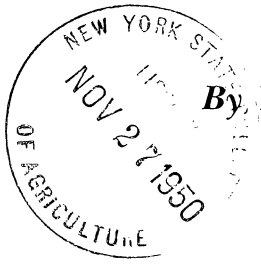
Changes In The Composition Of Maple Sap During The Tapping Season



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CHANGES IN THE COMPOSITION OF MAPLE SAP DURING THE TAPPING SEASON

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ABSTRACT

MAPLE SYRUP produced entirely by a freeze concentration procedure does not have characteristic color, flavor, or aroma. It is grayish and has a pungent sweetish taste. The true maple flavor and amber color are developed by boiling.

The constituents of maple syrup that are responsible for its pleasing qualities are not present as such in the sap but are developed by the action of heat on one or more sap components.

A sterile technique which makes it possible to collect sap free of the action of microorganisms throughout the season was used to demonstrate certain physiological processes in the maple tree.

It was found that organic nitrogen is present in maple sap throughout the season, and that it increases markedly as the season draws to a close. Appearance of buddy flavor was noted in syrup made from sterile sap at about the time the nitrogen content began to increase.

Various compounds were tried to inhibit the growth of microorganisms in sap. Objections were found to all, although carefully controlled applications of chemically pure hydrogen peroxide offered some success.

INTRODUCTION

THE physiological processes of the hard or sugar maple tree, *Acer Saccharum,* and the chemistry of maple sap and its products have been the subject of numerous investigations during the first half of this century. These investigations were undertaken for various purposes, but the majority may be roughly classified into the following groups: (A) A basis and methods of analysis for the detection of adulteration in maple products; (B) the identification of the constituents of maple sap and its products, particularly those related to flavor; (C) tree metabolism as reflected by seasonal changes in the constituents of sap or wood extracts; and (D) sugar bush culture and the physical aspects of sap collection and syrup production.

ADULTERATION AND FLAVOR STUDIES

The past research that has been performed accounts for substantial attainments in maple chemistry, though many of the ultimate goals

have not yet been achieved. Much of the analytical work on maple products to establish a basis for detecting adulteration was done in the early part of the century. As a result there are a number of tests available for detecting adulteration, such as Winton or Canadian lead number, conductivity value, ash analysis, and Cowles' malic acid value. But even with subsequent improvements such as manganese determinations, Riou and Delorme (15)[1] wrote in 1941 that, "In many cases it can be stated that the product has been adulterated, but it can never be stated on a scientific basis, that the product is 100 per cent pure."

A great deal of effort has been expended in attempting to determine the basic constituents of maple sap which are responsible for characteristic flavor and aroma in maple products. Nelson (12) found that the maple flavor appears to depend to a great extent upon an unstable phenolic substance associated with a vanillin-like aldehyde.

Sair and Snell (16) devised a method for fractionating the chloroform-soluble constituents of maple syrup and found that the substance chiefly responsible for maple odor is indicated to be an enolic viscous oil which is present in very small quantities.

Barnes and Kaufman (1), in their study of the browning reaction in foods, observed that ∝amino n–butyric acid when heated with glucose yields a product which has a flavor and odor which is strongly suggestive of maple.

Many other suggestions can be found, but positive identification of the various specific substances involved in producing characteristic maple flavor and aroma have not yet been made.

FREEZE CONCENTRATION OF SAP

It has been demonstrated that the characteristic flavor of maple syrup and sugar is not present in the sap but is developed during boiling. Findlay and Snell (6) confirmed this fact by showing that syrup produced by vacuum distillation or by partially freezing out the water and completing the concentration in a vacuum was lacking in maple flavor. When such syrups were boiled, however, the characteristic maple flavor developed.

In the course of the work reported here it was decided to see if a similar situation might exist for a syrup not subjected to a vacuum but produced entirely by freeze concentration. A 62° Brix syrup was made by numerous repetitions of a process of freezing the sap, chop-

[1]Reference is to Literature Cited, page 14.

ping the frozen sap to snow in a hammermill, centrifuging the free liquor, and refreezing the liquor thus obtained. The color of the unheated syrup was very light and somewhat gray in appearance. This changed to the usual amber color when boiled. The flavor of the unheated syrup, while not suggesting maple, was quite pungent. A fine maple flavor developed on boiling.

