sugars, organic acids, total polyphenols, total nitrogen, abscisic acid and auxin (Indol-3-acetic acid) showed no major differences between high vacuum technology and the control with values remaining within ranges previously published.
Conclusion: Results showed that a use of high vacuum systems increased sap yield and had no major impact on the quality and purity of maple sap and syrups compared with the control systems.

1. Introduction

Maple syrup is produced by evaporation of maple sap exuding from maple trees during the spring season. In this period, cold nights induce absorption of ground water by roots due to negative pressure within the tree. Warm days promote a natural exudation of sap at the tap when the internal pressure of the tree becomes greater than the external or atmospheric pressure [1]. Traditionally, sap was collected in buckets by gravity flow. When full, buckets were manually emptied in a collection tank, but this approach was laborious and produced poor sap yields. Around 1960, with the advent of plastic tubing system, many producers have adopted the use of vacuum pumps to intensify sap flows and increase sap yield [2, 3]. Indeed, according to Walters and Smith [4], vacuum applied at a tap enables to artificially reduce the external pressure leading to an increased differential pressure and allowing greater volume of sap to be extracted from the tree. It is worth mentioning that the unit of vacuum generally used by producers is the inch of mercury,

where one unit corresponds to 3.39 kPa. A study from Smith and Gibbs [5] reported that trees pumped at 5 inches of mercury (InHg) yielded twice as much sap as trees with sap collected by gravity flow. They also reported that the use of 10 InHg increased sap yield 2.5-fold compared to gravity flow. This suggested that increase of vacuum level led to increase in maple sap yield. Thus, vacuum levels used by producers tended to gradually increase and 15 InHg at the pump was, at that time, considered as a high vacuum level [6]. However, concerns started to rise about sap quality. Producers were worried that the vacuum utilization only increased the water content leading to a dilution effect of sap constituents. Laing *et al.* [6] reported that despite the 73 % increase measured of sap volume by using 15 InHg over gravity flow, sucrose and other solutes content in sap remained equivalent to gravity control.

Nowadays, it has become more common for producers to use 20 InHg as a standard of vacuum level and many tend to go higher. The advent of high-performance equipment and improved tubing system installation and design, provided access to higher vacuum level. Wilmot, Perkins and

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van den Berg reported that a maximum of 30 InHg can be reached at sea level [7]. This whole new level of high vacuum collection rose the same concerns as 50 years ago about sap and syrup quality as well as maple trees health. Wilmot *et al.* [7] studied the effect of different levels of vacuum (from 0 to 25 InHg) on sap sugar (°Brix) and mineral content. They found that increasing vacuum levels as high as 25 InHg had no consequences on some sap nutrients and led to no effect on the amount of visible internal damage to trees after one year.

Maple syrup composition has been extensively studied in the past. Physicochemical and rheological properties, minerals, organic acids, amino acids, polyphenols, volatile aroma compounds or sugar composition have been reported in numerous studies [8, 9, 10, 11, 12, 13, 14, 15]. While most studies on vacuum technology focused on sap yield and composition, to our knowledge, no studies have ever reported the impact of this new level of vacuum (25 InHg and more) on maple syrup constituents other than color grade [6]. Recently, polyphenols were reported to provide functional properties such as antioxidant as well as antimicrobial activity to maple syrup and its derivatives [16, 17, 18]. Therefore, it is of interest to investigate the impact of high vacuum sap collection on the value-added compounds of maple syrup. Concerning trees health, phytohormones like abscisic acid (ABA) while regulating plant growth and bud dormancy, are also secreted when the tree is facing an environmental stress such as a heat or a water stress [19, 20]. Furthermore, ABA is suspected to be a promising anti-diabetic molecule with studies showing a protective effect against type II diabetes in mice [21]. Additionally, auxin is another phytohormone secreted by plants in response to a wound to stimulate regeneration of vascular tissues [22]. Thus, phytohormone content in maple syrup could be good stress indicators of trees subjected to high vacuum utilization and should be investigated.

Therefore, the aim of this study was to evaluate the impact of the use of high vacuum level on sap yield and chemical composition as well as syrup composition and organoleptic quality.

2. Materials and methods

2.1. Study area

Experiments were conducted in Centre ACER's sugarbush, located at Saint-Norbert-d'Arthabaska in Quebec. The specific study was carried out on a 2 ha area with a 4 % slope and an elevation of about 600 feet in an uniform micro-climate. Prior to the study, a forest inventory was conducted with measurements of tree height, diameter at breast height (DBH), live-crown ratio, crown classification and location in order to select a homogeneous population of trees.

