MAPLE SAP AND SYRUP

STUDIES ON MAPLE SAP AND SYRUP

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I N T R O D U C T I O N

Maple syrup and sugar belong to North America and are produced only in limited areas of this continent, namely Eastern Canada and adjacent States, New England and Middle-West States. The manufacture of maple syrup probably dates back to a period before the advent of the white man because Sy (22) in a review of the writings of early discoverers, notes many allusions to the preparation by the Indians of a kind of sugar and syrup from the sap of the maple tree. There appears to be some difference of opinion as to whether the French settlers taught the Indians the process or the Indians taught the white people, but the latter alternative seems to be the most probable. Since the discovery, there has been a steady improvement in the methods of preparation of syrup and sugar from maple sap and they have become staple articles, concerning whose character and constitution there is considerable discussion.

(a) Botany of the Maple

The maple belongs to the genus '*Acer*' and for a time some confusion existed regarding the classification of the various species. The famous Swedish taxonomist, Linnaeus,, called the species with which he was most

familiar, namely, the silver or soft maple, '*Acer saccharinum*'. Wangenheim in 1787 started the confusion by calling the sugar or hard maple '*Acer saccharinum*', thinking that Linnaeus meant the sugar maple by this name. This went on for several years and was ultimately corrected to the classification to-day as adopted by careful writers and botanists:

(a) *Acer saccharum*, Marsh -- hard, rock or sugar maple.

(b) *Acer saccharinum*, Li -- soft or silver maple.

(c) *Acer rubrum*, Li -- red maple.

(d) *Acer nigrum*, Michx -- black maple.

All the species of *Acer* have sweet sap, and while the hard maple furnishes by far the largest part of maple sugar, the sap from the other species is used occasionally, especially that from the soft or silver maple.

It will be of interest to outline briefly the general structure and physiology of the sugar maple in order to facilitate the discussion of the experimental work to be described later. The trunk of the maple is divided into two parts:

(a) The reddish-brown heart wood -- duramen,

and (b) The yellowish white sap wood -- alburnum.

The alburnum is thicker than in many other trees, and this fact may partially account for the fact that the maple produces such a flow of sap, because as its name

'sap-wood' indicates, it is the sap conducting tissue. It consists of closed cells and vessels packed closely together without any intercellular spaces such as occur in the stems of some species of plant. The food substances which are contained in these vessels may be classified as living matter (protoplasm), water, solid foods (chiefly starch), soluble matters (sugars), and gas. From the point of view of sap flow and maple syrup production we are concerned merely with the water, carbohydrate foods, and minute amounts of undetermined material which cause the flavour of the finished product.

The carbohydrate foods are manufactured by the tree during the summer and stored for use in the early growth of the following spring. During the late winter or early spring, the ascent of sap begins. This is most active when the tree is in full foliage, but if the maple is wounded or tapped while it is still in the leafless stage, the sap may exude or 'run' from the wounded tissue during favorable weather. This exudation is known by plant physiologists as 'bleeding', but the sugar-makers always use the term 'running', although certain French Canadian farmers refer to it as "leaking".

It is evident that this flow of sap must be the result of some pressure within the tree. Jones, Edson and Morse (5) in 1903 presented a report of

several years' study of the causes and significance of sap pressure and movement, and while they did not reach any definite conclusions, they obtained good evidence for a probable mechanism. However, there still exists a great difference of opinions as to the explanation of these phenomena. Perhaps the best summary of theories as to the cause of sap flow is that given by L. R. Jones and quoted by Sy (22).

(1) High water content of the tree. This does not offer a satisfactory explanation because the tree actually contains more water several weeks after the flow stops.

(2) Root pressure. The work of Jones, et.al. (2) showed conclusively that there is rarely any root pressure.

(3) Alternate thawing and freezing. Jones showed that the sap in a maple tree very seldom freezes so this theory is of no value.

(4) pressure caused by expansion of gas and liquid in the tree due to rise and fall of temperature. Jones attributes some value to this, though he found that the rate of sap flow was not proportional to rise

in temperature and also observed sudden increases in pressure which could not be accounted for by the expansion of gas.

(5) Jones was inclined to believe that the flow of sap is due to the activity of living cells under certain stimuli or conditions. The principal stimulus is undoubtedly the more or less sudden fluctuation of temperature from slightly below 0^{o} to slightly above 0^{o}. This is in accord with the fact that during the sap season, the sap flows best on bright days preceded by nights during which there was freezing.

(b) Composition of Maple Sap.

The first reference to investigation on this subject is that of Wiley (26) in 1885, who determined the percentage sucrose -- presumably by direct polarization -- in the sap of 15 maple trees over one season's run. The average content of sucrose ranged from 2.33% to 5.01%. He found that the concentration of the sap varied when taken: (1) from different sides of the same tree; (2) from different heights on the tree; (3) at

different times of the day.

Following this, Morse and Wood (10) in 1895 published a bulletin on maple sap, but reported little more than the sucrose content for one season, just as Wiley had done. They found that the percentage sucrose varied in saps from different trees from 1.3 to 5.6%, with sap from the outer wood of the trees invariably richer in sucrose than that from the inner wood. The average of a number of analyses of sap shows that the sucrose content is about 3%, but it also contains traces of reducing sugars (invert sugar), organic acids, chiefly malic, mineral matter (inorganic salts, and small amounts of protein substances.