There seems to be little doubt that the flavor of maple products is not present as such in maple sap but is brought about by the action of heat upon one or more constituents of the sap during the concentrating process.

PHYSIOLOGICAL AND CHEMICAL CHANGES IN SAP

There is still much to be learned about the physiological changes that take place in a maple tree not only with the change of seasons but also during the tapping season itself. Variations of a profound nature occur and have great influence upon the quality of maple products.

In a study of the chemical differences between maple saps collected at different stages and which are directly the result of tree physiology, the effects of the action of microorganisms upon the sap must be differentiated. A thorough investigation was made some years ago at the Vermont Agricultural Experiment Station (5) of the influence of microorganisms upon the color, flavor, and chemical composition of syrup. The causes of so-called "buddy sap" were also studied (4).

It was demonstrated that while bacteria are responsible to a large degree for dark-colored or poor-flavored maple syrup, normal tree physiology engaged in the renewal of vegetative activity is also concerned in changing the sap so that late in the season it is no longer suitable for making maple products. Actually, the most common form of microorganisms present in maple syrup have a greater effect upon the color than upon the flavor, and while there are types that seriously affect the flavor, the responsibility for characteristic "buddy flavor" must be placed upon the tree itself.

Comparable observations were made at this Station (8) when it was shown that syrup made from late season sterile sap was as light in color as first run sap. In another investigation of the role of bacteria, Hayward and Pederson (7) have shown that the degree of darkening of maple syrup depends upon the alkalinity of the sap and the invert sugar content, both of which are increased by bacterial action.

CHEMICAL DETERMINATIONS ON ASEPTIC SAP

One of the constituents of sap which has not received much attention is nitrogen. There are reports in the literature indicating that analyses were made for nitrogen and the results reported on the basis of protein (N × 6.25). In 1904, Hortvet (9) determined the extent of the protein in syrup to range from 0.223 to 0.334 per cent.

Jones and Bradlee (10) more recently established a range of 0.0088 to 0.02 per cent in sap also expressed as protein.

It seemed worthwhile to study the nitrogen content of sap further; therefore, during the 1947 and 1948 seasons, the quantitative changes occurring in the nitrogen content of aseptic maple sap throughout the season were determined. Changes in the total sugars, reducing sugars, tannins, and acidity were also included.

APPARATUS FOR STERILE SAP COLLECTION

In order to eliminate the influence of bacteria, a sterile collecting device similar to that shown in Fig. 1 was used. The tapping tools, a ⅜-inch bit, and a wood chisel were wrapped in paper and sterilized in the autoclave. The spout apparatus consisted of a rubber stopper for a 4-liter Erlenmeyer flask which had an inverted glass U-tube plugged with cotton to equalize air pressure and a piece of straight glass tubing which was connected to a spile by a short length of rubber tubing. The spile used was a Willis closed type which was ground smooth on the end so as to obtain a snug fit in the rubber tubing. This assembly was also wrapped in paper and sterilized in the autoclave. The Erlenmeyer flask was plugged with cotton and sterilized in a similar manner. (A piece of ½-inch copper tubing, tapered at the tapping end,

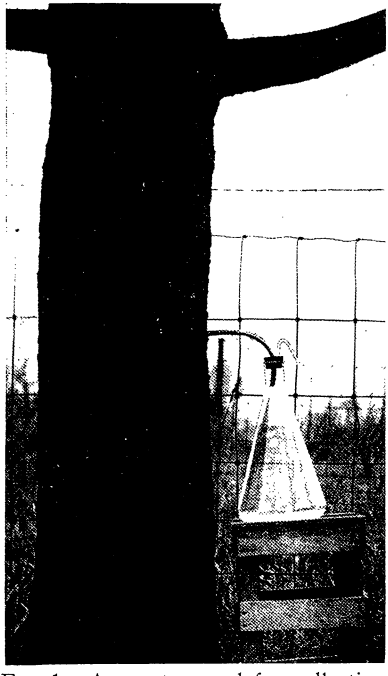

FIG. 1.—Apparatus used for collecting maple sap under sterile conditions.

may be used in lieu of the spile and rubber tubing.)