2.2. Experimental design and vacuum installation

Experiments were conducted over two sugar seasons in a homogeneous section of the sugarbush. Two groups of sugar maple trees (*Acer saccharum)* were randomly selected for each season and vacuum treatments were applied using a new tubing system (TS) for sap collection, according to the following design. The 2013 collection season started on March 7 and continued until April 29 with 97 control trees tested with a 20 InHg (~68 kPa) vacuum level and 99 treatment trees tested with a 25 InHg (~85 kPa) vacuum level. The 2014 collection season started on March 31 and continued until May 2 with 109 control trees tested with a 20 InHg and 109 treatment trees tested with a 28 InHg (~95 kPa) representing the highest vacuum level achievable at this particular location. The daily sap flow was monitored using a water meter installed at the end of tubing lines for each treatment. Each TS subjected to each treatment was linked to an independent extractor to receive fresh sap. Vacuum levels were maintained throughout the season with daily inspections of potential leaks. A maximum loss of 0.5 InHg was measured at the furthest taphole from the vacuum pump. At the extractors, a 1-L sample of sap was retrieved every day for each applied treatment for further analysis.

In order to evaluate the intra-group variability, a design adapted from Blum and Koelling [2] was applied. Twenty additional trees were randomly selected each year to individually record data on according control and treatment vacuum levels. In these cases, sap was collected via a barrel system (BS) where barrels were individually installed at the foot of each tree with a manual sap collection system allowing sampling without interrupting the vacuum. This system consisted of a stainless steel barrel connected to the tree with plastic tubing and valves, and sap was collected under the selected vacuum level independent from the main TS.

Each tree connected to the TS or BS received one tap 2 inches deep with installation of new spouts (19/64") and droplines (24 inches) prior to sap collection in 2013.

2.3. Syrup processing and sampling

All saps collected during the 2014 season from the TS were concentrated using a commercial membrane separation with FilmTec membranes NF90-2540 (Dow Chemical Company) to reach a concentration of 14 °Brix. Sap concentrates were stored at -18 °C until maple syrup production. Before maple syrup production, containers of sap concentrates were thawed overnight in a recirculating water tank at temperatures ranging from 4 to 8 °C depending on the thawing stage, allowing a slow and uniform process and to prevent microbial growth. Six syrups were produced from several sap concentrates originating from different flow days evenly distributed throughout the season and from both control and high vacuum systems. A pilot-scale electric evaporator (Centre ACER) was used for the evaporation of sap concentrate until a 66 % total soluble solids concentration (66 °Brix) was reached in conditions similar to those of a commercial syrup production process [23]. About 8 L of concentrate were used to obtain about 1.3 L of syrup. Syrups were then sampled for chemical composition, physicochemical and organoleptic analysis.

2.4. Physicochemical, microbial count and organoleptic analysis

For each sap and syrup samples, pH and conductivity were measured and microbial counts (total aerobic bacteria and fungal counts) were conducted according to previously published methods [24]. The total soluble solid content (TSS) in sap and syrup samples was measured with an AR200 Digital Hand-Held refractometer of Reichert Scientific Instruments (Buffalo, NY, USA) with automatic temperature compensation (ATC) at 20 °C. Syrup's color intensity was measured by reading the percentage of light transmittance at 560 nm (%T) in a Genesys 20 visible Spectrophotometer (ThermoFisher Scientific, Canada) using pure glycerol as a 100 % transmittance reference. Transmittance values were then used for color grading in accordance with the up-to-date provincial regulations [25]. Maple syrup organoleptic evaluations were conducted according to grading system standards in Quebec [26].

2.5. Sugars and organic acids analysis

Sugars and organic acids analysis in sap were conducted according to Lagace *et al* and adapted to the syrup matrix [24]. Sugars and organic acids were analyzed using a Shimadzu Prominence liquid chromatographic (LC) system purchased from Mandel Scientific (Guelph, ON, Canada). The LC system was equipped with binary pumps LC-20AB, degasser DGU-20A5, column oven CTO-20 AC and an autosampler SIL-2.

Sugars (sucrose, glucose and fructose) were analyzed by using 10 μL of filtered maple sap or diluted syrup (1.5 g in 50 ml of dionized water) that were injected into the LC system. Separation of sugars was done on a Sugar-Pak I column (Waters, Mississauga, ON, Canada) maintained at 84 °C and by using a calcium di-sodium EDTA solution at 0.05 g/L as eluent with a constant flow of 0.5 mL/min. Detection was carried out with a refraction index detector RID-10A (Shimadzu, Mandel Scientific).

