Effects of Sap Concentration with Reverse Osmosis on Syrup Composition and Flavor

A summary of experiments conducted at the University of Vermont Proctor Maple Research Center

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Concentrating maple sap with reverse osmosis (RO) significantly increases the efficiency and profitability of processing sap into syrup by reducing the amount of both fuel and time required to concentrate the material to syrup density in the evaporator. However, because this also reduces the amount of time sap is processed in the evaporator, and since most of the reactions from which the flavor and color properties of maple syrup are ultimately derived occur as sap is processed with heat in the evaporator, it is possible that this could result in impacts on the finished syrup, causing it to differ from syrup produced with raw sap. Previous investigations of the effects of RO were limited to small quantities of syrup produced through batch boiling in the laboratory, conditions which do not necessarily well replicate those that occur during processing in an evaporator (Morselli *et al.* 1982, Sendak and Morselli 1984). It is imperative to determine the effects that RO might have under realistic processing conditions, as any gains in efficiency would be nullified by any possible negative impacts on syrup quality. Thus, between 2008 and 2011 we conducted a series of controlled experiments performed with commercial maple equipment to investigate the potential effects of the use of RO on the composition, properties, and flavor of the maple syrup produced. The following is a brief, general summary of these experiments and the results observed. More comprehensive descriptions of each study can be found in the scientific journal articles published for each.

All experiments were conducted in the Maple Processing Research Facility at the University (MPRF) of Vermont Proctor Maple Research Center in Underhill Center, Vermont (UVM-PMRC) (Figure 1).

Experiment 1 – Comparison of syrup made from raw sap and 8% concentrate

This experiment was conducted to investigate the fundamental effects of concentrating sap with RO on the properties, composition, and flavor of maple syrup, and to determine if any significant differences exist in syrup made from raw and concentrated sap. To accomplish this, we conducted an experiment in which syrup was produced simultaneously from raw sap and from

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8% concentrate made from this same raw sap, using identical equipment and processing conditions.

Methods

During each trial of the experiment, raw sap flowing into the sap collection area was collected and segregated into a separate tank. Once collected, 300 gallons of this raw sap was set aside, and another portion was immediately concentrated to generate 300 gallons of 8% concentrate using a CDL RO unit equipped with seven, 8″ × 40″ Fluid Systems TFC 8923 S-400 membranes. The raw sap and concentrate were then immediately transported to the MPRF at UVM-PMRC and placed into separate stainless steel tanks that each fed one of two identical, 3 × 10′ Dallaire Model Deluxe, raised-flue, oil-fired evaporators (Figure 1). Both evaporators were equipped with automatic draw-offs and sap level regulation, and were configured to process sap as similarly as possible, with equal oil burner and exhaust draft settings. The evaporators were started simultaneously and run continuously until the supply of available material for each was consumed (~3-3.5 hrs). All syrup produced by each evaporator after the first hour of processing was collected separately and filtered through synthetic cone filters. After the experiment was complete, samples of syrup produced with each treatment were frozen for subsequent analyses. This experiment was repeated on 6 days during the 2011 maple production season (3/18, 3/21, 4/1, 4/2, and 4/8). At the end of each trial, connections between evaporator pans were plugged with rubber stoppers to minimize disruption of the concentration gradient between trials.

Figure 1. Research evaporators at the University of Vermont Proctor Maple Research Center Maple Processing Research Facility. Evaporators are oil-fueled, 3 x 10’ with cross-flow pans and raised flues.

The color, pH, conductivity, mineral, carbohydrate, and volatile flavor compound contents were determined for each syrup produced during the experiments. Syrup color was determined by measuring the percentage of light transmittance at 560 nm with a spectrophotometer using glycerol as a 100% transmittance standard. Electrical conductivity and pH were determined with a bench-top meter with an epoxy body electrical conductivity cell and a solid-state pH probe, respectively, both equipped with automatic temperature compensation. Density of sap and syrup was measured with a digital refractometer (Misco PA203X). The composition of inorganic mineral elements was determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The composition of sucrose, glucose, and fructose in each sample of syrup was determined by high-performance liquid chromatography (HPLC), and the composition of volatile flavor and aroma compounds in each syrup sample was determined by solid-phase microextraction (SPME) and gas chromatography time-of-flight mass spectrometry (GC-TOF-MS). Detailed descriptions of the analytical methods used can be found in van den Berg *et al.* (2014). For each parameter, a paired *t*-test was used to determine if averages differed significantly in the syrup produced simultaneously from raw sap and from the same sap concentrated to 8%. (Paired *t*-tests compare the composition of the pairs of syrup produced simultaneously with the two treatments.)

Sensory evaluation experiments were also conducted to examine potential effects on flavor. For sensory evaluation, triangle tests were conducted to determine if an overall difference could be detected in the flavor of syrup produced simultaneously with raw sap and the same sap concentrated to 8%. Triangle tests are a sensory evaluation method used to determine whether an overall difference in flavor exists between two samples. For example, in a triangle test designed to determine if an overall difference in flavor exists between the syrup produced with raw sap and the same sap concentrated to 8% during the experiment trial conducted on 3/18, each panelist would be presented with three samples of syrup. Two of the bottles would contain the syrup produced with raw sap during the 3/18 trial, and one of the bottles would contain the syrup produced with 8% concentrate during the 3/18 trial. (An equal number of panelists would receive two bottles of the syrup produced with concentrate and one bottle of the syrup produced with raw sap.) The panelists would then be asked to taste all three bottles of syrup and write down the number of the bottle they believe contains the one syrup that is different from the other two. If enough panelists correctly identify the 'odd' sample in the trio, then it is concluded that there is a difference in flavor between the pairs of syrup produced simultaneously with the two treatments.

Individual triangle tests were conducted for four of the six pairs of syrup produced during the experiment trials (to avoid sensory fatigue in the panelists) following the procedures described by Meilgaard *et al.* (2006). Twenty-six adult panelists with experience tasting and grading maple syrup participated in the test. Panelists were separated by cardboard partitions under fluorescent light during administration of the test, and sample presentation order was randomized for each panelist. Opaque sample bottles were used to eliminate any influence of syrup color on the panelists' perceptions. Pairs were considered significantly different ($p < 0.01$) if 15 of the 26 panelists positively identified the odd sample (Meilgaard *et al.* 2006).