In attaching the apparatus to the tree, a smooth section of the tree was selected and a *thin* layer of outer bark removed with the wood chisel for about 4 square inches around the site selected. The area was then swabbed with phenol, saturated with alcohol, and ignited. While still burning, a ⅜-inch hole was drilled with the sterile bit and the spile inserted as rapidly as possible. The tree end of the spile was poked through the paper and hammered into place by means of pounding on a screwdriver held against the shoulder of the spile. The cotton was then removed from the flask and the rest of the paper from the spile assembly, whereupon the rubber stopper was quickly inserted in the flask. A support for the flask was provided. When the flask was about two-thirds full, it was removed and replaced with another which had been sterilized. Sterile technique and precautions were used throughout, and in this manner it was possible to collect essentially aseptic sap during the entire season.

METHODS OF ANALYSIS

Total sugar and reducing sugars.—Because of the relatively small amounts of reducing sugars present in maple sap, a colorimetric method such as used for blood glucose worked to the best advantage. Nelson's colorimetric method as modified by Moyer and Holgate (11) was used on a 5–cc sample of sap.

Total nitrogen.—Since a qualitative test for nitrates on all sap samples was negative, the Kjeldahl-Gunning method for the determination of nitrogen in the absence of nitrates was used. Five hundred cubic centimeters of sap were placed in an 800–cc Kjeldahl flask, acidified with H_2SO_4, and boiled until about 95 per cent of the water had been driven off. The regular method was then followed.

Tannins and coloring matter.—The method used is that given for wine in the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, using a 10–ml filtered aliquot of the sap in question.

METHOD OF OBTAINING SAMPLES

During the 1947 and 1948 seasons, the use of a local maple sugar bush was obtained and 12 trees of an average diameter of 15 inches selected. Each one of these trees was tapped, using the aseptic method, and at the same time tapped in the regular manner, using Grimm spiles, buckets, covers, and cheesecloth to keep out insects.

The sap was collected at least once a day, and more frequently if necessary to keep the sterile bottles from overflowing. Bacterial counts were made on all individual samples, and the remainder of the sterile and regular sap composited separately. About 1 gallon of the sap was frozen at — 10°F for later analysis and the rest made into syrup in a small laboratory steam kettle. The syrup was examined for color and flavor only. Results of the analyses made on the various sap samples are shown in Table 1 for the 1947 season and in Table 2, for the 1948 season.

DISCUSSION OF RESULTS

Bacterial Growth.—The counts given in the tables are averages of the 12 separate counts. As indicated by the growth found in some of the regular sap samples, maple sap is an ideal medium for bacterial growth. Whenever the average temperature of the weather begins to approach 40°F, the growth will become very heavy. This growth becomes apparent as a cloudiness in the sap, and unless the sap is collected at frequent intervals may actually continue until the buckets become slimy and the sap putrid. It is interesting to note that the growth occurs not only in the sap in the bucket but in the tap hole as well. For instance, at one time during the 1948 season it was found that the bacterial count of sap in the bucket was 30,700,000 per cc and of the sap taken directly from the tap hole 26,900,000 per cc. Because of this it can be seen that rapid handling and thorough cleaning of buckets and spiles should be accompanied by frequent reaming of the tap holes. The counts obtained from the aseptic collection bottles are sufficiently low to indicate that these samples were essentially sterile, and that the organisms which were found entered at the time of emptying the filled flask and obtaining the samples for analysis.

Hydrogen-ion concentration.—Bois and Dugal (2) studied the hydrogen ion concentration of maple sap and found that it varies with the season, the day, and the individual trees. They placed the extreme values at pH 6.0 to 7.3. Similar results were noted in work reported here, although in very few instances were values of pH 6.50 to 7.1 exceeded, with an average of pH 6.8. Except for cases where the sap had badly spoiled, there was no marked difference between the aseptic and regular sap. However, Hayward and Pederson (7) determined that a difference would become manifest on boiling of the sap since growth of microorganisms in maple sap increases the alkalinity.