For organic acids, 5 ml of maple sap or diluted syrup (1 g in 5 ml of dionized water) were acidified by adding 25 μL of concentrated sulphuric acid. Then, samples were filtered with a 0.45 μM PDVF filter and 10 μL

was injected in the LC system equipped with a Synergi Hydro-RP column (250 × 4.6 mm, Phenomenex, Torrance, CA, USA), maintained at 25 °C and by using potassium monobasic phosphate (KH2PO4) at 0.02M and 0.5 ml/min as eluent. Detection of organic acids (oxalic, quinic, pyruvic, malic, fumaric and succinic) was done using a diode array detector UV SPD-M20A (Shimadzu, Mandel Scientific) and the obtained chromatograms were analyzed at 210 nm for quantification.

2.6. Phytohormone analysis

Phytohormone standards ABA and auxin (indole-3-acetic acid) as well as Methanol (HPLC grade) were purchased from Sigma Aldrich (Oakville, ON, Canada). Trifluoroacetic acid (TFA) and acetic acid were purchased from American Chemicals Ltd (Saint-Laurent, QC, Canada). Solid-phase extraction (SPE) Cartridge, Sep-Pak C18 6cc-500mg sorbent was purchased from Waters Canada. Phytohormone contents were determined according to the method developed by Ma *et al.* with some modifications [27]. For the SPE procedure, a volume of 5.0 g of each maple syrup sample was weighted and diluted in 10.0 g of demineralized water. The pH was adjusted to 3.0 with addition of acetic acid. Loaded samples and SPE Sep-Pak C18 were subsequently washed according to Ma *et al.* method [27]. Afterwards, each sample was eluted with 2 ml of methanol then evaporated under dry nitrogen before dissolution into 250 µl of methanol. An aliquot of the obtained extract was used for the LC-PDA analysis conducted on the LC System described previously for the analysis of organic acids. A volume of 20 µl was injected into a Zorbax SB-C18 column (4.6 × 250 mm, 5 mm, Agilent). Oven temperature was set at 30 °C and the mobile phase comprised 0.2 % TFA in water for solvent (A) and methanol for solvent (B). The elution gradient was started with 20 % B, increased to 98 % B at 17 min, and hold at 98 % B for 1 min. The flow rate was set at 0.75 ml/min. Auxin and ABA phytohormones were characterized at 261 and 273 nm respectively. For method validation, the linearity of the external calibration curve (1–110ppm) was evaluated according to the correlation coefficient R^2 which was higher than 0.995 for ABA and auxin. For recovery, maple syrups spiked at 25 ppm with analytical standard solution showed a recovery of 100.4 % and 81.4 % for ABA and auxin, respectively. Finally, LOQ were estimated according to signal to noise ratio method (S/N*10) and was about 0.09 and 0.15 ppm for ABA and auxin respectively.

2.7. Mineral analysis

Minerals analysis was performed by the IRDA laboratory (Institute of Research and Development in Agri-Environment, Quebec, Canada), which is accredited under ISO 17025:2005. The analytical procedure consisted in acidifying 2.5 g of maple sap or 1 g of syrup sample with 0.5 mL of HNO_3 70 % and then the volume was adjusted to 50 mL with Milli-Q system filtered water. Samples were centrifuged and filtered through 0.45 µm membrane and analyzed by ICP/OES, optima 430 from PerkinElmer (Woodbridge, ON, Canada). The wavelengths (nm) applied for different elements were as follows: K (766.490), Ca (315.887), Mg (279.077), Mn (257.610), Na (589.592) and Zn (213.857). The standard solutions Multi-Element (900-Q21-002 Plasma CAL), Plasma CAL-Fe (10000 mg/L) and Plasma CAL-S (1000 mg/L), purchased from SCP Science (Canada), were used for calibration curves.

2.8. Total phenolic, nitrogen and protein content analysis

The total phenolic content determination was carried out spectrophotometrically according to the Ma & Cheung method and results are expressed as vanillic acid equivalent (VAE) per volume of syrup [28].

Total nitrogen was analyzed according to the Dumas combustion method [29]. For each sample, 0.2 g of syrup was weighted and analyzed with an elemental analyzer (Vario Max-Cube, Elementar Analysensysteme GmbH, Hanau, Germany) using as a standard 0.05 g of a rice flour calibration sample containing 1.13% of Nitrogen (LECO

Corporation, Canada).

Total protein content was estimated by multiplying the total nitrogen content obtained by the Dumas method with Jones's factor (6.25) for each syrup sample [30].