Results

Syrup produced simultaneously with raw sap and the same sap concentrated to 8% sugar was very similar (Table 1). The most notable difference observed was with respect to syrup color: syrup produced with raw sap was slightly, but statistically significantly lighter in color than the syrup produced simultaneously with the same sap concentrated to 8%. The average difference in light transmittance between the pairs of syrup produced simultaneously with raw sap and concentrate was 11.8 percentage points, however only one

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pair fell within different grade classifications from one another (Figure 3). This result is particularly interesting, since it is the opposite of what might be expected: it is commonly hypothesized that syrup produced from concentrate will be lighter in color than syrup produced from raw sap due to the shorter processing times required with concentrate.

Besides color, few other statistically significant differences in composition or properties were observed between syrup produced with raw sap and concentrate. The conductivity did not differ significantly between the two types of syrup and, accordingly, almost no differences were observed in the composition of dissolved minerals between the syrup types (Table 1). The exception to this was magnesium (Mg), which was found in slightly lower quantities in syrup produced from raw sap, by approximately 20 ppm (Table 1). This is most likely due to the slight permeability of this membrane to Mg, which would result in the concentrate containing proportionally slightly less Mg than the raw sap.

Parameter measured	Raw Sap (2%)	Concentrated Sap (8%)	*p*-value
Brix (°)	67.1 ± 0.2	67.2 ± 0.1	0.4142
Conductivity (μS cm^{-1})	171.6 ± 11.5	162.9 ± 9.1	0.1572
Light transmittance (%)	57.7 ± 4.4	45.9 ± 3.8	**0.0001**
pH	7.1 ± 0.05	7.5 ± 0.09	**0.0091**
Calcium (ppm)	946 ± 77	939 ± 23	0.9164
Phosphorous (ppm)	2.3 ± 0.7	4.1 ± 1.2	0.1424
Potassium (ppm)	1948 ± 37	2009 ± 48	0.2694
Magnesium (ppm)	153.2 ± 7.7	133.1 ± 8.4	**0.0063**
Iron (ppm))	1.9 ± 0.5	1.9 ± 0.6	0.9171
Manganese (ppm)	27.2 ± 5.1	16.0 ± 2.4	0.1097
Boron (ppm)	1.2 ± 0.2	0.9 ± 0.1	0.0625*
Copper (ppm)	0.9 ± 0.1	1.0 ± 0.1	0.6274
Zinc (ppm)	3.2 ± 0.1	3.6 ± 0.2	0.1084
Sulfur (ppm)	17.5 ± 1.2	18.7 ± 1.2	0.5443
Sucrose (%)	65.4 ± 0.9	64.2 ± 0.7	0.2175
Glucose (%)	0.11 ± 0.004	0.09 ± 0.006	**0.0125**
Fructose (%)	0.69 ± 0.02	0.67 ± 0.02	0.4607
Total invert sugar (%)	0.79 ± 0.02	0.75 ± 0.02	0.0938*
Volatile flavor compounds (millions of peak area count)	2.4 ± 0.3	2.3 ± 0.3	0.9166

Table 1. Chemical composition and properties of maple syrup produced simultaneously from raw maple sap and the same sap concentrated to 8% with RO. Values are averages (± standard error) of 6 experiment trials. *p*-values are for statistical tests to determine whether average values for the two treatments were equal. Bold *p*-values indicate statistically significant differences (α = 0.05).

The pH of syrup produced with raw sap was slightly lower than that of syrup produced with concentrate, though the difference was small and the average values for both syrup types were near neutral (Table 1). Glucose was the only sugar that differed significantly between the two types of syrup, however the difference was also numerically quite small (0.11 vs. 0.09%, Table 1). From a practical perspective, these differences were very small, and would not impart any functional or quality difference between the syrups.

It is possible that the difference in the pH of syrup produced from raw sap and concentrate is simply a residual effect of the slight difference in pH between raw and concentrated sap – in reverse osmosis, the pH of the concentrate will always be slightly higher than the incoming material being concentrated. (This is because RO membranes are permeable to carbon dioxide gas, but not to the bicarbonate ions which typically balance the effects of CO2 on pH.) This can be seen in Table 2, which shows the composition and properties of the sap, concentrate, and permeate used in each trial of this experiment, and the average composition of the sap and concentrate. This slight difference in pH might also explain the small differences observed in glucose – sucrose, the primary sugar found in maple syrup, is comprised of a molecule each of glucose and fructose bonded together. The rate that sucrose is split (hydrolyzed) into glucose and fructose increases as the pH of a solution gets lower. Thus, it's possible that the slightly lower pH of the syrup produced with raw sap might result in slightly increased rates of sucrose hydrolysis, and thus also result in slightly higher concentrations of glucose relative to the syrup produced with concentrate.

Although color differed between the syrup made with the two treatments, the relative quantity of volatile flavor compounds did not differ significantly between syrup made simultaneously with raw sap and the same sap concentrated to 8% (Table 1). This suggests that

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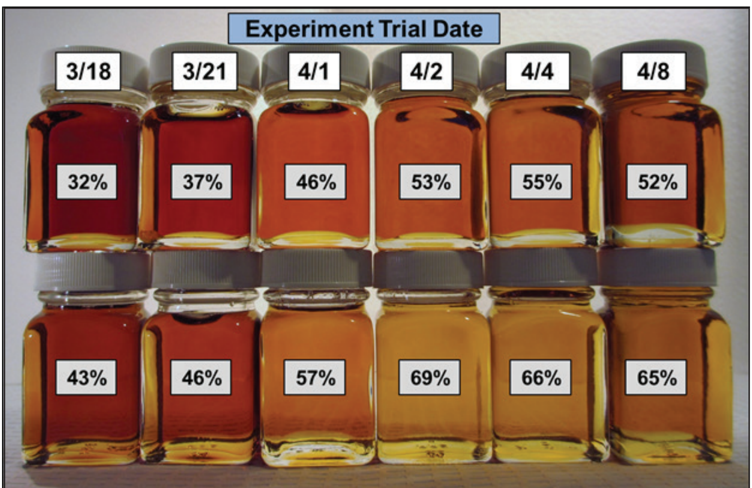

Figure 2. Samples of maple syrup produced simultaneously with raw maple sap (bottom row) and the same sap concentrated to 8% by RO (top row) during 6 experiment trials. Percent values indicate the light transmittance (560 nm) of the samples. Light transmittance ranges for international standard maple syrup grades: ≥75.0% = Golden Color with Delicate Taste, 50.0-74.9%% = Amber Color with Rich Taste, 25.0-49.9% = Dark Color with Robust Taste, <25% = Very Dark Color with Strong Taste.

