

Research: Sap beverages

Determining Shelf Life and Consumer Acceptability of Processed Maple Sap Beverages for Small Businesses

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Abstract

Consumer interest in local products and functional beverages has increased markedly in recent years. Several beverages composed primarily of maple sap are currently available in the marketplace. These products are sold at ambient temperature, and are considered shelf stable. Prior investigation has demonstrated the need for specialized processing equipment in order to achieve shelf stability of maple sap beverages. The current study investigated the feasibility of producing a refrigerated beverage without the use of commercial processing equipment for small businesses. Acidification with either citric acid or lemon juice did not increase shelf life in heat treated samples and was associated with changes in the visual properties of the beverages. Although boiling was sufficient to produce a beverage with greater than thirty days of refrigerated shelf life, samples were highly susceptible to recontamination, which resulted in spoilage in under one week. All processing methods investigated led to formation of a cloudy precipitate. Several filtration methods were unsuccessful in efficiently removing this precipitate. Consumer acceptability of the visual characteristics of the beverages was

also tested, and revealed that the acidified samples were significantly less acceptable than the unacidified control sample. This study suggests that production of a refrigerated sap beverage is possible at small scale, but cannot be recommended due to the difficulty of maintaining a suitably sanitary environment and product. Ongoing experimentation will focus on the feasibility of sap as a substrate for fermented beverage products, which may overcome some of the limitations highlighted in the current report.

Introduction

The market for functional beverages in the U.S. has experienced immense growth in recent years, currently comprising an estimated 59% of the complete functional foods market (Corbo and others 2014). Maple sap is known to be a rich source of minerals and other health-promoting compounds, including antioxidants (Yuan and others 2013), and has potential for use in functional beverage applications, which would allow maple syrup producers to generate additional revenue from their raw material. A number of maple sap beverages are currently available nationally, including Maple Water and

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Sap! maple soda water. These products are aseptically packaged, allowing them to be sold without refrigeration. While this method offers a safe and stable product, it also requires use of very costly processing lines.

Before processing, maple sap is expected to contain a number of different microorganisms originating in the environment, collection system and/or storage vessels. Prior work by researchers in this group showed over one million bacteria per milliliter of unprocessed sap (Calder and others 2016). The types of microorganisms present can change depending on time of season, how sap is collected, how long it is stored and the temperature of storage, but often includes bacteria such as *Pseudomonas* spp., which can grow even during refrigeration (Filteau and others 2011). In order to produce a commercially viable product, most, if not all of these microorganisms, need to be inactivated by processing. Common methods for accomplishing this goal include heat, addition of salt or acid, and exclusion of air. Even with the correct use of these strategies, many microorganisms are still capable of survival, and their growth during storage leads to microbial spoilage.

A shelf life of greater than thirty days is needed to allow adequate time for processing, transport, merchandising, purchasing and consumption of a typical food product. This project investigated the feasibility of producing a perishable maple beverage using processing methods and equipment available to very small producers. The study also sought to clarify the effects that individual treatments, including heating time and acidification have on microbial populations, as well as the

acceptability of the product's visual appearance to consumers.

Research Objectives:

1) Study 1: To determine if boiling at 212°F for different time intervals would extend the shelf life of sap.

2) Study 2: To determine the combined effects of boiling time and acidulant on the microbial shelf life of refrigerated maple sap beverages.

3) Study 2: To identify the primary types of microorganisms responsible for sap beverage spoilage.

4) Study 3: To gain insight into precipitate formation and removal in processed maple sap.

5) Study 4: To assess consumer acceptance of the appearance of sap beverages.

6) To help establish guidance for small maple producers interested in producing sap beverages.

Materials and Methods:

Sap was collected in Somerset County, Maine and transported in clean and sanitized (100ppm bleach) food-grade, plastic buckets to the University of Maine () School of Food & Agriculture's Matthew E. Highlands Pilot Plant. Sap was frozen and held at -10 to -12°C for several months. Prior to the studies, sap was thawed under refrigeration at 3-4°C and then immediately used.