2.9. Statistical analysis

Data analysis was done using the R software version 3.4.3 [31]. Welch's t-test was applied to compare yields collected by the BS and a bootstrap simulation was done to resample the data from the BS to match the distribution of trees linked by the TS. To assess significant differences between chemical content in samples collected from the different vacuum levels, ANOVA was performed, normality and homogeneity of variance were evaluated with Shapiro-Wilk's test and Levene's test respectively [32]. In cases of non-normally distributed data, a non-parametric Kruskal-Wallis one-way ANOVA was performed.

3. Results and discussion

3.1. Impact of high vacuum levels on sap yield

In order to evaluate the impact of high vacuum levels on sap yield per tap, volume of sap collected with the BS on different vacuum levels were compared. Average sap-yield volumes per tap in the BS varied with vacuum levels. Table 1 presents results of sap yield comparison analyses. High vacuum levels permitted greater yields with a 35.7 % increase at 25 InHg and 65.8 % increase at 28 InHg compared to control when volumes of 10 trees per treatment were averaged together. During the 2013 season, one tree out of ten didn't produce any sap and was considered as abnormal data and wasn't integrated in the statistical analysis. Sap volumes collected with high vacuum levels (25–28 InHg) were significantly greater than volumes at 20 InHg control vacuum level ($p < 0.05$). Ten samples per treatment being quite small to make solid assumptions about the effectiveness of high vacuum levels on sap yields, the bootstrap simulation was used to resample data at a larger distribution, herein, equal to the number of trees linked with the TS. In accordance with the regular analysis, the comparative analysis from the bootstrap simulation showed that yields obtained with high vacuum pumping are significantly greater than control vacuum level ($p < 0.001$).

Fig. 1 presents the cumulative sap volume per tap collected in the TS for each treatment level. At a 25 InHg vacuum level, the volume of sap per tap was 166.19 L while the control indicated 139.47 L/tap representing a yield increase of 19.2 %. The difference was visible after 5 days of flow, where the high vacuum pumping started to yield more sap than control. The same tendency was observed at the 28 InHg vacuum level. Indeed, the cumulative sap yield at the end of the season was 163.13 L/tap while the control was 118.06 L/tap representing a yield increase of 38.2 %. A difference became notable from the 9th day and remained for the rest of the season. The 25 InHg and 28 InHg vacuum levels increased the sap collection by 5.3 and 5.6 L/tap per inch of mercury respectively, suggesting a linear correlation between the amount of sap collected and the level of applied vacuum. These results are in accordance with the previously reported work of others [4, 7, 33]. Therefore, the increase of sap yield with high vacuum level is of economic importance to the producer considering that, with proper equipment and installation, a more abundant and consistent harvest can be obtained year after year compared to a 20 InHg vacuum level.

3.2. Impact of high vacuum levels on sap composition

In order to seek if high vacuum collection of sap has an impact on its quality, chemical composition, microbial counts and physicochemical analysis were performed on sap collected daily in the TS for the control and the 28 InHg high vacuum treatment through the 2014 season and results are presented in Table 2.

Mineral composition showed no significant differences between the

Table 1
Sap yields collected with two high vacuum levels (25–28 InHg) compared to control (20 InHg) in the barrel system and summary on analyses for comparing yields from different vacuum levels using Welch's t-test on original dataset and with a bootstrap simulation.

Vacuum level	Original analysis					Bootstrap simulation			
	N[a]	Mean (L/tap)	SD[b]	95% CI[c]	*p* value*	N[d]	Simulated Mean	95% CI	*p* value*
Control (20 InHg)	10	144.11	34.8	(-90.9; -11.8)	0.01428*	97	141.78	(-42.2; -33.1)	2.10^{-16}***
25 InHg	9	195.50	44.82			99	179.42		
Control (20 InHg)	10	114.69	35.12	(109.0; -41.8)	$1.7.10^{-04}$***	109	113.82	(-79.4; -73.4)	2.10^{-16}***
28 InHg	10	190.11	36.49			109	190.23		