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producing syrup from sap concentrated by RO to 8% sugar does not significantly affect the overall development of flavor in maple syrup. However, because the total quantity of volatile flavor compounds doesn't always equate with the perceived level of flavor (Belitz *et al.* 2004), a sensory evaluation experiment was also conducted to investigate the potential impacts of RO on syrup flavor. For this experiment, we hypothesized that if concentrating sap with RO significantly affected syrup flavor, then an overall difference should be evident in the flavor of the syrup produced simultaneously with raw sap and the same sap concentrated to 8% with RO. We conducted triangle tests on the pairs of syrup produced during four of the experiment trials (3/18, 4/2, 4/4, and 4/8). However, for each of the four pairs of syrup tested, panelists did not detect a difference in the flavor of the syrups produced simultaneously with raw and concentrated sap (Table 3). For the syrup pair produced on 3/18, the number of panelists who correctly identified the odd sample was close to the critical value necessary to conclude that a difference existed, suggesting that some differences in flavor might be present, but that if present they are likely very subtle. Thus, taken together with the results of volatile flavor compound analysis, these results suggest that concentrating sap to 8% with RO does not significantly impact syrup flavor.

In conclusion, the results of this study indicate that concentrating maple sap to 8% with RO does not substantially affect syrup composition, properties, or flavor. Syrup produced simultaneously from raw maple sap and the same sap concentrated to 8% had similar properties and composition, and had flavor that was indistinguishable by both chemical profiles and by panelists in sensory experiments. The most notable effect observed was on syrup color, which was slightly darker in syrup produced with concentrate. However, the differences observed in color were quite small, with only one of the six sample pairs differing in color grade. Any decrease in syrup value due to this minor reduction in color would be more than offset by the lower fuel and labor costs required for processing concentrate.

Experiment 2 – Comparison of syrup made from sap at 2, 8, 12, and 15% concentration

Another experiment was conducted to investigate the effects of the level of concentration with RO on the properties, composition, and flavor of the syrup produced. In this experiment, syrup was produced simultaneously with sap at 2, 8, 12, and 15% sugar concentration, and its composition, properties, and flavor analyzed and compared.

Methods

Specifically, sap concentrated to 15% using a Springtech 1600 unit with a Hydranautics LSY-PVD-1 membrane and the permeate generated during concentration were obtained. Three hundred gallons of the 15% concentrate were retained, while the remainder was diluted with the appropriate quantity of the permeate necessary to generate 300 gallons of sap at each of three different sugar concentration levels: 2, 8, and 12%. Each treatment was placed in a separate stainless steel tank which fed one of four identical, 3 × 10′ evaporators (as described above), and the evaporators were started simultaneously and allowed to continue processing until

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																			Averages	
Experiment trial date	3/18/2011			3/21/2011			4/1/2011			4/2/2011			4/4/2011			4/8/2011			Treatment	
Material type	P	S	C	P	S	C	P	S	C	P[†]	S	C	P	S	C	P	S	C	Raw Sap	8% Concentrate
Brix (°)	0.0	2.3	8.4	0.0	2.4	7.7	0.0	2.4	8.1		2.5	7.8	0.0	2.5	8.2	0.0	2.2	8.0	2.4 ± 0.05	8.0 ± 0.11
pH	6.3	7.5	7.7	5.8	6.7	7.1	6.0	7.0	7.3		6.6	6.9	6.0	7.0	7.4	6.0	6.7	7.1	6.9 ± 0.09	7.2 ± 0.10
Conductivity (µS cm^{-1})	6.3	460.7	1218.0	6.5	504.7	1217.0	3.2	398.0	987.8		460.0	1083.0	3.3	456.6	1105.0	4.6	464.5	1203.0	457 ± 14	1136 ± 38
Calcium (ppm)	0.09	49.9	196.0	0.12	60.0	198.0	0.04	41.8	141.0		47.5	160.0	0.11	50.5	173.0	0.11	52.1	193.0	50.3 ± 2.4	176.8 ± 9.4
Phosphorous (ppm)	bdl	0.5	3.4	bdl	0.5	2.2	bdl	0.4	1.9		0.2	0.9	bdl	0.2	1.1	bdl	0.3	1.4	0.4 ± 0.1	1.8 ± 0.4
Potassium (ppm)	1.1	58.8	256.0	1.0	62.8	257.0	0.5	49.9	212.0		56.1	189.0	0.5	56.1	202.0	0.7	56.7	276.0	56.7 ± 1.7	232.0 ± 14.5
Magnesium (ppm)	0.012	5.6	20.5	0.013	6.7	20.5	0.004	4.5	14.5		5.4	16.8	0.009	5.7	18.1	0.010	6.0	20.6	5.6 ± 0.3	18.5 ± 1.0
Iron (ppm)	bdl	0.1	0.2	bdl	0.1	0.2	bdl	0.0	0.1		0.1	0.2	bdl	0.1	0.1	bdl	0.1	0.2	0.1 ± 0.01	0.2 ± 0.01
Manganese (ppm)	bdl	5.5	22.0	bdl	5.6	18.6	bdl	4.2	14.3		4.8	16.2	bdl	5.3	17.9	bdl	6.0	21.8	5.2 ± 0.3	18.5 ± 1.2
Boron (ppm)	bdl	0.1	0.1	bdl	0.1	0.1	bdl	0.0	0.1		0.1	0.2	bdl	0.1	0.1	bdl	0.0	0.1	0.05 ± 0.004	0.12 ± 0.009
Copper (ppm)	bdl	0.1	0.2	bdl	0.1	0.2	bdl	0.0	0.2		0.1	0.2	bdl	0.0	0.2	0.03	0.0	0.1	0.05 ± 0.01	0.18 ± 0.02
Zinc (ppm)	bdl	0.3	1.2	bdl	0.3	1.0	bdl	0.2	0.8		0.2	0.8	bdl	0.2	1.0	bdl	0.3	1.0	0.2 ± 0.0	1.0 ± 0.1
Sulfur (ppm)	bdl	0.8	3.7	bdl	1.0	3.9	bdl	0.7	2.5		0.9	3.7	bdl	0.9	3.5	bdl	1.0	4.0	0.9 ± 0.05	3.5 ± 0.22
Sucrose (%)	nm	1.8	7.1	nm	1.8	6.1	nm	2.0	7.0		1.9	5.4	nm	1.9	6.4	nm	1.6	6.4	1.8 ± 0.1	6.4 ± 0.2
Glucose (%)	nm	0.07	0.24	nm	0.09	0.25	nm	0.07	0.22		0.10	0.29	nm	0.10	0.26	nm	0.09	0.23	0.09 ± 0.006	0.25 ± 0.010
Fructose (%)	nm	0.04	0.12	nm	0.04	0.14	nm	0.03	0.11		0.04	0.16	nm	0.04	0.13	nm	0.04	0.13	0.04 ± 0.002	0.13 ± 0.007
Total invert sugar (%)	nm	0.1	0.4	nm	0.1	0.4	nm	0.1	0.3		0.1	0.5	nm	0.1	0.4	nm	0.1	0.4	0.13 ± 0.01	0.38 ± 0.02