STUDY 1: Heating study

The sap treatments were selected based on an initial study that found higher pasteurization temperatures were more adequate in lowering the microbial load of sap (Calder and others 2016). Boiling temperature (212°F) was selected based on the processing/visual ease for producers inherent in

the fact that 212°F can be observed as a rolling boil. Treated sap was immediately poured into sanitized glass containers, capped, inverted for 5 minutes and allowed to cool for 30 minutes at room temperature. The containers were then held at refrigerated temperatures (3-4°C) until further analyses. Sap was tested for pH, Brix and also aerobic plate counts (bacteria), yeast and molds using 3M™ Petrifilms™. Analyses occurred on days 0, 3, 7, 14, 21, and 28. The pH and Brix values were analyzed in triplicate replications, while microbial testing was conducted in duplicate reps.

Sap Treatments:

- 1) Control fresh sap, no treatment

Boiled sap time:

- 2) 2 minutes
- 3) 5 minutes
- 4) 10 minutes
- 5) 15 minutes

STUDY 2: Heat and acidification study

In order to investigate the interaction of boiling and acid addition to sap, two extended boiling times (10 and 15 minutes), and two commercially available acid sources (citric acid and lemon juice) were combined. Sap was tested for pH, Brix and also for initial microbial population counts including aerobic mesophiles, lactic acid bacteria, fungi and coliforms. All microbial tests were conducted on cultural media, (tryptic soy agar; deMann, Rogosa, Sharpe agar; acidified potato dextrose agar; lauryl sulfate tryptose broth, respectively). Sap was treated as outlined below, stored at 4°C and analyzed periodically during storage for 42 days.

Sap Treatments:

- 1) Control; unacidified, not heated (C0), boiled for ten (C10) or 15 minutes (C15)

- 2) Acidified to pH 4.0 with lemon juice, not heated (LJ0), boiled for ten (LJ10) or 15 minutes (LJ15)

- 3) Acidified to pH 4.0 with citric acid, not heated (CA0), boiled for ten (CA10) or 15 minutes (CA15)

Heating temperature was selected due to ease of measurement, acidulants were selected according to availability and consumer familiarity. Processing was carried out in UMaine's Food Microbiology Laboratory, a BSL (biosafety level) II facility. Sap was boiled in stainless steel pots, samples were immediately transferred into separate, sterile, disposable 15ml conical tubes, such that each sampling was conducted from a previously unopened tube. Tubes were allowed to come to room temperature and were refrigerated for the duration of the study. Three full, independent study replicates were conducted.

STUDY 3: Precipitate Formation

In a separate set of experiments, the effects of acidification, heating and cooling methods on precipitate formation (previously observed to be associated with processing) were assessed. Samples were heated by boiling for 10 or 15 minutes and were acidified to pH 4.0 by addition of citric acid.

Sap Treatments:

- 1) Control; unacidified, not heated (UN)

- 2) Unacidified, heated and cooled at room temperature (UHRT), refrigerator (UHF), or on ice (UHI)

- 3) Acidified, not heated (AN)

- 4) Acidified, heated and cooled at room temperature (AHRT), refrigerator (AHF), or on ice (AHI)

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Various filtration methods including the use of cone filters, cheesecloth, centrifugation, and vacuum filtration were assessed. Sample clarity was measured in a spectrophotometer at 580nm.

STUDY 4: Consumer sensory testing

In order to gauge consumer acceptability of the appearance of processed sap products, a consumer test was conducted at the University of Maine Sensory Testing Center. Approval was obtained from the Institutional Review Board for the Protection of Human Subjects. Panelists were recruited by email or through use of flyers posted near the sensory testing center. Random three digit codes were generated for each sample, samples were presented simultaneously in randomized order to each panelist on a white background. Bev-

erages were presented in clear or blue glass bottles (4oz each), panelists were instructed not to open bottles. All samples were boiled for 15 minutes. Treatments assessed were:

- 1) Unacidified (clear bottle)
- 2) Acidified to pH 4.0 with citric acid (clear bottle)
- 3) Acidified to pH 4.0 with lemon juice (clear bottle)
- 4) Acidified to pH 4.0 with lemon juice (blue bottle)

A blue bottle was used to obscure presence of precipitate, lemon juice-acidified sap was filled into this container because it appeared the cloudiest of treatments assessed. Panelists were asked to rank liking on a scale of 1-9 where 1 corresponded with "dislike extremely" and 9 corresponded with "like extremely". Attributes assessed included clarity, thickness, color and



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overall appearance. Testing was conducted using SIMS software, version 6. Results were analyzed in the same program, compared using analysis of variance followed by Tukey's Honest HSD.

Color was measured instrumentally using a LabScan XE colorimeter (Hunter Associate Laboratory, Inc., Reston, VA). Sap was tested at room temperature in a glass sample cup topped with a white disk. An opaque cover prevented ambient light interference. Reflectance was measured with the white disk backing, an illuminant of D65, 10° standard observer angle, and a 1.75-inch-diameter viewing area. The CIELAB L_ab values were collected. Individual samples were measured in triplicate and sap treatments were analyzed in triplicate to obtain the averages.

Results and Discussion

STUDY 1: Heating study

The untreated sap had a high bacterial load, which ranged from 5.66 log CFU/ml on Day 0 to the highest level detected on Day 21, which was 6.74 log CFU/ml (estimated over 1,000,000 bacterial cells per ml of sap). These counts

were similar to bacterial results from our initial study (Calder and others 2016). However, heat treatments were quite effective in lowering bacteria to non-detectable levels, even after only two minutes of boiling (Table 1). During refrigerated storage, untreated sap samples maintained consistent bacterial counts, while the heat-treated sap samples showed slight increases in bacterial growth on Day 21 and 28. By Day 28, the 2 minute sap treatments had TNTC (Too Numerous To Count) levels. Yeast and mold counts were quite similar and ranged from 3.02 to 3.77 log CFU/ml in the untreated sap samples (results not shown). Similar to the bacteria results, the heat treatments were effective in lowering the yeast and mold to non-detectable levels. However, heat treatments were even more effective as yeast and molds were not detected in any of the heat-treated samples during the entire 28 days storage time.

The sap pH levels ranged from 6.0 to 8.0 for all treatments. The untreated sap appeared more acidic than the heat treated samples, demonstrating a pH level decrease during storage from an

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Table 1: Average Aerobic Plate Counts (Bacteria, Log CFU/ml) in Maple Sap During Refrigerated Storage

<i>Treatment</i>	<i>Day 0</i>	<i>Day 3</i>	<i>Day 7</i>	<i>Day 14</i>	<i>Day 21</i>	<i>Day 28</i>
Control	5.66±0.07	6.03±0.06	5.99±0.01	6.58±0.02	6.74±0.02	6.48±0.02
2 Min	ND	ND	ND	ND	2.37±0.41	TNTC
5 Min	ND	ND	ND	ND	<1.0	ND*
10 Min	ND	ND	ND	ND	ND	1.22±0.77
15 Min	ND	ND	ND	ND	<1.0	1.12±0.71

Heat treatment was 212° F at the following treatment times:

2 Min = 2 minutes; 5 Min = 5 minutes; 10 Min = 10 minutes; 15 Min = 15 minutes

These sap treatments were not acidified.

After capping, heat treated containers were inverted for 5 min, cooled to room temp and stored at 3-4 deg C.

ND = Not detectable (≤10 CFU/ml); TNTC = Too Numerous to Count

Averages n=6; *Day 28, n=4 for the 5 min treatment

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initial value of 6.34 to 5.28 on Day 28. The decreasing pH values over time could be due to the microbial activity in the sample jars, which increased during storage. Heating seemed to have an unexpected effect on sample pH levels. The higher the treatment time, the higher the pH levels, and this trend stayed consistent over storage time. The 2 and 5 minute heat treated samples had pH levels > 7.40, while the 10 and 15 minute heat treated samples had pH levels >7.70.