Total and reducing sugars.—As the bacterial population becomes

TABLE 1.—ANALYSES OF MAPLE SAP, 1947 SEASON.

DATE	REGULAR SAP						STERILE SAP					
	Bacterial count per cc	pH	Total sugar, per cent	Reducing sugar, mg per cent	Total N, mg per cent	Tannin	Bacterial count per cc	pH	Total sugar, per cent	Reducing sugar, mg per cent	Total N, mg per cent	Tannin
Mar. 13	7,000	6.95	2.33	2.5	0.43	Negative	18	6.95	2.25	1.5	0.30	Negative
14	56,000	6.95	2.08	4.5	0.40	Negative	10	7.00	2.70	0.5	0.44	Negative
17	———	——	——	——	——	———	12	7.00	2.26	1.0	0.39	Negative
19	1,000	6.80	2.60	8.0	0.74	Trace	—	——	——	——	——	———
20	900	6.68	2.73	4.0	0.50	Negative	8	7.02	2.60	0.5	0.17	Negative
21	1,000	6.50	2.63	3.0	0.46	Negative	2	6.85	2.51	1.0	0.23	Negative
22	600	6.52	2.46	4.0	0.58	Negative	—	——	——	——	——	———
24	2,500	6.98	2.33	4.0	0.49	Negative	77	6.65	2.53	1.5	0.28	Negative
29	———	——	——	——	——	———	25	6.62	2.43	1.5	0.22	Negative
Apr. 1	15,000	6.60	2.40	13.0	1.04	Negative	2	6.62	2.43	0.5	0.23	Negative
2	94,000	6.72	2.33	7.0	0.80	Negative	4	6.75	2.18	1.5	0.40	Negative
4	90,000	6.65	1.70	49.0	1.48	Negative	13	——	——	——	——	———
7	31,000,000*	6.90	1.71	11.0	2.23	Negative	31	——	——	——	——	———
10	8,700,000*	6.20	1.80	91.0	1.79	Negative	100*	6.82	2.30	2.5	5.48	Negative
17	27,000,000*	——	——	——	——	———	5*	6.75	2.50	1.0	5.37	Negative
22	4,500,000*	——	——	——	——	———	200*	6.70	2.34	2.1	8.19	Negative

*True buddy flavor developed in syrup made from this sap.

TABLE 2.—ANALYSES OF MAPLE SAP, 1948 SEASON.

DATE	REGULAR SAP						STERILE SAP					
	Bacterial count per cc	pH	Total sugar, per cent	Reducing sugar, mg per cent	Total N, mg per cent	Tannin	Bacterial count per cc	pH	Total sugar, per cent	Reducing sugar, mg per cent	Total N, mg per cent	Tannin
Mar. 16	130	7.10	2.67	5.0	0.77	Negative	4	6.90	2.52	3.5	0.84	Negative
18	600	7.00	2.42	5.8	0.76	Negative	2	6.95	2.29	4.0	0.80	Negative
19	400	7.15	2.05	6.8	1.04	Negative	4	7.10	2.12	2.5	1.25	Negative
22	8,200,000	7.00	1.99	95.0	1.07	Negative						
24							2,700	7.10	1.98	3.0	1.44	Negative
26	6,200,000*	6.50	1.85	38.8	1.98	Trace	200*	7.10	1.82	3.3	2.76	Negative
30	1,500*	7.10	1.95	13.0	3.23	Negative	41*	7.05	2.01	4.0	2.76	Negative
Apr. 2	14,800,000*	5.90	1.75	90.0	4.33	Negative	3*	7.10	1.90	4.5	3.30	Negative
5	5,000,000*	6.40	1.27	10.0	6.20	Negative	7*	6.90	1.72	4.5	3.46	Negative

*True buddy flavor developed in syrup made from this sap.

greater in the sap, the amount of sucrose decreases and through inversion the reducing sugars increase. There is a tendency for the sucrose to decline as the sap season progresses. This phenomenon is associated with physiological processes of the tree and has been reported by other observers (10). In the sap collected aseptically it is apparent that there was a greater quantity of reducing sugars present in the 1948 season than in 1947. In both cases, however, there was little significant change in the amount present throughout the season.