The occurrence of invert sugar in the sap was not noted by early investigators, who maintained that it was formed during evaporation, but Sy (22) obtained wide ranges on different syrups and sugars, which could not be accounted for by differences in the method of preparation. Other investigators noted this, and subsequent analyses of fresh sap showed that reducing sugars do occur in the sap to an extent of about 0.02%. Thus syrup made from such sap would contain about 0.50% reducing sugars even if none were formed during evaporation, but Bryan (1), in an analysis of 481 maple syrups, reported several

which had an invert sugar content below 0.5% and one which had none.

The amount of invert sugar present is greater at the end of the season than at the beginning, and this naturally gives rise to the question:

"What causes the formation of invert sugar?"

It might be supposed that it is formed from sucrose by the malic acid also present, but if this were the case, then since all genuine maple products contain malic acid, we would expect considerable, and about the same amount, in the different syrups or sugars. Actually the invert sugar content varies considerably and it is known that fruit acids cause very little hydrolysis of cane sugar, therefore we must look elsewhere for the cause of inversion.

Now towards the end of the sugar season when the weather is warmer and the buds begin to swell, the sap gradually undergoes certain changes involving a deterioration in color and flavour. It is popularly believed that the swelling of the buds, associated with the renewal of vegetative activity in the tissues of the tree is accompanied by a change in the composition of the sap within the trunk, and that the alteration in color and flavour are manifestations of this change. The term 'buddy' is universally used to describe this

sort of sap, and the syrup prepared from such sap has a characteristic strong taste. It is known, however, that inferior products result from carelessness in handling the sap, and since the proteid, carbohydrate and mineral contents of sap make it a fairly good medium for the development of bacterial life, the activities of large numbers of micro-organisms would presumably affect the flavour and quality of the syrup produced under such conditions.

In 1910, Edson (3) demonstrated by a large number of field and laboratory tests that:

(a) Maple sap as it occurs within the tree is free from bacteria and other micro-organisms.

(b) As the sap flows from the tree it becomes infected, in the taphole, spouts and buckets with wild yeasts, spores of molds, and countless numbers of bacteria. This infection becomes increasingly heavy with the advance of the sugar season and is the cause of the 'souring' of sap.

Edson investigated the effect of micro-organisms on color and flavour only, but Jones (6), following up this work, prepared syrups from sap which he had

inoculated with various bacteria and molds and analyzed them completely. The most interesting variations were to be observed in the invert sugar content -- extremes, calculated to a moisture-free basis, of 0.12 to 28.35% being recorded. The average invert sugar figures for the various organisms used showed a fairly regular gradation from 0.41 to 11.03%, but the maximum and minimum figures among the different groups exhibit wide variations. These differences were doubtless due to the fact that certain strains produced a more complete infection and were better inversion agents for sucrose than were others. Certain of the organisms used, notably those of the fluorescent type group of bacteria, had but little effect on the sucrose. Generally speaking, however, the yeasts and molds, which often but not always thrive well in a slightly acid medium, together with the spore-bearing bacteria, had the most pronounced inverting action on sucrose, either through the production of invertase, or by the formation of acid, or both. In many cases they likewise seriously affected color and flavour.

Therefore, from the above considerations it can be seen that the invert sugar in sap is largely the product of the activities of micro-organisms, though it is quite possible that a very small amount is present in the sap in the tree. The process of boiling pure sap

may produce no marked inversion of the sucrose as Jones showed by evaporating a syrup at a very slow rate, when a low invert sugar content, 0.66%, together with a satisfactory flavour and color are obtained.

(c) Manufacture of Maple Products.

In the early days of the manufacture of maple syrup, the processes and apparatus were very crude. The North American Indians used two methods to obtain a concentrated sugar solution from the sap -- allowing the sap to freeze and removing the ice, and evaporating the sap by placing it in clay or bark vessels and dropping in heated stones. These methods were replaced by boiling in copper or iron kettles, and the practice has been continued in some parts of Quebec even up to the present time. In this primitive kettle process, the sap is boiled continuously during the day, fresh sap being added from time to time, and the syrup is removed at night. This long boiling produces a very dark product with a strong flavour containing more reducing sugars and impurities than the product from modern evaporators. With the latter form of apparatus the process is continuous, the sap being admitted at one end of a pan divided into several compartments and allowed to flow slowly from one to the other in such a way as to

traverse a distance of about 90 feet or more of heating surface. It is 'finished off' in a small compartment by boiling to a temperature of 105°C. or 219°F., when the syrup has a density of 1.32 corresponding to a water content of 35%.

During the boiling process, the separation of two types of substances is to be noted.

(a) After the sap has begun to boil, there is soon observed upon the surface of the liquid a scum which increases in amount as the sap is concentrated. This scum, generally dark brown in color, is believed to be due to a considerable extent to the coagulation of part of the proteins of the sap. A scum, obtained by reboiling syrup which had begun to 'sour', i.e. diluted with water and boiled down to syrup again, was examined by Lochead in this laboratory, and found to contain a large amount of suspended dirt which evidently found its way into the sap from various causes. Besides this, the analysis showed that certain mineral salts of the sap, originally in solution, but precipitated on concentration, must be caught and suspended in the scum,

while the vigorous boiling of the sap, and continual frothing, lead to the accumulation at the surface of a considerable amount of sugar.