Table 2. Composition of the sap and 8% concentrate used in each of the 6 experiment trials, and the permeate generated during sap concentration. Overall averages (± standard errors) of the composition and properties of sap and concentrate used in the 6 trials are also shown. S= raw sap, C= sap concentrated to 8%, and P = permeate generated during sap concentration.

Table 3. Number of correct responses in triangle tests conducted with 26 panelists to determine if differences in flavor were detectable between pairs of maple syrup produced simultaneously from raw sap and the same sap concentrated to 8% during 4 experiment trials. The flavor of each pair was considered significantly different ($p < 0.01$) with 15 or more correct responses

Experiment trial date	Number of correct responses
3/18	14
4/02	12
4/04	7
4/08	12

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the supply of available liquid for each was consumed. After the first 1.5 hours of processing, all syrup produced by each treatment evaporator was collected separately. After the experiment was complete, the syrup produced with each treatment was filtered with a plate filter press, then packaged and frozen for subsequent analyses. The experiment was repeated on four days during the 2008 production season (4/2, 4/3, 4/5, and 4/8).

Sample analyses for color, conductivity, pH, carbohydrate, mineral and flavor composition were performed as described in the previous experiment. Data were compiled and for each parameter, repeated measures analysis of variance procedures were used to determine if significant differences existed between the averages of syrup produced simultaneously with sap at the four treatment levels of sugar concentration. If an overall significant difference was found between the four treatments for a parameter, pairwise comparisons between the individual treatments were performed with orthogonal contrasts to determine if significant differences existed between any of the individual sap concentration treatment levels. For sensory evaluation, triangle tests were conducted to determine if an overall difference could be detected in the flavor of syrup produced simultaneously with sap at 2% and 15% concentration. Individual triangle tests were conducted for each of the syrup pairs produced during the four experiment trials, using 22 adult panelists with experience tasting and grading maple syrup. Pairs were considered different ($p < 0.01$) if 14 of the 22 panelists correctly identified the odd sample (Meilgaard *et al.* 2006). More detailed descriptions of the methodology and analytical methods used can be found in van den Berg *et al.* (2011).

Results

As in the first study, very few differences were observed in the composition or properties of syrup produced with the different sap concentration levels. The conductivity of syrup differed significantly between syrup produced with the four treatments, with a pattern suggesting lower conductivity in syrup produced with more concentrated sap. This pattern followed and was likely driven by that of the composition of manganese (Mn) in the samples, which was lower in syrup produced with more concentrated sap, and was the only mineral which differed significantly between the treatments (Table 4). Similar to Mg in the first study, the differences between the treatments were small, averaging approximately 30 ppm. Also similar to the first study, the pH differed significantly between the treatments, with slightly lower values in syrup made from less concentrated sap. In the first study, the quantity of glucose was slightly higher in syrup made from raw sap than in syrup made from concentrated sap; similar to this, in this study the quantity of total invert sugar (glucose + fructose) and fructose differed significantly between the treatments, also with slightly higher amounts found in syrup made from less concentrated sap. Like the first study, though statistically significant, all of these differences were numerically quite small, and unlikely to impart any functional or practical difference between the syrup made with the different concentration levels.

Syrup color also differed slightly, but significantly, between the treat-

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ments. However, the general pattern appears to be the opposite of what was observed in the first study – syrup produced with more concentrated sap tended to be *lighter* in color than syrup produced with less concentrated sap. However, looking at the data more closely, although this pattern is evident when comparing syrup made from 2% versus 15% sap, and 8% versus 12 and 15% sap, syrup made from 8% sap was actually *darker* than syrup made from 2% sap, the same result observed in the first study. Thus, although the data generally show a pattern of increasingly lighter syrup with increasing sap concentration, the results again show that syrup made from 8% concentrate was slightly darker than syrup made from 2% sap. It is important to emphasize that the differences in color between the treatments were extremely small in this study. For example, the difference between the average light transmittance of syrup produced with 2% and 8% sap was 3.1 percentage points, and the average difference in light transmittance between syrup samples produced with the different treatments in each trial was approximately 4 percentage points. Further, in three of the four trials, syrup produced with all four treatments fell within the same color grade classification (Figure 3).

Although an overall significant difference was detected in the total quantity of volatile flavor compounds between the syrup produced with the four concentration levels of sap, no significant differences were observed in the quantity of flavor compounds in pairwise comparisons between the individual treatments (2% *vs.* 8%, etc.)