*One star denotes significance at the 0.05 level, two stars denotes significance at the 0.01 level and three stars denotes significance at the 0.001 level.
  [a] Number of taps used to measure average sap volume per tap (one tap per tree), collected in barrels.
  [b] Standard deviation.
  [c] 95 % confidence interval obtained by Welch's two samples t-test.
  [d] Number of taps based on the corresponding number of trees actually used to measure total volume with the tubing system.
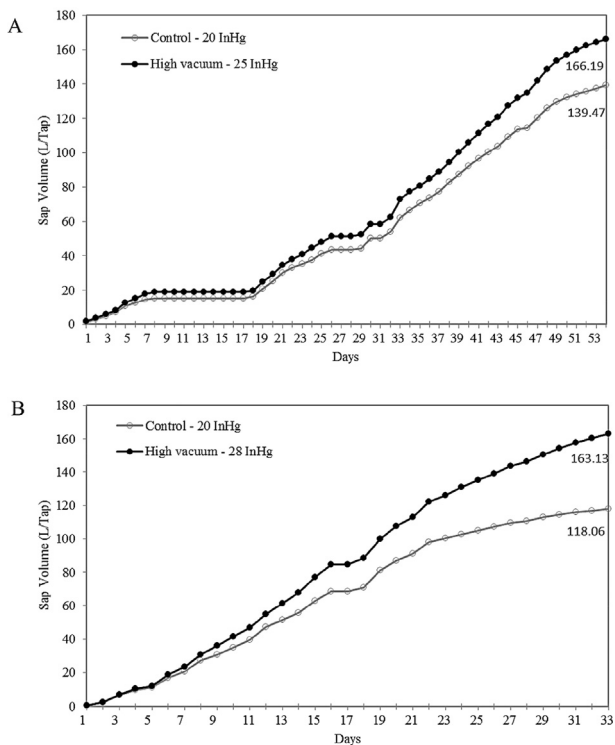

Fig. 1. Influence of two different vacuum levels (a) 25 InHg and (b) 28 InHg on sap volume per tap (L/tap) compared to control (20 InHg) during two sugar seasons collected with the tubing system.

Table 2
Means of main minerals, organic acids, sugars, physico-chemical characteristics and microbial counts in maple sap collected with control vacuum level (20 InHg) and high-vacuum level (28 InHg) in the tubing system with respective P-values.

Analysis	Control - 20 InHg		High vacuum - 28 InHg		
	Mean[a]	SD[b]	Mean	SD	*p* value*
Minerals (µg/g)					
Potassium	69.55	6.70	72.99	7.49	0.053
Calcium	117.12	24.72	112.94	25.55	0.38
Magnesium	9.64	1.74	9.79	1.88	0.83
Sodium	0.03	0.04	0.03	0.03	0.69
Zinc	0.28	0.05	0.28	0.05	0.92
Manganese	2.28	0.50	2.39	0.59	0.44
Organic Acids (µg/g)					
Oxalic	0.54	0.41	0.33	0.14	0.0495*
Quinic	45.44	48.47	34.41	45.62	0.15
Pyruvic	5.96	5.60	5.38	5.89	0.41
Malic	274.18	114.86	300.92	87.29	0.45
Fumaric	6.55	5.59	6.94	5.72	1.00
Succinic	52.82	80.70	52.43	82.34	0.76
Sugars (%)					
Sucrose	1.98	0.42	1.96	0.36	0.50
Glucose	0.03	0.03	0.01	0.01	0.005**
Fructose	0.04	0.07	0.01	0.01	0.02*
Physico-chemical					
pH	7.08	0.83	7.17	0.88	0.64
TSS (Brix)[c]	2.16	0.22	2.06	0.30	0.15
Conductivity (uS/cm)	585.21	84.44	578.94	87.51	0.77
Microbial counts (log CFU/ml)					
Bacteria	5.73	1.09	5.46	1.03	0.26
Fungi	5.07	1.56	4.49	1.38	0.07

*One star denotes significance at the 0.05 level, and two stars denotes significance at the 0.01 level.
  [a] *N* = 33.
  [b] Standard deviation.
  [c] Total Soluble Solids.

control and the 28 InHg vacuum level. However, potassium showed a *p* value near α = 0.05, implying a certain relationship with high vacuum pumping but not statistically significant. But it is worth mentioning that potassium content in sap from each treatment level remained within ranges previously published [24]. Concerning organic acids, high vacuum pumping had no significant differences with control except for oxalic acid concentrations. The latter was significantly lower in sap collected with high vacuum than control and comparison analysis showed a *p* value of 0.0495 which is near the significance level α, indicating a low but significant difference. Sugar analysis showed that fructose and glucose are significantly lower in high vacuum collection sap than control (*p* < 0.05). Although their means stood in the average distribution found in sap [24], this difference can also be explained by the fungi count found greater in control sap than in high vacuum sap with 5.07 and 4.49 log CFU/ml respectively. Fungi, specifically yeasts, are commonly known for hydrolyzing sucrose due to the action of extracellular invertases, releasing fructose and glucose into the media [34]. While the *p* value from fungi count comparisons indicated no significant

differences, its value was still near the α = 0.05, suggesting that this hypothesis cannot be totally dismissed.

In addition, high vacuum collection did not influence physicochemical characteristics of sap such as pH, conductivity and TSS showing no significant differences which is in agreement with previously reported works of others [6, 35].