(b) As the density of the boiling sap becomes greater, a precipitate is formed, some of which settles to the bottom of the evaporator, but a great deal is filtered off by pouring the hot syrup through a felt 'strainer'. This precipitate is known as 'sugar sand', or 'niter', and consists chiefly of the calcium salts of organic acids, calcium malate being present to the extent of 65-80%. (Snell (21)). Von Lippmann (9) has reported finding tartaric acid in notable quantities, and a small amount of tricarballylic acid, but Snell, though obtaining tartaric acid, states that: "Though our search for other acids has not been thorough, it is reasonably certain that these cannot be present in material quantities." Nelson (14) made an investigation of a sample of maple sugar sand and was

able to identify formic, acetic, fumaric, succinic and citric acids in addition to -malic as well as to obtain evidence of traces of d-tartaric and tricarballylic acids. The lack of concordance among the results of these investigators, points to a variation in the composition of sugar 'sand' which in turn is obviously due to a difference in the history of the syrups from tree to finished product. Then since Jones (6) has shown that micro-organisms have a pronounced effect on the composition and flavour of maple syrup it is quite possible that these acids may be formed as a result of their action on the carbohydrates and other substances present in the sap. Therefore, it is not safe to conclude that all the organic acids reported in the 'sand' are present as such in the original sap.

(d) The Finished Product.

Maple syrup, according to "The Maple Sugar Industry Act, 1930" is defined as: "the syrup made by the evaporation of maple sap, or by the solution of maple sugar in water. Maple syrup shall not contain more than thirty-five per cent of water. A gallon of maple syrup shall weigh not less than thirteen pounds, two ounces and shall contain 277.274 cubic inches".

The high price of maple syrup and sugar is not due to their sweetening power or nutritive value but rather to certain aromatic and condimental bodies which impart to them an agreeable flavour. This flavour is very characteristic and though it varies to some extent in products from different sections of the country, this variation seems to be due, according to Jones (7) and Bryan (1) to the manner of collection of sap and the manufacture of the syrup rather than to peculiarities of the sap of the locality. At any rate, the flavour is developed or intensified during the process of manufacture and it is the purpose of this thesis to follow it from sap to syrup.

THE FLAVOURING PRINCIPLE

(a) Early Investigations.

The first reference to the chemical composition of the flavouring principle of maple products was made by Wiley (25) in 1905, who attributed it to an ether or aldehyde possessing a high boiling point. However he claimed that some of the flavouring matters present in the natural sap of the maple, were volatile, basing his claim on the strongly aromatic odors pervading the air in the vicinity of a maple sugar camp. The really volatile part is probably exceedingly small and Sy (22) believes the odors in question are due mostly to fine droplets of sap or syrup thrown into the air during the boiling or carried with the steam in the same manner as salt is found in sea air and caustic soda in the air over a vessel in which a solution of the caustic has just been made.

There is no question that a characteristic aroma is given off by boiling genuine maple syrup, and Jones (7) makes this the basis of a test to distinguish pure and adulterated products: Dissolve about 20 g. of syrup or sugar in 40 c.c. of hot water, boil, and note odor, either while still boiling or immediately after removal from the heat. This test would not be sufficient

to condemn a sample but considered in connection with other analytical data would be of some value.

Sy (22) carried out an experiment in an attempt to isolate the flavouring matter and was unsuccessful, but proved that very little, if any, of it was volatile. He distilled fresh sap into a closed container (A) connected through two vertical condensers with a flask (B) containing water. The contents of A had a slight maple odor resembling that of corn cobs but had no flavour while those of B had no odor or flavour of any kind. He obtained only a trace of residue from an ether extraction of the distillate in B and concluded that "if the flavouring substances are esters or aldehydes, they are present in but very small amounts or are but little volatile".

In 1911, McGill (11) discussing the range of analytical values of maple syrups, paid some attention to the flavour and suggested that if we knew to what it is due we might quantitatively determine the constituent and make this the basis of a test for genuine products. He believed that certain esters of malic and other acids have most to do with maple syrup flavour, the acids in question yielding calcium salts that are comparatively insoluble in dilute alcohol.

Robison (17) was the first to attempt the isolation of the flavouring principle from maple syrup

by means of extracting agents. He did not extract the syrup directly, but concentrated it under reduced pressure, so that no caramelization would occur, and prepared sugar from it. Extracting this sugar in a Soxhlet extraction apparatus with the following organic solvents: Benzene, chloroform, carbon tetrachloride, ether, ethyl alcohol, acetone and ethyl acetate, -- it was found that benzene extracts gave the most natural maple odor; ether, carbon tetrachloride and chloroform gave fair results, but ethyl alcohol, acetone and ethyl acetate dissolved too much sugar to permit any observations on other substances present. The first group of solvents yielded resinous residues and a small amount of non-resinous material which resembled an oil. The same results were obtained by extracting the sugar with the cold solvents.