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Parameter measured	Treamtent 2%	8%	12%	15%	Overall *p*-value	*p*- values for pairwise comparisons 2% vs 8%	2% vs 12%	2% vs 15%	8% vs 12%	8% vs 15%	12% vs 15%
Brix (°)	66.8 ± 0.4	67.1 ± 0.4	67.6 ± 0.1	67.2 ± 0.2	0.2107						
Conductivity (μS cm^{-1})	120.6 ± 5.7	106.0 ± 1.2	95.2 ± 4.5	98.0 ± 5.0	**0.0014**	0.0984	**0.0016**	**0.0109**	0.1352	0.2466	0.3078
Light transmittance (%)	46.4 ± 6.2	43.3 ± 6.9	46.4 ± 7.3	51.2 ± 6.1	**0.0088**	0.0659	0.9902	0.0630	0.1171	**0.0438**	**0.0419**
pH	6.0 ± 0.0	6.3 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	**0.0001**	**0.0100**	**0.0047**	**0.0008**	0.3654	0.0985	0.8518
Calcium (mg kg^{-1})	1045 ± 59	1011 ± 86	944 ± 106	870 ± 141	0.3218						
Phosphorous (mg kg^{-1})	bdl	bdl	bdl	bdl							
Potassium (mg kg^{-1})	768 ± 47	764 ± 47	740 ± 47	751 ± 42	0.9271						
Magnesium (mg kg^{-1})	115.1 ± 8.6	120.5 ± 3.1	118.2 ± 3.3	114.9 ± 3.0	0.6540						
Iron (mg kg^{-1})	bdl	bdl	bdl	bdl							
Manganese (mg kg^{-1})	75.9 ± 7.0	39.1 ± 1.7	31.4 ± 1.8	26.4 ± 3.0	**0.0001**	**0.0168**	**0.0121**	**0.0118**	**0.0014**	**0.0073**	**0.0449**
Boron (mg kg^{-1})	bdl	bdl	bdl	bdl							
Copper (mg kg^{-1})	bdl	bdl	bdl	bdl							
Zinc (mg kg^{-1})	2.3 ± 0.3	2.3 ± 0.4	2.0 ± 0.4	1.8 ± 0.3	0.4660						
Sulfur (mg kg^{-1})	bdl	bdl	bdl	bdl							
Sucrose (%)	58.9 ± 0.5	59.1 ± 0.3	59.1 ± 0.4	59.1 ± 0.5	0.9021						
Glucose (%)	0.28 ± 0.05	0.24 ± 0.02	0.22 ± 0.02	0.23 ± 0.03	0.1084						
Fructose (%)	0.30 ± 0.04	0.24 ± 0.03	0.23 ± 0.03	0.23 ± 0.03	**0.0001**	**0.0184**	**0.0199**	**0.0097**	0.0747	0.0587	0.8033
Total invert sugar (%)	0.58 ± 0.09	0.48 ± 0.06	0.46 ± 0.06	0.46 ± 0.06	**0.0103**	0.1053	0.0568	0.0821	**0.0372**	**0.0153**	0.5668
Volatile flavor compounds (millions of peak area count)	9.1 ± 2.2	13.2 ± 0.9	18.3 ± 2.9	18.6 ± 2.1	**0.0494**	0.2417	0.1449	0.1093	0.1429	0.0557	0.9034

Table 4. Chemical composition and properties of maple syrup produced simultaneously from sap at 4 concentration levels: 2, 8, 12, and 15%. Values are averages (± standard error) of 4 experiment trials. Overall p-values are for statistical tests to determine whether the average values of the 4 treatments were equal and, where significant overall differences were detected, for pairwise comparisons to determine if significant differences existed between the averages of the individual treatments. Bold p-values indicate statistically significant differences ($\alpha = 0.05$).

Experiment trial date	Number of correct responses
4/02	10
4/03	6
4/05	10
4/08	7

Table 5. Number of correct responses in triangle tests conducted with 22 panelists to determine if differences in flavor were detectable between pairs of maple syrup produced simultaneously from sap at 2% and 15% concentration during 4 experiment trials. The flavor of each pair was considered significantly different ($p < 0.01$) with 14 or more correct responses.

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(Table 4). This suggests that the overall statistical difference is not indicative of any actual differences between the individual treatments. Interestingly, the data suggest a trend for syrup made from more concentrated sap to contain more volatile flavor compounds than syrup produced with less concentrated sap (Table 4), which contrasts with the common anecdotal hypothesis that syrup produced from more concentrated sap might be less flavorful than syrup made from less concentrated sap. This also contrasts with the common assumption that the level of flavor is positively correlated with syrup color – in these syrups, lighter colored syrups contained *more* flavor compounds than darker colored syrups. Sensory evaluation experiments were conducted to further examine the flavor of the syrup produced with the different treatments. We hypothesized that if differences in flavor existed in syrup produced with the different concentration levels, that they would be most readily detectible in syrup produced with the two most extreme treatment levels, 2 and 15%. Thus, we conducted triangle tests to determine if differences could be perceived in the flavor of the pairs of syrup produced simultaneously with 2 and 15% sap on each of the four experiment trial dates. In the triangle tests, panelists were not able to detect differences in the flavor of syrup produced simultaneously with these treatments for any of the four pairs tested (Table 5). Together, these results suggest that producing syrup with different concentrations of sap did not result in significant impacts on syrup flavor.

In conclusion, the results of this study indicate that producing syrup with sap concentrated to between 8 and 15% does not substantially affect syrup composition, properties, or flavor. Syrup produced simultaneously from sap at 2, 8, 12, and 15% sugar concentration had similar properties and composition. In addition, no difference was detected in the flavor of syrup produced simultaneously with the same sap at 2 and 15% concentration. Very small

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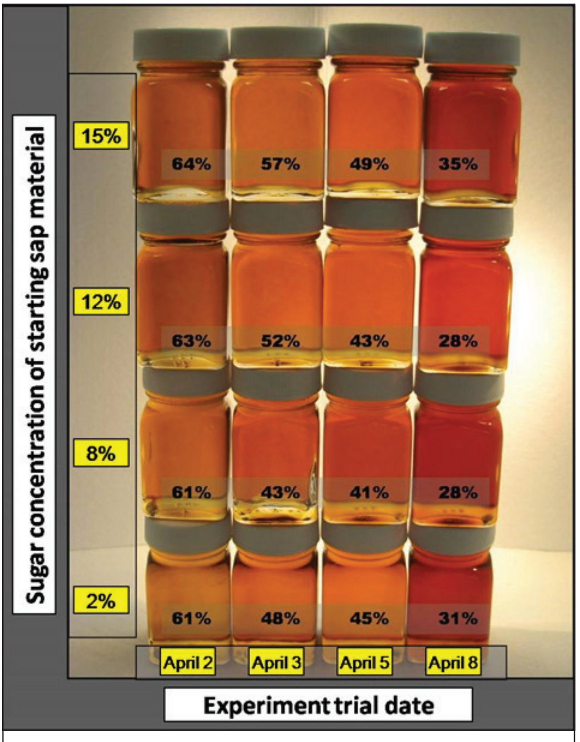