3.3. Impact of high vacuum levels on syrup composition and properties

Chemical composition and physicochemical properties of 6 syrups produced during the 2014 season by evaporation of sap collected through the TS for each treatment were evaluated and results are presented in Table 3. There were no significant differences among vacuum levels for minerals, organic acids as well as total protein and nitrogen content in syrups. Phytohormones (ABA and Auxin) and total phenolic content also showed no significant differences between vacuum levels and

Table 3

Means of main minerals, organic acids, sugars, physico-chemical characteristics and other chemical compounds in maple syrups produced from sap collected with control vacuum level (20 InHg) and high-vacuum level (28 InHg) in the tubing system with respective P-values.

Analysis	Control - 20 InHg		High vacuum - 28 InHg		
	Mean[a]	SD[b]	Mean	SD	*p* value
Minerals (µg/g)					
Potassium	1670.10	213.95	1701.13	296.55	0.84
Calcium	1691.08	1191.53	1618.20	1078.37	1.00
Magnesium	191.88	67.32	195.70	87.32	0.93
Sodium	10.58	1.66	12.35	3.52	0.63
Zinc	3.79	0.45	4.77	1.75	0.42
Manganese	13.50	20.07	24.84	36.26	0.34
Organic Acids (µg/g)					
Oxalic	28.73	12.68	23.36	7.23	0.39
Quinic	84.03	69.61	184.77	381.97	0.11
Pyruvic	471.66	50.14	380.13	136.80	0.11
Malic	5278.90	1490.48	5611.82	2238.20	0.87
Fumaric	291.74	91.39	287.60	146.15	0.95
Succinic	1624.00	1631.12	1754.91	1700.16	0.63
Sugars (%)					
Sucrose	64.37	3.79	64.37	3.76	0.63
Glucose	0.44	0.60	0.26	0.34	0.20
Fructose	0.33	0.48	0.43	0.76	0.42
Physico-chemical					
pH	7.30	0.52	7.09	1.04	0.67
Conductivity (uS/cm)	212.32	37.80	220.63	41.57	0.38
Transmittance (%; 560 nm)	63.49	13.08	74.38	11.40	0.15
Other analysis					
ABA[c] (ng/g)	212.10	85.10	161.00	56.00	0.25
Auxin (ng/g)	730.30	129.00	804.00	160.60	0.35
Total protein (%)	0.25	0.15	0.27	0.21	0.57
Total nitrogen content (µg/g)	308.60	72.89	304.74	135.13	0.35
Total phenolic content (µg/g)	62.33	5.18	64.08	5.99	0.60
Organoleptic evaluation					
Taste defects[d]	3/6	—	3/6	—	

[a] *N* = 6.
[b] Standard deviation.
[c] Abscisic acid.
[d] Number of syrups out of 6 with unidentified defect corresponding to the symbol √R4 (according to grading standard system in Quebec). Remaining syrups were free of defects.

concentrations were close to those reported in literature [8, 17, 36].

The significant differences of invert sugars (fructose and glucose) previously observed in sap were no longer present in syrups and their means correspond to contents generally found in maple syrup [9]. High vacuum collection of sap also had no incidence on pH, light transmittance and conductivity of syrups produced. Organoleptic evaluation of syrups for each treatment level was performed and 3 out of 6 syrups presented taste defects and were graded as unidentified defects (Table 3). According to Quebec's grading standard system, unidentified defect can be attributed either to an unidentified set of bad tastes and/or odors or to an absence of flavor. This type of defect occasionally occur during the sugar season. The proportion in each group being equal, it can be concluded that levels of vacuum had no impact on the organoleptic properties of syrups in terms of taste defects. These results suggest that the use of high vacuum sap collection does not significantly affect properties and most of the major and minor chemical compounds of maple syrup.

4. Conclusion

Results obtained in this study indicate that current high vacuum collection of sap has no major incidence on the sap nutrient content except a low but significant reduction of glucose and fructose content in the sap when high vacuum level (28 InHg) is used compared to control