Now it had been noted that maple syrup, which had been decolorized with charcoal, lost some of its strength of flavour so Robison applied this fact to separate the flavouring principle. After allowing syrup to remain in contact with charcoal for some time in order for maximum adsorption to take place, the charcoal was filtered off and treated with ethyl alcohol to remove the adsorbed flavour. On evaporation of the alcohol extract a bitter resinous residue was obtained similar to those from extractions of the sugar.

No crystalline substances were obtained from any of the extractions quoted above but in most cases, after driving the solvent from the sugar, it was found that the maple flavour had disappeared, hence it must have been in the solvent. Robison, therefore, applied a number of tests to a concentrated benzene extract of maple sugar, the results of which may be briefly noted here:

(a) Bases tend to precipitate the material in the benzene extract.

(b) Acids in general give color reactions.

Making use of (b), extractions of caramel with the same solvents were tested with acids and it was found that a chloroform extract gave no color with nitric acid, while an orange color was produced with a chloroform maple extract. This difference suggested a colorimetric method for detecting adulteration -- the extent of which could be determined by comparison with suitable color standards. Robison applied the method to a number of pure and adulterated syrups but obtained 'color values' which varied within wide limits and seemed to depend on the character of the original syrup rather than on the extent of adulteration.

The next reference in the literature to flavour

of maple products is a United States Patent of a 'Process for Manufacturing a True Maple Flavoring Product' -- Sale and Wilson (20). Use is made of the fact that sucrose can be removed from the syrup with suitable reagents, leaving most of the flavouring material in solution, (though Robison (17) found that most bases formed a precipitate with flavour extracts). The process consists in adjusting a maple product (sap, syrup or sugar) to a density of about 28°B., heating it and precipitating the sucrose with a solution of barium hydroxide. The precipitate is filtered off, and after neutralizing the excess barium in the filtrate, the liquid is concentrated to a density of 34°B. The product is a concentrated true maple flavour, one part of which is claimed to contain "all or nearly all of the flavour in 40 parts more or less of maple sirup".

(b) Nelson's Analysis of the Flavour.

In 1928, Nelson reported an investigation on the nature of maple flavour in which he used three methods to isolate the unknown substance:

(1) The barium process of Sale and Wilson.

(2) Adsorption of flavour on norite.

(3) Extraction of syrup directly with ether.

Method (1). The concentrated solution of flavouring material obtained from the Sale and Wilson process was extracted thoroughly with ether and the ether solution treated with dilute ammonia. The ammonia extract on acidification and extraction with ether gave a residue which was stirred with water and lead acetate solution, filtered, and the filtrate was extracted with ether. On evaporation of the ether a reddish oil was obtained which was dissolved in dilute sodium carbonate and extracted with ether. The ether residue had a phenolic odor and gave a green color reaction with ferric chloride.

The ether solution left after extraction with dilute ammonia yielded a residue which became partly crystalline. The crystals were separated and recrystallized from ethyl acetate, when white, spindle-shaped crystals melting at 212° were obtained. These were insoluble in water, but dissolved in sodium hydroxide solution. The substance reduced copper acetate and Fehling's solution and gave a crystalline derivative on boiling with ferric chloride. These reactions indicated a quinone derivative but hydrophlorone, which melts at 212°, proved to be crystallographically different.

Method (2). Maple syrup was treated with norite, which removed the flavour, but it was found that the

latter could not be recovered as such from the norite.

Method (3). The work of Nelson on the flavour by this method will be described in detail because his procedure has been followed by subsequent investigators.

Ether extractions were made of 38 l. of both Vermont and Michigan maple syrups and after the acids had been recovered from the extracts with sodium bicarbonate, they were treated with a strong solution of sodium bisulphite. The sodium bisulphite solutions, containing the aldehydes were acidified and extracted with ether.

A. After evaporation of the ether, the residues were boiled out several times with petroleum ether, which on evaporation gave residues with a distinct vanillin-like odor. The substance from the Vermont syrup showed a tendency to crystallize but was not sufficiently pure for examination.

The substance from the Michigan syrup, however, was obtained in crystalline form in a yield of 127 mg. This gave a blue color with ferric chloride and responded to the resorcinol and phloroglucin tests for vanillin, but crystallized in plates (vanillin-needles) and melted at 74-76°. Again Nelson concluded from crystallographic examination that it was neither

vanillin nor ethyl vanillin, though the odor was very similar, and a crystalline derivative corresponding to dehydrodivanillin was obtained by boiling with ferric chloride.

The residues left after boiling with petroleum ether (A) were not crystalline and had an odor like maple.

B. After removing the aldehydes, Nelson treated the ether extracts with dilute ammonia to separate phenols, pouring the resultant ammonia solutions into sulphuric acid and extracting with ether. On evaporation of the ether, reddish-yellow residues were obtained in comparatively large quantities -- 0.4 g. from Vermont and 0.5 g. from Michigan syrup. These were resinous and had an intense odor of maple but resisted all efforts to induce crystallization, changing easily to a darker colored resin, at the same time losing its characteristic maple odor. It was noted that this change seemed to take place to some extent on evaporating the ethereal solutions, though it was not determined whether this was due to polymerization or oxidation. Boiling with ferric chloride failed to yield a volatile, quinone-like substance such as was observed in the crystalline material isolated in (a).