The Brix levels in the untreated sap samples were found to be 2 Degrees Brix (an estimated 2% sugar level), which would be expected in maple sap. However, Brix levels slightly decreased in the 2 and 5 minute treated samples, which was unexpected. For the 10 and 15 minute treated samples, the Brix levels increased, which would be expected

since the longer the boiling time, the more water is evaporated from the sap to concentrate the sample sugars. The 15 minute heat-treated sap samples had Brix values >2.50.

During the course of the study, we experienced cloudy sap samples along with a white precipitate across all treatments. These observations were also noted in our initial study (Calder and others 2016). The precipitate could be from denatured amino acids or a mineral precipitate bound to amino acids. Although the Control samples were not heated, the components in sap may be susceptible to cold and/or heat shock which could cause the precipitate to form. These aspects were investigated further in Studies 2 and 3.

STUDY 2: Heat and acidification study

Study 2 took a more in-depth look at the microbial populations present in



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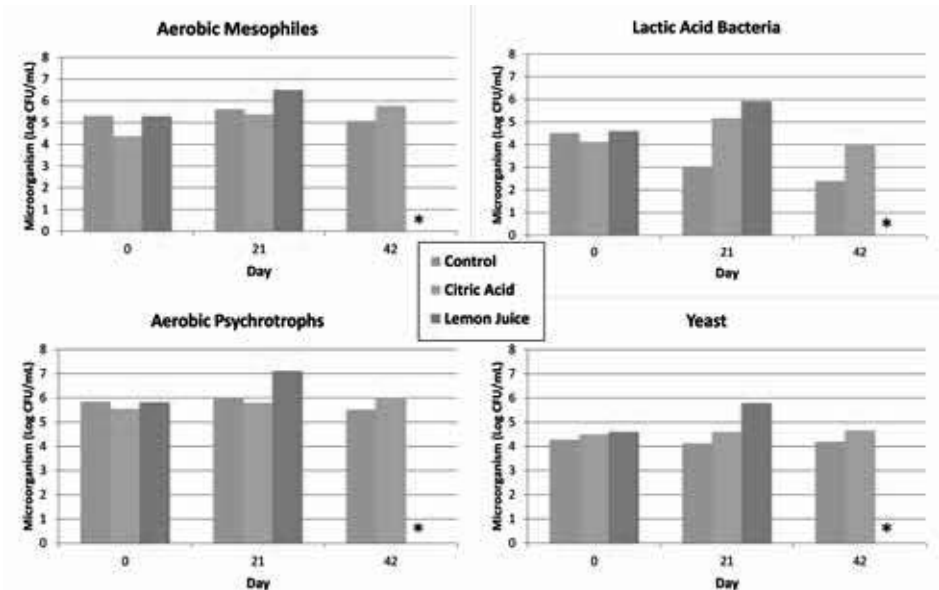


Figure 2: Predominant Microbial Populations in Unheated Maple Sap During Refrigerated Storage. n = 3, citric acid and lemon juice samples acidified to pH 4.0. * This sample was only evaluated for 21 days due to extremely high counts

sap during storage. Coliforms, an indicator of potential fecal contamination and/or poor sanitation, are commonly enumerated in agricultural commodities. Using a standard Most Probable Number (MPN) method, we were unable to detect any coliforms during this study (< 3 MPN/ml). Consistent with the results of Study 1, boiling, whether for 10 or 15 minutes, regardless of the presence or type of acidulant, was sufficient to reduce microbial populations by greater than 5 log CFU/ml. No significant increases in population were observed in any of the heat treated samples (data not shown).

Notably, the addition of acid was not associated with a significant decrease in any microbial populations on Day 0 (Figure 2). In unheated samples, unacidified sap maintained lower counts for yeast and lactic acid bacteria throughout 42 days of storage when compared to sap containing either citric

acid or lemon juice. Samples acidified with lemon juice also demonstrated the highest levels of aerobic mesophiles (total bacteria growing at body temperature) and psychrotrophs (total bacteria growing at refrigeration temperature) by Day 21 of storage. Levels of psychrotrophic bacteria were higher in unprocessed sap than any of the other populations tested, and were highest in samples acidified with lemon juice.