Tannins and coloring matter.—According to Bryan (3), tannins are probably not normal constituents of sap but enter from washings from the tree. A qualitative test for tannins was made because from time to time reference is made in the literature indicating their presence. At no time was a positive test given by any of the closed aseptic systems and only once during each of the two seasons was a trace reported in the regular samples. It was felt that these two cases are probably a result of rain water washing down the tree and following the spout into the pail, or possibly bits of bark falling off and finding their way into the sap.

Total nitrogen.—As a result of negative reactions in all the qualitative tests made for proteins and nitrates, including Millons' reaction, xanthoproteic reaction, glyoxylic acid reaction, the Biuret test, and the diphenylamine test for nitrates, the nitrogen discussed in this paper is assumed to be nonprotein but organic in character. Nitrogen analyses of the scum which rises to the surface of sap during boiling did not give results significantly different than the sap itself, and in analyzing finished syrup the nitrogen content was found to be nearly proportionate to the degree of concentration of the sap, as shown in Table 3.

TABLE 3.—RELATION OF NITROGEN CONTENT TO CONCENTRATION OF MAPLE SAP IN 1947.

DATE	SUGAR, PER CENT		NITROGEN, PER CENT		RATIO OF CONCENTRATION	
	Sap	Syrup	Sap	Syrup	Sugar	N
Mar. 13	2.25	66	0.00031	0.0091	29:1	29:1
Mar. 29	2.43	66	0.00022	0.0066	27:1	29:1
Apr. 1	2.43	66	0.00023	0.0069	27:1	29:1

As shown in Tables 1 and 2, the organic nitrogen tends to remain fairly constant until late in the season when it suddenly increases to a comparatively high level in both the regular and aseptic sap. Our

observation from organoleptic tests of the syrup made from these sap samples is that as the amount reached a level of about 2 mg per cent nitrogen the true buddy flavor began to become evident. As the nitrogen level increased, the buddy flavor became more intense. In view of the small amounts of nitrogen involved the quantity contributed by the cells of a large number of bacteria growing in the regular sap was estimated. Assuming that 1 billion bacteria weigh 0.8 mg (14) and that a microorganism such as *Pseudomonas aeruginosa,* similar to the predominating organisms found in maple sap, contains 2.7 per cent nitrogen (13), 10 million microorganisms would contribute 0.0216 mg per cent nitrogen. This indicates that the nitrogen from the cellular structure of microorganisms would not in itself be significant. However, the microorganisms growing in sap can very well bring about changes in the structure of the organic nitrogen substances so that the total nitrogen found in regular sap may differ in some way from that in aseptic sap.

INHIBITION EXPERIMENTS

Any method whereby the microorganisms which will grow in maple sap can be kept to a minimum is of great value in producing maple syrup with a high-scoring color and flavor grade. Aseptic methods of collection are not practical for commercial operations, and furthermore, it was felt that other methods of inhibiting bacteria, particularly with chemical agents, should be investigated in the event that a simpler method could be found to obtain bacteria-free sap for experimental purposes.

After considering a large number of compounds, objections were found to nearly all. Either they constituted health hazards or impaired the quality of the syrup. Others were highly impractical. Willets and Tressler[2] found that 50 ppm of SO_2 held back the growth of organisms, but that there was so much SO_2 fixed as SO_3 that it could not be removed by boiling. Thus an undesirable flavor was imparted to the finished syrup. If smaller concentrations of SO_2 were used to eliminate this bad feature, the bacteria were not inhibited.