Figure 3. Samples of maple syrup produced simultaneously from sap at four sugar concentration levels (2, 8, 12, and 15%) during 4 experiment trials. Samples in vertical columns are the syrups produced simultaneously with the different concentration levels during each trial of the experiment. Percent values indicate the light transmittance (560 nm) of the samples.

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differences were observed in the color of syrup produced with the different treatments – the general, overall trend observed was for lighter colored syrup to be produced from more concentrated sap. However, consistent with the results of the first study, syrup produced from sap at 8% concentration was slightly darker than syrup produced from sap at 2%.

Experiment 3 – Comparison of syrup made from sap concentrated to moderate and high levels with RO

Although the first studies demonstrated that concentrating sap with RO up to 15% does not result in substantial impacts on the syrup produced, one remaining question was whether concentrating sap to higher levels might have greater impacts on syrup properties, composition, or flavor. Concentrating sap to levels greater than 15% increases efficiency and profitability by further reducing the time and cost of processing sap to syrup density. However, since it also further reduces the length of time sap is processed with heat in the evaporator, it is possible that syrup could be affected to a greater degree than when processing sap concentrated to more moderate levels (8-15%). Thus, a third study was conducted to investigate the potential impacts of concentrating sap with RO to a level higher than 15% on the properties, composition, and flavor of the syrup produced, and determine if any significant differences existed in syrup produced simultaneously with the same sap concentrated to either moderate or high levels.

Methods

To accomplish this, we conducted a study in which syrup was produced simultaneously from the same sap concentrated to either 8 or 21.5% sugar. Specifically, for each trial of the experiment, a common source of maple sap was concentrated sequentially to generate 300 gallons each of 8 and 21.5% concentrate using a CDL RO unit equipped with 8 × 40 Mark I membranes (Dow FilmTec, Midland, MI, USA). Each treatment was placed in a separate tank that fed one of two identical, 3 × 10′ evaporators (as described previously). The evaporators were started simultaneously and run continuously until the supply of concentrate for each was consumed. All syrup produced with each treatment after the first 1.5 hours of processing was collected. After processing was complete, the syrup produced with each treatment was filtered separately with a plate filter press, and was then kept frozen until subsequent analyses. Separate trials of the experiment were conducted on five individual days during the 2009 maple production season (3/17, 3/19, 3/28, 3/29, 4/2).

Sample analyses for color, conductivity, pH, carbohydrate, mineral, and flavor composition were performed as described in the previous experiment. Data were compiled and for each parameter, a paired Student's *t*-test was used to determine if significant differences existed in the means of the syrup produced simultaneously from the same sap concentrated to either 8 or 21.5%. For sensory evaluation, individual triangle tests with 28 panelists with experience tasting and grading maple syrup were conducted for each pair of syrup produced during the first four trials of the experiment to determine if an overall difference could be detected in the flavor of maple syrup produced simultaneously with the same sap concentrated to 8 and 21.5% sugar. Pairs

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were considered different ($p < 0.01$) if 16 of the 28 panelists correctly identified the odd sample (Meilgaard *et al.* 2006). More detailed descriptions of the methodology and analytical methods used can be found in van den Berg *et al.* (2012).

Results

Syrup produced with sap concentrated to 21.5% was significantly lighter in color than the syrup produced simultaneously with the same sap concentrated to only 8% (Table 6). The difference in percent light transmittance ranged from 2.0 to 17.7 percentage points, with an average difference of 11.1 (Figure 4). Three of the five syrup pairs fell within different grade classes. Besides the difference in syrup color, there were no other significant differences in the properties, mineral, or carbohydrate composition of the syrup produced simultaneously with sap concentrated to either 8 or 21.5% sugar (Table 7).

The quantity of volatile flavor compounds also did not differ significantly between syrup produced simultaneously from the same sap concentrated to 8 and 21.5% sugar (Table 6). Further, in triangle tests, panelists did not de-

Parameter measured	8%	22%	*p*-value
Conductivity (μS cm^{-1})	222.7 ± 18.6	235.3 ± 14.8	0.1805
Light transmittance (%)	34.1 ± 6.5	45.2 ± 4.3	**0.0160**
pH	6.9 ± 0.1	7.1 ± 0.1	0.2640
Calcium (ppm)	1027 ± 170	862 ± 140	0.3016
Phosphorous (ppm)	bdl	bdl	
Potassium (ppm)	2138 ± 94	2142 ± 83	0.8975
Magnesium (ppm)	143.6 ± 21.9	128.6 ± 23.0	0.1492
Iron (ppm))	5.1 ± 1.4	2.9 ± 0.7	0.2864
Manganese (ppm)	19.7 ± 10.41	12.8 ± 5.3	0.1250 *
Boron (ppm)	bdl	bdl	
Copper (ppm)	bdl	bdl	
Zinc (ppm)	3.6 ± 0.1	3.6 ± 0.2	0.7917
Sulfur (ppm)	18.8 ± 3.2	15.5 ± 3.4	0.1875
Sucrose (%)	65.6 ± 0.4	64.9 ± 1.1	0.5298
Glucose (%)	3.0 ± 0.2	3.2 ± 0.3	0.6031
Fructose (%)	2.1 ± 0.2	1.6 ± 0.2	0.1210
Total invert sugar (%)	5.1 ± 0.2	4.8 ± 0.4	0.4902
Volatile flavor compounds (millions of peak area count)	5.0 ± 0.4	5.0 ± 0.6	0.9727

** Indicates mean comparison made with nonparametric Wilcoxon Signed Rank tests.*

Table 6. Chemical composition and properties of maple syrup produced simultaneously from the same sap concentrated with RO to either 8 or 21.5% sugar. Values are averages (± standard error) of 5 experiment trials. p-values are for statistical tests to determine whether average values for the two treatments were equal. Bold p-values indicate statistically significant differences (α = 0.05).