STUDY 3: Precipitate Formation

In all experiments conducted to this point, we have observed the presence of haze or snowy precipitate in sap samples during storage. Study 3 was undertaken to assess the factors that contribute to the lack of clarity, and to see whether practical methods for clarification could be employed. Regardless of filtration method, precipitate removal was unsuccessful. While no clear clarity

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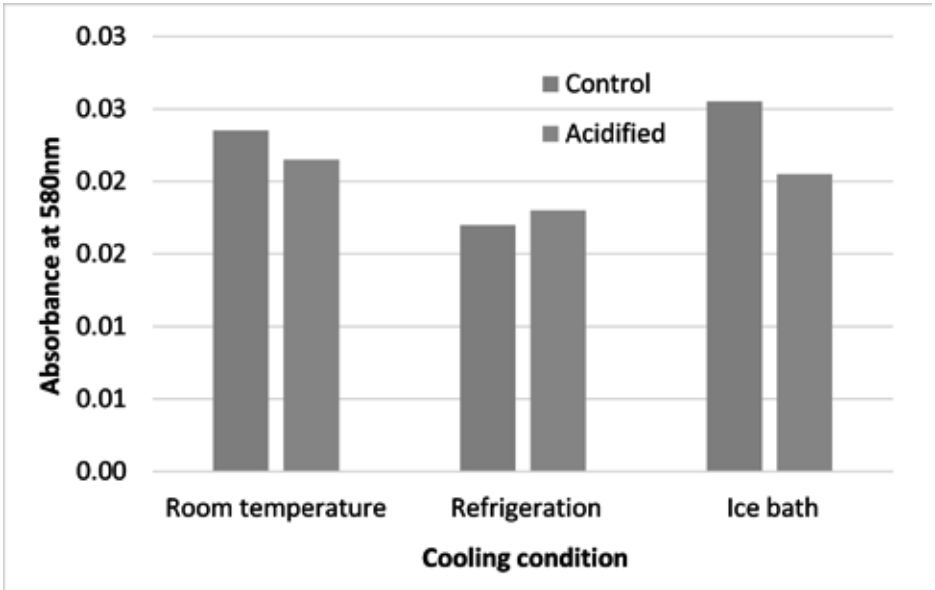


Figure 3. Clarity of Boiled Maple Sap Cooled at Various Temperatures. n = 4. Boiling for 10 minutes. Acidification to pH 4.0 with citric acid.

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trend was observed according to cooling methodology used, samples cooled under refrigeration demonstrated the least cloudiness (lowest absorbance, Figure 3), but values were highly variable. While investigators suspect that more costly methods, such as membrane filtration, would be successful in clarifying sap, the expense associated with such processing places those methods out of scope for this study. Since it is unlikely that small producers will be able to successfully clarify sap beverages, the effect of appearance on consumer acceptance was investigated (Study 4). Mineral composition analysis of this precipitate is ongoing.

STUDY 4: Consumer sensory testing

In a visual sensory test conducted at the UMaine Sensory Testing Center, 63 panelists rated their liking of the appearance of various sap beverage prototypes. Panelist demographics are dis-

played in Table 2.

Of the panelists who participated, 64% said they would be interested in trying a maple sap beverage and 83% would consider purchasing the control sample. The maple beverages that were acidified with either acid were liked significantly ($p < 0.05$) less than the control (Table 3). Panelists liked the color of the control and citric acid samples significantly more than the samples acidified with lemon juice.