During the 1948 season hydrogen peroxide was used as an inhibitory agent. Thirty per cent CP H_2O_2 in a dilution of 1–7,500 was used. This quantity when added to maple sap being held at room temperature until it could be evaporated, successfully inhibited bac-

[2]Willets, C. O., and Tressler, C. J., Jr. Unpublished progress report of the second season's operation of an experimental maple sugar bush, New York State Agricultural Experiment Station, Geneva, N. Y., 1940.

terial growth for a period of 24 hours and could not be detected by a potassium dichromate test after the sap had boiled for a few minutes. It was found that the quantity of H_2O_2 is quite critical since any more than about a 1–7,500 dilution appeared to have an oxidizing effect on the sap and imparted an undesirable flavor to the syrup. Much less than this dilution will not inhibit bacterial growth satisfactorily. Experiments adding the peroxide to the buckets were not particularly successful for the aforementioned reasons, and in view of the fact that some of the sap would be in contact with a concentrated solution for a long time before the bucket was filled and a proper dilution achieved. Furthermore, commercial hydrogen peroxide usually contains a stabilizer, such as acetanilid, and the chemically pure peroxide is unstable and difficult to handle which makes the application quite complicated.

SUMMARY

Maple syrup produced by freeze concentration methods does not have the characteristic maple color, flavor, or aroma until it has been boiled under atmospheric pressure.

A method of collecting sap aseptically was devised so that the respective roles of bacterial action and tree physiology could be further studied and differentiated. By use of this system it was definitely determined that late in the season the tree in its physiological processes brings about a change in the character of maple sap which makes it unsuitable for maple products.

This sap is commonly referred to as "buddy sap", and as it begins to manifest itself it is accompanied by a definite increase in the organic nitrogen content of the sap.

Dark-colored syrup, stringy, or colored saps are a result of bacterial action, and although bacteria may have their own adverse effect on the flavor of syrup they do not cause the physiologically induced buddy sap.

No readily applicable methods of inhibiting bacterial growth were found. Hydrogen peroxide will accomplish inhibition, but it can also oxidize the sap to produce off-flavored syrups, and it is difficult to apply and handle.

LITERATURE CITED

1 Barnes, H. M., and Kaufman, C. W. Industrial aspects of browning reaction. *Ind. and Eng. Chem.,* **39,** *1167. 1947.*

2 Bois, E., and Dugal, L. C. La sève d'érable et son pH. *Le Naturaliste Canadien,* **67,** *137–141. 1940.*

3 Bryan, A. Hugh. Maple sap sirup. *United States Dept. Agr. Bur. Chem., Bul. No. 134. 1910.*

4 Edson, H. A. Buddy sap. *Vermont Agr. Exp. Sta. Bul. No. 151. 1910.*

5 ————, Jones, C. H., and Carpenter, C. W. Microorganisms of maple sap. *Vermont Agr. Exp. Sta. Bul. No. 167. 1912.*

6 Findlay, G. H., and Snell, J. F. Some constituents of the sap of the sugar maple. *Canadian Jour. Res.,* 13, *Sect. B, 269–275. 1935.*

7 Hayward, F. W., and Pederson, C. S. Some factors causing dark-colored maple syrup. *New York State Agr. Exp. Sta. Bul. No. 718. 1946.*

8 Holgate, K. C. Sterile maple sap prolongs yield, protects quality. *Farm Res.,* **14,** *No. 1, 15. 1948.*

9 Hortvet, J. The chemical composition of maple-syrup and maple-sugar, methods of analysis, and detection of adulteration. *Jour. Amer. Chem. Soc.,* **26,** *1523–1545. 1904.*

10 Jones, C. H., and Bradlee, Jennie L. The carbohydrate contents of the maple tree. *Vermont Agr. Exp. Sta. Bul. No. 358. 1933.*

11 Moyer, J. C., and Holgate, K. C. Determination of alcohol-insoluble solids and sugar contents of vegetables. *Anal. Chem.,* **20,** *472–474. 1948.*

12 Nelson, E. K. The flavor of maple sirup. *Jour. Amer. Chem. Soc.,* **50,** *2009–2012. 1928.*

13 Porter, J. R. Bacterial Chemistry and Physiology. *New York: John Wiley & Sons. 1946.*

14 Rahn, O. Physiology of Bacteria. *Philadelphia: Blakiston Company. 1932.*

15 Riou, P., and Delorme, J. The analytical problem of maple sap products. *Canadian Jour. Res.,* **19,** *Sect. B, 68–72. 1941.*

16 Sair, L., and Snell, J. F. Fractionation of the chloroform extract of maple syrup. *Canadian Jour. Res.,* **17,** *Sect. B, 281–289. 1939.*