C. A very small residue which was partly crystalline was obtained by evaporating the ether solutions remaining after the removal of acids, aldehydes and phenols.

Recrystallized from alcohol, spindle-shaped crystals (M. P. 210-212°) were formed, but these failed to give a pungent, volatile substance when boiled with ferric chloride as in (A).

From his results, Nelson concluded that the maple flavouring principle depends largely on an unstable phenolic substance which was associated in all probability with the crystalline aldehyde melting at 74-76° and similar in odor and properties to vanillin. He also stated that maple syrup may contain minute quantities of other aldehydic substances, but did not attribute any influence on the flavour to the phlorone-like substance he obtained in crystalline form other than that it may be related to vanillin.

(c) Skazin's Work.

Skazin's work (23) on the flavour of maple syrup may be discussed under two headings:

A. Concentration of sap,

B. Extraction of the flavour.

A. Three methodsof concentration were used.

(1) Ordinary evaporation at atmospheric pressure. This yielded a syrup of good flavour.

(2) Evaporation under reduced pressure. The sap was boiled in 22 l. pyrex flasks heated on a

water bath by means of steam, so that the temperature during concentration was never above 52°C. No caramelization took place at this temperature, producing a syrup much lighter in color than syrups prepared as in (1). It was noted that there was not the characteristic maple taste in syrups prepared in this manner but that it could be developed by boiling vacuum concentrated syrups in the air or under a reflux. Skazin therefore concluded that the flavour is present in maple sap but is only brought out by heating to a certain temperature.

(3) Concentration by freezing. This method consisted in freezing the sap in an ice-cream machine of small capacity, removing the ice which formed on the walls of the container and repeating the freezing, each freezing leaving the liquid more concentrated in sugar. However, the results were not satisfactory as a large quantity of sap had to be frozen about 30 times in order to obtain a syrup of 24% total solids. The flavour

was that of watermelons rather than of maple syrup.

B. Extraction of the flavour.

After a preliminary study of the efficiency of solvents for the extraction of the flavouring materials in maple syrup -- namely: carbon tetrachloride, petroleum ether, carbon disulphide, benzene, ether and chloroform -- chloroform being found the best, Skazin undertook the extraction of about 10 gallons of maple syrup. The process consisted in diluting the syrup with an equal volume of water and shaking two gallon portions with one litre of chloroform for six hours in a churn. The combined chloroform extracts were conventrated to a volume of 150 c.c., containing 0.975 g. of solids, and Nelson's (13) procedure for the analysis was employed, resulting in a final separation into portions as follows,

1.	Sodium bisulphite extract		
	(a) Soluble in petroleum ether	0.0133 g.	1.4%
	(b) Insoluble in petroleum ether	0.0353 g.	3.6%
2.	Ammonia extract	0.0622 g.	6.4%
3.	Resin	0.6598 g.	67.7%
4.	Unknown parts (by difference)	0.2044 g.	20.9%
	Total	0.9750 g.	100.0%

A brief discussion of the nature of the various portions is essential in order to relate the results to Nelson's findings.

1. (a) was obtained in crystalline form -- "needles with some yellow drops in them" -- obviously these should have been recrystallized before a combustion analysis was performed on them. These crystals had a melting point of 77-79°C. (Vanillin 80-81°C.) and gave the phloroglucinol, resorcinol, and ferric chloride tests for vanillin. One combustion analysis, "carried out more or less successfully" gave an empirical formula of $C_8H_6O_3$, which is reasonably close to that of vanillin.
2. This residue was reddish-yellow, resinous and had an agreeable odor, but no crystallization could be induced.
3. This residue comprised the greatest proportion of the material extracted but was not examined carefully. It was a dark brown resin with a faint maple odor; insoluble in water, slightly soluble in ether, and quite soluble in alcohol and chloroform. The

following values were obtained:- saponification number 433.5, acid number 58.7, ester number 347.8. The saponification number is very high and must correspond to an acid with a comparatively low molecular weight but no acid could be separated.

Microcombustion analyses were carried out on 2. and 3., but the data obtained are of no value because of the impurity of the material.

Making a comparison of the results obtained by Nelson and Skazin on practically the same amount of syrup, we find that the former separated about five times as much vanillin-like substance, and though these were similar in reaction, they differed in crystalline form and melting points. It does not seem to have occurred to Nelson to compare his unknown (M. P. 74-76°) with coniferyl alcohol, which melts at 73-74°C. and crystallizes in rhombic prisms.

Nelson also obtained a higher yield of resin from the ammonia treatment, but so far as can be ascertained the properties of the substances were the same in both cases. On the other hand, Skazin found a much larger proportion of final residue after removal of aldehydes and phenols. We are therefore justified in saying that either:

(a) The nature of the flavouring substances

in maple syrup varies under different conditions of environment or of preparation.

or (b) That the method of separation of constituents of the extract is faulty.

At present it is not possible to choose between these two alternatives.