As shown in Figure 4, the lemon juice sample had the highest yellow value of the three samples and most likely the color disliked most by consumers. The primary feedback was the precipitate in the sap was unappealing. The feedback on using a colored bottle to mask the texture of the beverage was mixed, which is likely a result of presenting both clear and opaque bottles in the same test. As previously

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Table 2. Sensory Evaluation Panelist Characteristics

<i>Attribute</i>	<i>Response</i>	<i>Percentage</i>
Gender	Male	41.3
	Female	57.1
	Other/Prefer not to say	1.6
Age	18-30	66.7
	31-40	9.5
	41-50	3.2
	51-60	11.1
	61-70	9.5
Functional beverage consumption	At least weekly	25.4
	At least monthly	57.1

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mentioned, instrumental analysis revealed that the primary color difference among samples was a higher b^* value for the sap acidified with lemon juice, which corresponds to a more yellow, less blue appearance (Figure 4).

Appendix: Fermented beverage feasibility

During the course of this project, a limited study was conducted to assess the potential for utilizing mixed cultures of yeast and bacteria to produce a fermented beverage from maple products. Several different kombucha culture systems were utilized for this testing. While the sugar concentration in sap alone is not sufficient to drive successful fermentation, kombucha was successfully prepared from a number of combinations of maple sap and syrup. Traditionally, kombucha is made from black tea sweetened with sugar. We compared beverages with the same total sugar, replacing water with sap, sugar with syrup, or both. During fer-

mentation, pH serves as the primary measurement of progress, with typical product fermentation taking 7 days and producing a product with a final pH of 3.5 – 2.5. Results for this experiment are displayed in Figure 5.

Experimental treatments were successfully fermented, as the substitution of syrup for sugar almost indistinguishable, with both entering the finished product pH range on day 7. The combination of maple sap and sugar fermented fastest, reaching a finished pH after only four days, while the sap and syrup samples fermented the slowest, finishing after 10 days.

Conclusions

Sap is highly perishable and contains a high bacteria and yeast load that could be a potential food safety issue for consumer health. While extended heating of sap appears to be effective in lowering the microbial levels for a shelf life of at least 42 days under refrigeration.

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Table 3. Average Hedonic Scores for Visual Attributes of Maple Sap Beverage Prototypes

<i>Treatment</i>	<i>Color</i>	<i>Thickness</i>	<i>Clarity</i>	<i>Overall liking</i>
Control	6.7	6.4	6.6	6.6
Citric acid	6.3	6.2	5.4	5.6
Lemon juice (clear bottle)	5.6	5.9	4.6	5.0
Lemon juice (blue bottle)	5.5	6.0	5.3	5.5

Ranked on 1-9 scale where 1 = Dislike extremely and 9 = Like extremely

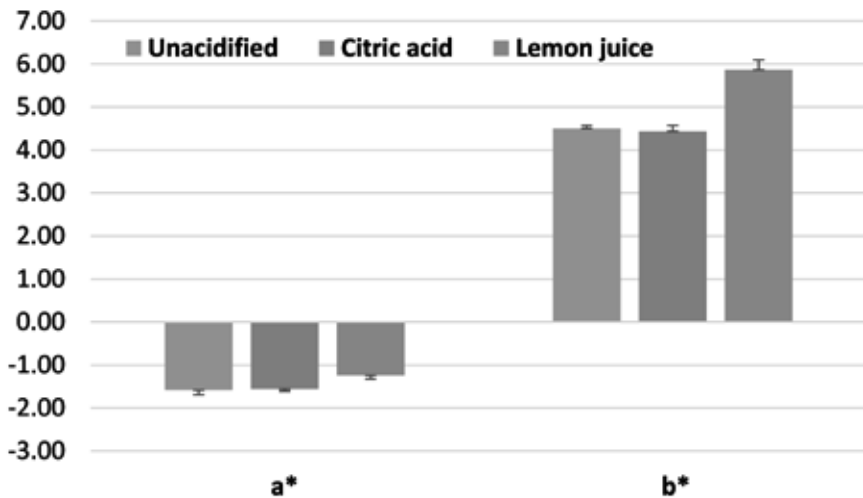


Figure 4. Color Values for Processed Sap Beverages. Error bars represent standard deviation, n = 9. a* ranges from red (positive) to green (negative). b* ranges from yellow (positive) to blue (negative).