Experiment trial date	Number of correct responses
3/17	14
3/19	8
3/28	13
3/29	10

Table 7. Number of correct responses in triangle tests conducted with 28 panelists to determine if differences in flavor were detectable between pairs of maple syrup produced simultaneously from the same maple sap concentrated with RO to 8 and 21.5% sugar during 4 experiment trials. The flavor of each pair was considered significantly different ($p < 0.01$) with 16 or more correct responses.

tect differences in the flavor of syrup produced simultaneously from sap concentrated to 8 and 21.5% for any of the four pairs tested (Table 7). That no differences were detected in either the perceived flavor or the quantity of flavor compounds between the syrup produced with the two treatments strongly indicates that producing syrup from highly concentrated sap does not significantly affect syrup flavor.

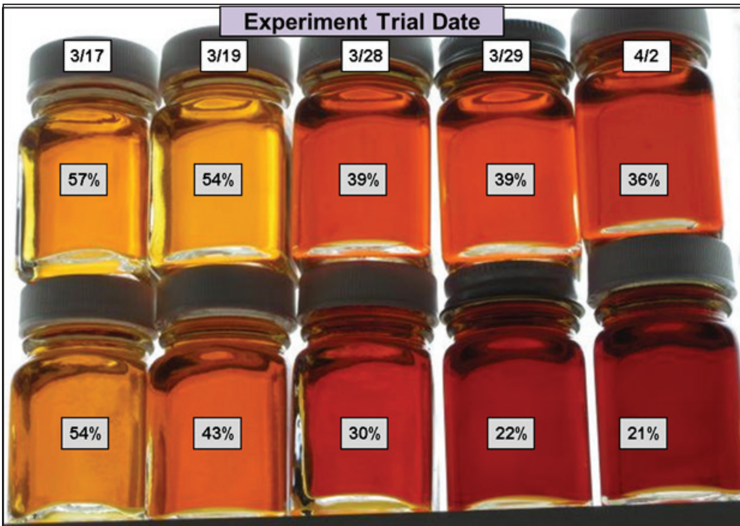

Figure 4. Samples of maple syrup produced simultaneously from the same sap concentrated with RO to either 8 (bottom row) or 21.5% (top row) sugar during 6 experiment trials. Percent values indicate the light transmittance (560 nm) of the samples.

In conclusion, the results of this study indicate that producing syrup with sap concentrated to very high levels by RO does not significantly affect syrup properties, composition, or flavor. Syrup produced with sap concentrated to 21.5% was slightly lighter in color than syrup produced from the same sap concentrated to only 8%, however there were no other significant differences in the composition, properties, or flavor of the two types of syrup.

Conclusions

Broadly, the results of these experiments indicate that the use of RO at any concentration level has no substantive impacts on the composition, properties, or flavor of the syrup produced. A few general observations of the results of these experiments can be summarized as follows:

Color: The most notable effect observed throughout the three experiments was on syrup color. In general, the results suggest that producing syrup from 8% concentrate is likely to result in slightly darker syrup than with raw sap, and that concentrating to levels above 8% is likely to result in progressively slightly lighter colored syrup. However, it's important to emphasize that the effects observed on syrup color were generally very small, often not large enough to cause the color grade to differ. Thus, the results of these studies suggest that producing syrup with concentrated sap is generally likely to have relatively minimal impacts on syrup color.

Flavor: The results of all three studies indicate RO does not significantly impact syrup flavor. No effects of RO on syrup flavor were observed, either

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by chemical composition analyses or panelists in sensory experiments, between syrup produced simultaneously from raw sap and the same sap concentrated to 8%, from the same sap at 2 and 15% concentration, or from the same sap concentrated to 8 and 21.5%. Thus, although given the slight differences in color observed it is reasonable to conclude that using RO has some impact on flavor development, these results indicate that any impact is quite subtle, and beyond what most people are able to perceive.

Other composition and properties: Very few other differences were observed in the composition of syrup. In all cases, the differences that were observed were numerically very small, and not likely to be of any practical significance. In addition, all values for all parameters measured in syrup produced with all treatments during the three experiments were within published ranges for the composition and properties of pure maple syrup (Stuckel and Low 1996, Perkins *et al*. 2006, van den Berg *et al*. 2006, Perkins and van den Berg 2009).

Concentrating sap with RO can provide very large savings in time, fuel, and energy use, increasing the profitability of syrup production substantially. The collective results of these experiments demonstrate that these benefits can be achieved without detriment to the quality of the syrup produced.

	Permeate	Sap	Concentrate	Calculated Concentration
Brix (°)	0.0	2.4	8.1	8.1
pH	6.0	7.0	7.3	
Conductivity (μS cm^{-1})	3.2	398.0	987.8	
Calcium (mg kg^{-1})	0.04	41.8	141.0	141.0
Phosphorous (mg kg^{-1})	bdl	0.4	1.9	1.3
Potassium (mg kg^{-1})	0.5	49.9	212.0	168.3
Magnesium (mg kg^{-1})	0.004	4.5	14.5	15.0
Iron (mg kg^{-1})	bdl	0.04	0.12	0.14
Manganese (mg kg^{-1})	bdl	4.2	14.3	14.2
Boron (mg kg^{-1})	bdl	0.04	0.09	0.14
Copper (mg kg^{-1})	bdl	0.04	0.15	0.14
Zinc (mg kg^{-1})	bdl	0.2	0.8	0.7
Sulfur (mg kg^{-1})	bdl	0.7	2.5	2.3
Sucrose (%)	nm	2.0	7.0	6.6
Glucose (%)	nm	0.07	0.22	0.24
Fructose (%)	nm	0.03	0.11	0.10
Total invert sugar (%)	nm	0.10	0.33	0.34

Table 8. Example composition of raw sap and the same sap concentrated to 8% with RO, the permeate generated during concentration, and the hypothetical concentration of the 8% concentrate when calculated by multiplying the composition of raw sap by the concentration factor used in the generation of the concentrate. In this sample, the concentration factor from raw sap to concentrate was 3.4. (Data from 4/1 trial of Experiment 1.)