E X P E R I M E N T A L

Considering the fact that syrup prepared from maple sap by:

1. Concentration under reduced pressure, or
2. Partial concentration by freezing, followed by further concentration under reduced pressure, does not possess the characteristic maple flavour, which is an intrinsic property of syrup prepared by evaporation of sap at atmospheric pressure, we are led to the assumption that at the temperature attained in ordinary evaporation, a chemical change of some kind takes place in certain substances present in the sap, producing the flavouring substances as such. Types of reactions which suggest themselves as possibly leading to the production of flavour, are:

   (a) Oxidation.
   (b) Hydrolysis.
   (c) Decomposition.

   At any rate, the change evidently takes place at a temperature higher than 60°C., the maximum boiling point of sap during

the process of vacuum concentration as carried out in this laboratory.

With the exception of Skazin's observations (23), data in the literature regarding the process of flavour development are lacking. Practically all the work has been carried out on the flavour as present in genuine maple syrups, so it was determined to study at least qualitatively the changes in flavour, during the complete process from sap to syrup, and by observing the conditions under which the flavour is developed to shed some light on the nature of the changes involved.

With this object in view, the experimental work falls naturally into four groups:

(a) Preparation of syrups.
(b) Development of the flavour.
(c) Biochemical examination for glucosides.
(d) Analysis of the flavouring principle of genuine maple syrup.

(a) Preparation of Syrups.

This was carried on largely during the course of the sap seasons in the springs of 1931 and 1932, fresh maple sap being delivered at the laboratory daily. The season of 1931 extended from March 17 -- April 9, and in

1932, from March 31 -- April 14. Data on the sap of 1931 are lacking but the total solids, color and rate of flow are reported on the 1932 sap as follows:

TABLE 1

Date 1932	Quantity	% Total Solids	Color	Rate of flow of Sap
March 31	8 gals.	3.0	Water-clear	Moderate
April 1	10 "	3.5	"	"
" 2	20 "	4.05	Slightly green	Slow
" 4	20 "	3.9	Water-clear	"
" 6	20 "	3.75	"	Moderate
" 7	20 "	3.6	"	Rapid
" 9	20 "	4.3	Yellow	Slow
" 11	20 "	4.8	"	"
" 13	20 "	4.1	Green	Rapid
" 14	20 "	4.35	"	"
Total	178 gals.			

Notes:

1. Total solids were determined by means of an Abbe refractometer graduated for sucrose. pH values, taken colorimetrically with chlorphenol red and bromthymol blue showed a range of 6.4 to 6.9, hence the sap is always slightly acid.

2. The amount of solids, chiefly sucrose, present in the sap seems to depend to some extent on the rate of

flow, since it is higher in general when the sap is 'running' slowly. We might expect this to be the case if the sucrose is brought into solution from the sap wood, because the longer time of contact would result in a more concentrated solution.

3. The weather became warm from April 8 to April 11 so that there was no freezing at night. At this time the sap became dark colored as at the end of the season. However, a cold spell followed this, and started another 'run', the sap being light colored but possessing a different taste from that of the first of the season.

4. To prevent the growth of micro-organisms in the sap, 5 c.c. of chloroform was added to each bucket before the day's run had commenced.

Concentration of sap.

(a) Concentration under reduced pressure. 370 litres of sap was concentrated by this method. The apparatus consisted of a 22 l. Pyrex flask attached to a water condenser, a large bottle to receive the distillate, a manometer, and a water pump. A thermometer was inserted in the rubber stopper of the flask, as well as a separatory funnel to permit the addition of sap without interrupting the boiling. The flask was surrounded by a water bath heated by leading in steam. Two sets of this apparatus were used.

The pressure in the apparatus during concentration varied from 4 - 8 cm. of mercury with a corresponding temperature range of 53 - 60°C. The sap was evaporated to the consistency of syrup, filtered while hot and bottled. For clearness, the various products may be classified as follows:

TABLE II

Syrup	Date Finished	Yield	% Total Solids	Remarks
A	April 4	3 litres	64.0	Golden yellow color
B	" 5	2 "	62.7	" " "
C	" 9	5 "	59.0	Dark yellow color
D	" 10	2.3 "	65.0	Brownish yellow color
E	" 13	2.75 "	66.0	" " "
F	" 15	2.05 "	61.0	Light brown, greenish tinge
G	" 16	2 "	64.2	" " "

Notes:

None of these syrups had the typical maple flavour, as a matter of fact, no distinct flavour could be detected. There was a progressive deepening in color as the season grew longer which is, according to Jones (6), attributable to the action of micro-organisms.

(b) Concentration by freezing. This method has been known for some time, but is of no value so far as production

of syrup is concerned. However, to obtain a more concentrated liquid than sap in which it is definitely certain no changes have taken place in the flavouring material, this method is useful. Accordingly, about 200 l. of fresh sap was treated in this way during the season. An ice cream cabinet with two compartments, -- capacity four gallons each -- was available for freezing the sap. The procedure consisted in removing the layer of ice which formed on the walls of the container after it had become about an inch thick and repeating the freezing operation. Naturally there was a considerable loss of sucrose when each portion of ice was removed, so that the final total yield of only 3 litres of concentrated sap was to be expected. This was made up of three portions:

Syrup	% Total Solids
H	30.1
I	23.0
J	19.0

These syrups were slightly yellow in color and tasted like a solution of sucrose, no flavour being noticeable.

(b) Development of the Flavour.