(20 InHg). Despite this minor difference found in sap samples, the end product (maple syrup) showed no differences in organoleptic quality, nutrient content (including glucose and fructose) as well as in compounds with functional properties such as phenolic compounds. Abscisic acid and auxin content were similar in syrup coming from high vacuum operations and control, suggesting that no increase of stress from trees was observed with increase of vacuum level. Considering that the maximum level of vacuum reachable is 30 InHg at sea level [7], producers using a vacuum level as high as 28 InHg can obtain significantly higher syrup yield and economic benefit without sacrificing quality. This can only be achieved by using good quality equipment and operation to assure the best performance of the vacuum system. Some concerns however still remain about sustainability of trees subjected to this level of high yield operations. Results from a previous study showed that healthy trees subjected annually to high-yield condition practices for 5–10 years, didn't exhibit any decrease in growth rates [37]. It was also reported that growth rates of trees subjected to high-vacuum operations for at least five years were sustainable as long as current tapping guidelines were respected [38]. However, vacuum utilization being present for more than 20 years, the question is still open as to whether a continuous and annual application of high vacuum might affect the tree carbohydrate reserves and ultimately, limit their lifespan. This question is particularly important for slow growing and stress affected trees. Other tests must also be performed under other conditions (different locations, seasons, etc.) to validate the results of the present study and to conclude on this technology on a larger scale.

Declarations

Author contribution statement

Luc Lagacé: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mariane Camara: Analyzed and interpreted the data; Wrote the paper.

Nathalie Martin: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Fadi Ali: Contributed reagents, materials, analysis tools or data.

Jessica Houde, Stéphane Corriveau: Performed the experiments.

Mustapha Sadiki: Analyzed and interpreted the data.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] J.W. Marvin, M.T. Greene, Temperature-induced sap flow in excised stems of Acer, Plant Physiol. 26 (3) (1951) 565.

[2] B.M. Blum, M.R. Koelling, Vacuum pumping increases sap yields from sugar maple trees. Res Pap US Dep Agric For Serv, 1968 (Reseach Note NE-106).

[3] D. Chapeskie, L.J. Staats, Design, Installation, and Maintenance of Plastic Tubing Systems for Sap Collection in Sugar Bushes. East Ont Model for Kemptv Ont Can 86pp, 2006.

[4] R.S. Walters, H.C. Smith, Sugar maple sap volume increases as vacuum level is increased, US Forest Service 213 (1975).

[5] H.C. Smith, C.B. Gibbs, Comparison of vacuum and gravity sap flows from paired sugar maple trees. US Dep Agric For Serv, 1970 (Research Note NE-122).

[6] F.M. Laing, J.W. Marvin, M. Morselli, D.W. Racusen, E.L. Arnold, E.G. Malcolm, Effect of high-vacuum pumping on volume yields and composition of maple sap, Res. Rep. 65 (1971).

[7] T.R. Wilmot, T.D. Perkins, A.K. van den Berg, Vacuum sap collection: how high or low should you go, Maple Syrup Dig A (19) (2007) 27–32.

[8] A.S. Singh, A.M.P. Jones, P.K. Saxena, Variation and correlation of properties in different grades of maple syrup, Plant Foods Hum. Nutr. 69 (1) (2014) 50–56.

[9] J.G. Stuckel, N.H. Low, The chemical composition of 80 pure maple syrup samples produced in North America, Food Res. Int. 29 (3–4) (1996) 373–379.

[10] T.D. Perkins, A.K. van den Berg, Chapter 4 maple syrup—production, composition, chemistry, and sensory characteristics, in: Advances in Food and Nutrition Research [Internet], Academic Press, 2009 [cited 2019 Mar 25]. p. 101–43. Available from: http://www.sciencedirect.com/science/article/pii/S1043452608006049.

[11] D.W. Ball, The chemical composition of maple syrup, J. Chem. Educ. 84 (10) (2007 Oct 1) 1647.

[12] M. Sadiki, N. Martin, Solid-phase extraction and procedure for determination of phenolic compounds in maple syrup, Food Anal. Methods 6 (3) (2013 Jun 1) 737–744.

[13] H. Sabik, J. Fortin, N. Martin, Identification of pyrazine derivatives in a typical maple syrup using headspace solid-phase microextraction with gas chromatography–mass spectrometry, Food Chem. 133 (3) (2012 Aug 1) 1006–1010.

[14] L. Li, N.P. Seeram, Maple syrup phytochemicals include lignans, coumarins, a stilbene, and other previously unreported antioxidant phenolic compounds, J. Agric. Food Chem. 58 (22) (2010 Nov 24) 11673–11679.

[15] L. Li, N.P. Seeram, Further investigation into maple syrup yields 3 new lignans, a new phenylpropanoid, and 26 other phytochemicals, J. Agric. Food Chem. 59 (14) (2011 Jul 27) 7708–7716.

[16] V.B. Maisuria, Z. Hosseinidoust, N. Tufenkji, Polyphenolic extract from maple syrup potentiates antibiotic susceptibility and reduces biofilm formation of pathogenic bacteria, Appl. Environ. Microbiol. 81 (11) (2015) 3782–3792.