The composition of invert sugar is of particular note. The concentrate does not contain any more glucose, fructose (or total invert sugar) than what is calculated by the concentration factor. One effect of concentrating sap with RO that is often presumed is that concentrate will have a proportionally greater invert concentration than the raw sap it was generated from (due to increased microbial activity with greater sugar concentrations). These data clearly demonstrate that this does not occur. Concentrate does not contain proportionally more invert than the raw sap; the invert content of concentrate was simply proportional to that of the sap.

Expanded Discussion

That the syrup produced with raw sap and sap concentrated to different levels with RO was very similar is not surprising, given that the primary difference between raw sap and concentrate is simply the concentration of the substances present. When RO's are functioning properly, very little else besides water passes through the membrane and into the permeate. This is illustrated well by the data in Table 8, which shows the composition of a sample of raw sap and the 8% concentrate made from that sap, along with the hypothetical composition of the concentrate calculated by multiplying the concentration of the substances in the raw sap by the concentration factor used in the concentration of the sap to 8%. The calculated concentrations are very similar to the actual concentrations measured in the 8% concentrate. So, RO concentrate is essentially concentrated sap. The differences between the actual and calculated concentrations of the concentrate are mostly in the ions that pass through RO membranes in very minute amounts, predominantly potassium (K), magnesium (Mg), and calcium (Ca). However, as you can see in Tables 8 and 2, permeate contains very little of even these ions; in fact, in the study of raw sap versus 8% concentrate, for example, over 99.8% of Ca and Mg, and 98.7% of K, were retained in the concentrate (Table 2). If we consider the reactions that occur during sap processing in a very simplified way, the rates of these reactions are influenced by temperature, the concentration of reactants, and the length of time they occur. With concentrated sap, the length of time for reactions to occur is reduced, but the concentration of reactants and temperature are both increased. Thus, it may simply be that the changes are somewhat balanced out, and processing raw sap or concentrate made from that sap, whether concentrated to 8, 15, or 20%, results in very similar syrup.

So why, then, do we see some differences in color between syrup produced with raw sap and different levels of concentrate? Concentrating sap to 8% is a much larger concentration step than concentrating from 8% to higher levels. Most of the water removed by RO, ~75%, is removed in in this step, and concentrating sap to higher concentrations than 8% removes proportionally much less water (Figure 5). It is possible that the large concentration step from 2 to 8% doesn't result in reductions in processing times large enough to balance the simultaneously large increases in concentration, and thus slightly more color development reactions occur with 8% concentrate than with raw sap. But when sap is concentrated to levels higher than 8%, the reductions in processing time may be sufficient to overcome the increased concentration, and fewer color development reactions occur during processing than with less concentrated sap.

This is a very simplified account of the reactions that occur as sap and concentrate are processed in the evaporator and doesn't take into account changes that the presence of particular types of reactants, like the reducing sugars glucose and fructose, or in processing conditions like pH, can have on the reactions that occur during processing. In reality, the reactions that occur when sap is processed with heat in the evaporator are so complex that it's unlikely to ever be possible to accurately or consistently predict the effects that changing a single variable will have on the syrup produced. In general, much of the color and flavor in maple syrup develops through reactions that occur when sug-

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ar solutions are processed with heat, termed "nonenzymatic browning reactions." These include sugar degradation reactions as well as Maillard reactions, which are reactions between reducing sugars, like glucose and fructose, and nitrogen-containing compounds, like amino acids. Nonenzymatic browning reactions yield colored pigments and flavor and aroma compounds, including both desirable and off-flavors. These reactions are enormously complex and occur both simultaneously and in series, so that a change in one variable at one point in processing is likely to result in a cascade of changes affecting all subsequent reactions. In addition, these reactions can be affected by even minute changes in numerous variables, including pH, moisture levels, and the concentration and types of reactants present. For example, different color and flavor compounds develop when glucose or fructose are heated with the same amino acid. Likewise, heating glucose with different types of amino acids results in a unique set of compounds for each of the different amino acids. Altering the pH of any one of these reactions would result in yet another unique set of colored pigments and flavor and aroma compounds. So even very small changes in processing conditions in the evaporator, such as pH, or in the types or proportions of reactants present, such as glucose and fructose (which are more reactive than sucrose in nonenzymatic browning reactions) can alter the nature and rate of reactions that occur during processing in very complex ways. However while this complexity makes specific prediction of the effects of any processing treatment on syrup difficult, despite this, the results of our experiments consistently demonstrated that effects of RO on syrup composition, color, and flavor were minimal.

Acknowledgements

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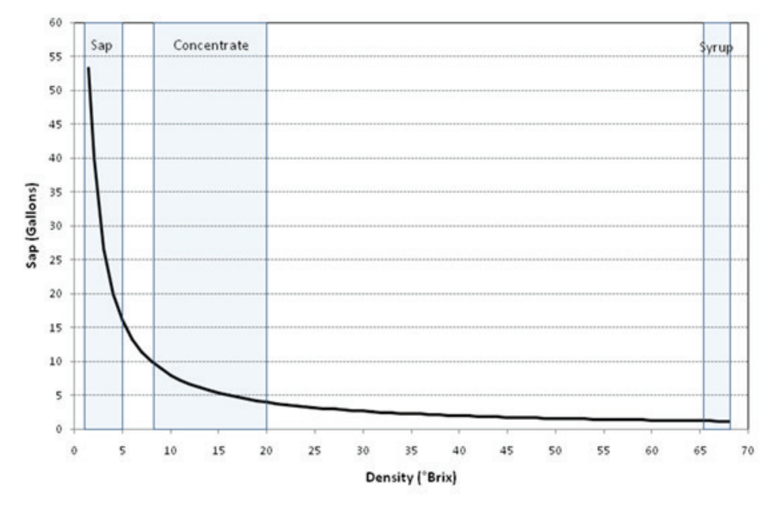

Figure 5. Relationship between sap sugar concentration (°Brix) and the number of gallons required to produce one gallon of maple syrup (Figure from Perkins and van den Berg 2009).

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