The facts at hand are:

(1) The flavour is not developed by boiling

below 60°C. under reduced pressure.

(2) It may be developed by boiling vacuum concentrated syrup under a reflux or in the open.

This was proved by refluxing 100 c c. each of syrups A -- 1 (Table II) for 1½ hours on an oil bath, eliminating danger of caramelization from local overheating. In every case the characteristic maple taste could be distinguished -- though not as pronounced as in maple syrups prepared in open evaporators, distinct nevertheless, and definitely not present in the vacuum concentrated syrup. Then if the change merely requires heating in the air, it should be possible to develop the maple flavour in the sap before concentration.

In order to prove this, two 3 l. portions of fresh sap were heated on a water bath under a reflux for 2½ hours, and then concentrated under reduced pressure. Both tasted strongly of maple. The experiment was repeated, substituting an oil bath for the water bath so that active boiling would take place; and again the syrup had a good maple flavour. Then since the change does not occur during concentration under reduced pressure, it must have been due to the heating of the sap.

(a) Oxidation. The first explanation to account for these observations that suggests itself, is that the development of the flavour is an oxidation of some constituents of the sap promoted by heating to about 100°C. If the process is oxidation, then no flavour should develop if air is replaced by some indifferent gas, and it should be intensified by the use of pure oxygen or the addition of an oxidizing agent. In 1930, a sample of syrup concentrated under reduced pressure in this laboratory, was treated by Massey in various ways to determine whether this was true. A summary of the treatments follows:

TABLE III

Treatment	Results
(a) Heated at 101° in a sealed tube for 1 hr.	Slight flavour.
(b) As (a) with 1/10 vol. of a 3% H_2O_2 solution added	Peculiar fruity flavour, not maple.
(c) Ordinary maple syrup treated as in (b)	Same flavour as (b).
(d) Boiled 1 hr. in casserole	Slight flavour.
(e) As (d) but H_2O_2 added	No flavour.
(f) Boiled under a reflux condenser for 3 hrs.	No flavour.
(g) As (f), boiled 1½ hrs.	Flavour of maple.
(h) As (g), but H_2O_2 added	Fruity flavour as in (b).
(i) As (h), boiled ½ hr.	Flavour as (h) but milder.
(j) Boiled under a reflux condenser for 1 hr. with a steady stream of air bubbling through	Maple flavour.
(k) As (j), sulphur dioxide substituted for air	Very light color, but difficult to remove SO_2 so flavour not determined.
(l) As (j), Carbon dioxide substituted for air.	No maple flavour.
(m) As (j), hydrogen substituted for air	Maple flavour.
(n) As (j), nitric oxide substitued for air	Slight maple flavour.
(o) As (j), hydrogen sulphide substituted for air, followed by 10 min. passage of hydrogen	Black precipitate formed. Flavour not determined.
(p) As (j), oxygen substituted for air	Slight flavour.

Notes

From the results it can be seen that oxygen is not necessary for the development of flavour, and that an oxidizing agent such as hydrogen peroxide destroys, rather than enhances, the flavour.

However, these observations were not made on the sap during evaporation, so the effect of passing various gases through the boiling sap in an open dish was tried. Air, oxygen, hydrogen, nitrogen and carbon dioxide were bubbled through sap during the whole time of its evaporation to syrup and it was found that in every case the product possessed a good maple flavour. Here we are by no means certain that the gas being passed through the sap is the only one that is in contact with the surface, so the following experiment was undertaken:

A distillation apparatus, consisting of a 3 l. Pyrex flask, condenser and receiver, was connected to a water pump and manometer. A glass tube, connected to a cylinder of carbon dioxide, was inserted in the stopper of the distilling flask so that it reached almost to the bottom. 3 l. of sap was added to the flask and the apparatus was evacuated as far as possible. Carbon dioxide was then allowed to fill the apparatus and a slow stream of gas was maintained by a slight suction while the flask was heated on an oil bath. This prevented caramelization while

the sap was being concentrated. The syrup obtained had a very good maple flavour. A check was run, using oxygen instead of carbon dioxide. The products from the two experiments were identical in flavour, therefore it is safe to conclude that the development of maple flavour is not a process of oxidation.

(b) To the next question: "Is heat the only necessary factor in the process?", the answer is given by the following experiment: portions of syrups A - I (Table II & ff.) were heated in sealed tubes at 105°C for 1½ hours. It was found that the maple flavour had been brought out in every case, though more distinct in A, B, D and H than in the others.

(c) Extractions of sap and vacuum concentrated syrup. If the change involved in the development of maple flavour is chemical, then it might be possible to find a solvent which would remove the substance before the change had taken place, but not after. If the flavour is removed from sap or vacuum concentrated syrup the syrup prepared by evaporating the sap will have no maple flavour; and similarly, the latter would not show a maple flavour when refluxed. Accordingly, fresh sap was extracted with a number of organic solvents, three litre portions with 750 cc. of extracting agent being shaken up from time to time during

the course of an hour. Then the two layers were separated, the sap was boiled down in an open evaporating dish and the extract was evaporated to dryness in vacuo on a water bath. the results may be presented most suitably in the form of a table.

TABLE IV

Extractions of maple sap.