[17] M. Thériault, S. Caillet, S. Kermasha, M. Lacroix, Antioxidant, antiradical and antimutagenic activities of phenolic compounds present in maple products, Food Chem. 98 (3) (2006) 490–501.

[18] Y. Liu, K.N. Rose, N.A. DaSilva, S.L. Johnson, N.P. Seeram, Isolation, identification, and biological evaluation of phenolic compounds from a traditional North American confectionery, maple sugar, J. Agric. Food Chem. 65 (21) (2017 May 31) 4289–4295.

[19] R. Finkelstein, Abscisic Acid synthesis and response, Arabidopsis Book 11 (2013) e0166.

[20] B. Steuer, T. Stuhlfauth, H.P. Fock, The efficiency of water use in water stressed plants is increased due to ABA induced stomatal closure, Photosynth. Res. 18 (3) (1988) 327–336.

[21] A.J. Guri, R. Hontecillas, H. Si, D. Liu, J. Bassaganya-Riera, Dietary abscisic acid ameliorates glucose tolerance and obesity-related inflammation in db/db mice fed high-fat diets, Clin. Nutr. 26 (1) (2007) 107–116.

[22] R. Aloni, E. Aloni, M. Langhans, C.I. Ullrich, Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism, Ann. Bot. 97 (5) (2006) 883–893.

[23] G.B. Allard, M. Belzile, 7.0. Évaporation de l'eau ou du concentré d'eau d'érable, in: Cahier de transfert technologique en acériculture, CRAAQ, 2004.

[24] L. Lagacé, S. Leclerc, C. Charron, M. Sadiki, Biochemical composition of maple sap and relationships among constituents, J. Food Compos. Anal. 41 (2015) 129–136.

[25] PPAQ, Classification [Internet], Producteurs et Productrices Acéricoles du Québec, 2019 [cited 2019 Mar 20]. Available from: http://ppaq.ca/en/producer/useful-information/classification/.

[26] P.P.A.Q. Classification [Internet], Producteurs et productrices acéricoles du Québec, 2018 [cited 2019 Feb 7]. Available from: https://ppaq.ca/en/producer/useful-information/classification/.

[27] Z. Ma, L. Ge, A.S.Y. Lee, J.W.H. Yong, S.N. Tan, E.S. Ong, Simultaneous analysis of different classes of phytohormones in coconut (Cocos nucifera L.) water using high-performance liquid chromatography and liquid chromatography–tandem mass spectrometry after solid-phase extraction, Anal. Chim. Acta 610 (2) (2008 Mar) 274–281.

[28] Y.-T. Ma, P.C. Cheung, Spectrophotometric determination of phenolic compounds by enzymatic and chemical methods a comparison of structure- activity relationship, J. Agric. Food Chem. 55 (10) (2007) 4222–4228.

[29] J.B.A. Dumas, Procedes de l'analyse organique, Ann. Chem. Phys. 47 (1831) 198–205.

[30] D.B. Jones, Factors for Converting Percentages of Nitrogen in Foods and Feeds into Percentages of Proteins, US Dept Agric Circ, 1931, p. 183.

[31] Team RC. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Austria, 2015, 2018, http://www.R-project.org.

[32] J. Fox, S. Weisberg, D. Adler, D. Bates, G. Baud-Bovy, S. Ellison, et al., Package 'car.', Vienna R Found Stat Comput, 2012.

[33] R.R. Morrow, C.B. Gibbs, Vacuum pumping doubles maple sap yield on flat land, US Dep Agric For Serv (1969) 5 (Research Note NE-91).

[34] S. Shall, A.W.A. Baseer, The mechanism of action of yeast invertase, Biochem. J. 122 (1) (1971) 19.

[35] J.W. Kelley, L.J. Staats, High-vacuum pumping effects on maple sap sugar yield, N. J. Appl. For. 6 (3) (1989) 126–129.

[36] Y. Desjardins, C. Ratti, I. Zaharia, S. Abrams, Maple sap and syrup are a rich source of abscisic acid with potential benefits to health, in: Acta Horticulturae [Internet], International Society for Horticultural Science (ISHS), Leuven, Belgium, 2012, pp. 129–136.

[37] A. van den Berg, T. Perkins, M. Isselhardt, T. Wilmot, Growth rates of trees tapped with high-yield sap collection practices – are conservative tapping guidelines sustainable? Maple Syrup Dig 25A (4) (2013) 27–34.

[38] A.K. van den Berg, T.D. Perkins, M.L. Isselhardt, T.R. Wilmot, Growth rates of sugar maple trees tapped for maple syrup production using high-yield sap collection practices, For Sci 62 (1) (2016 Feb 22) 107–114.