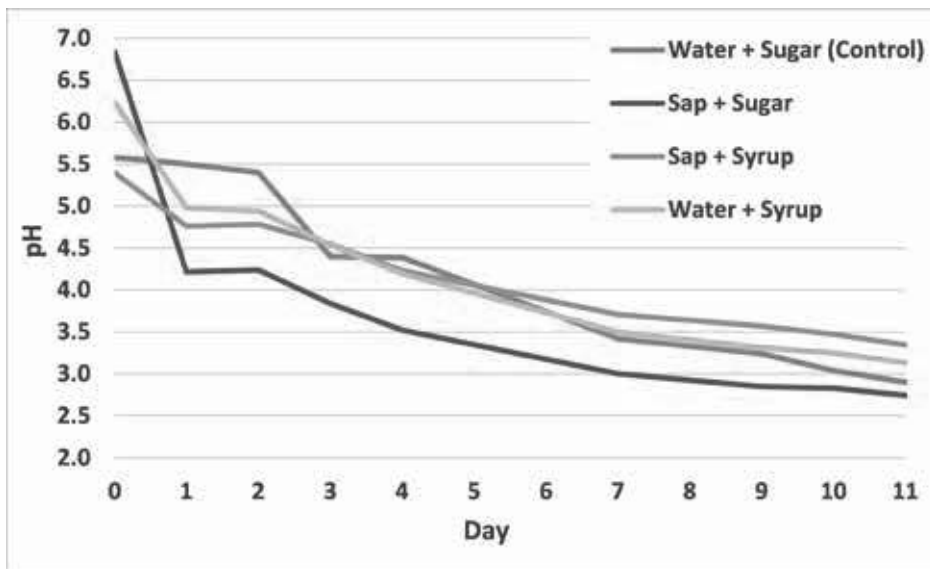


Figure 5. pH of Experimental Maple Kombucha Beverages During Fermentation. n = 3. Initial sugar concentration 10% in all treatments, fermentation carried out at ambient temperature.

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tion, any sanitation failure resulting in introduction of bacteria from air or packaging is likely to result in rapid spoilage, even when the product is promptly refrigerated. Initial consumer data suggests that people are willing to try maple sap beverages and would consider buying them at retail locations. The acidification of the sap led to a significant decrease in consumer acceptability of the beverages' overall appearance, as lemon juice significantly decreased consumer acceptability of the beverage color.

The production of a refrigerated maple-based beverage at very small scale cannot be recommended due to lack of microbial stability and unresolved issues of product clarity. Initial investigations into the fermentation of sap show promising results, and would likely overcome the main hurdles associated with producing a heat treated, acidified product. More research is warranted to explore options for value-added production from maple sap at this scale, particularly with regard to fermented beverage concepts.

Acknowledgments

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References

- Calder BL, Davis-Dentici K, Hopkins KM. 2016. Evaluating Processing Methods to Produce Safe, Shelf-Stable Maple Sap Beverages for Small Operations. *Maple Syrup Digest*. February, 30-39.
- Corbo, M.R., A. Bevilacqua, L. Petruzzzi, F. P. Casanova, & M. Sinigaglia. 2014. Functional Beverages: The Emerging Side of Functional Foods. *Compr Rev Food Sci Food Saf* 13: 1192-1206. doi: 10.1111/1541-4337.12109.
- Filteau M, Lugacé L, LaPointe G, Roy D. 2011. Correlation of maple sap composition with bacterial and fungal communities determined by multiplex automated ribosomal intergenic spacer analysis (MARISA). *Food Microbiol* 28(5): 980-989.
- Yuan, T., L. Li, Y. Zhang, N.P. Seeram. 2013. Pasteurized and sterilized maple sap as functional beverages: Chemical composition and antioxidant activities. *J Funct Foods* 5: 1582-1590. doi: 10.1016/j.jff.2013.06.009.