Solvent	Nature of residue	Flavour of prepared syrup
1. Ethyl acetate	Small, yellow in color Needle-shaped crystals formed.	Sweet, but not maple.
2. Amyl alochol	Resinous.	Maple.
3. Chloroform	Resinous, but contained white crystals.	Slight maple.
4. Ether	Slight sediment.	Maple.
5. Petroleum ether	Resinous.	Slight maple.
6. Benzene	Reddish brown, resinous odor.	Slight maple.

Notes.

The ethyl acetate was purified by neutralizing the acid present, and redistilling. Ethyl acetate was the only solvent which removed the precursor of the flavour completely. The residue from the ethyl acetate, chloroform, and petroleum ether extracts were most promising both in quantity and from the slight flavour remaining in the syrup,

so it was resolved to repeat the extractions with a larger amount of sap. The method consisted in shaking 5 l. portions of sap with 1 l. of solvent in a large bottle packed firmly into a butter churn. The churn was turned by an electric motor and each portion was shaken for 30 minutes. The rest of the treatment was the same as above.

15 l. of sap was extracted in this way with chloroform, ethyl acetate, and petroleum ether, but the residues obtained were very small, weighing 0.0600 g., 0.0313 g. and 0.0172 g. respectively. Those from ethyl acetate and petroleum ether were much lighter in color than that from chloroform, and did not possess the same resinous odor. However, the quantity available did not permit any careful examination.

Extractions of vacuum concentrated syrup. Ethyl acetate and chloroform were used to extract syrups concentrated under reduced pressure. 200 c.c. of the syrup was shaken up for 15 minutes with 100 c c. of the solvent in a separatory funnel, separated and the extract evaporated to dryness in vacuo. The syrups were heated on a water bath until all the dissolved extracting liquid had been driven off and then refluxed for 1½ hours. By this method, the flavour was developed in the syrup extracted with chloroform but not in that extracted with ethyl acetate.

In the latter case, a slight sour taste could be detected — probably due to the hydrolysis of ethyl acetate during the heating. The residues were very small and partly crystalline, and had a resinous odor. No identification was possible.

Now since ethyl acetate had been found to remove the material from sap and vacuum concentrated syrup, which is the precursor of the true maple flavour, it was decided to compare its effect on ordinary maple syrup. The extraction was carried out in the same way as above, and it was found that the syrup still retained its original flavour after driving off the ethyl acetate. The residue from evaporation of the ethyl acetate extract was a strong smelling resin.

Discussion of results.

From the fact that ethyl acetate removes some substance from sap and syrup concentrated under reduced pressure, preventing the development of flavour, and that it does not remove the flavour from genuine maple syrup, it is probable that a chemical change is involved in the production of the true maple flavour.

Since it has been shown that the process is not oxidation but is brought about by heat, it must be decomposition, hydrolysis or polymerization.

(c) Biochemical Examination for Glucosides.

Glucosides occur widely in the plant kingdom and are the basis of a large number of drugs and other substances with physiological properties. They are most commonly found in very small amounts in the fruit, bark or roots of plants, accompanied by enzymes which hydrolyze them when the plant tissue is destroyed. They are usually levo-rotatory, crystalline, colorless, bitter, soluble in water or alcohol, and so far as is known the naturally-occurring members are of the β-class and are hydrolyzed by emulsin. The hydrolysis may be carried out with water and acids as well as with enzymes.

Now it is quite possible that glucosides are present in the wood of the maple tree, and a small amount may be dissolved in the sap. During the process of evaporating the sap, prolonged exposure to heat and the small amount of acid present may cause the hydrolysis of these glucosides. Rosenthaler (18) states that 'If the hydrolysis is to be performed with water alone, a temperature above 100°C. is usually necessary'. The temperature attained during evaporation of sap is 105°C. If such hydrolysis of a glucoside took place, glucose would be formed, and the non-sugar part of the glucoside molecule would impart the characteristic flavour to the syrup. On this assumption, reducing sugars should always be present in maple syrup,

though obviously a certain amount is formed from inversion of sucrose by boiling and in some cases by the action of micro-organisms (6). In an analysis of 481 syrups, Bryan (1) as mentioned before reports only one with 0.00% invert sugar and this did not seem to be normal as it was cloudy in appearance, had a fair taste, and gave an invert polarization of -1.8°V. at 87°C., while practically all other syrups were 0°V. at the same temperature. Again, Bryan, et al. (2) report a higher percentage of invert sugar in syrup prepared from maple sugar than from sap, but this is usually attributed to the inversion of sucrose due to the higher temperature employed in making the sugar, compared to that reached during the concentration to the syrup stage.

General methods for the isolation of glucosides are not known but the process in most cases is begun from an aqueous or alcoholic extract. If the substance is obtained in crystalline form it may often be identified by the vacuum sublimation method of Niethammer (15). He used the method to detect glucosides in tissues. It consists in heating the tissue in a vacuum and condensing any sublimate on a watch glass fastened with water-free glycerin to a water-cooled receiver fitted into the evacuated tube. The crystalline form of the sublimate is compared with that of the sublimate obtained from the pure glucoside, and a

test with KBr_3 is applied. Data are supplied on aesculin, syringin, saponarin, digitonin, salicin, and rhinanthrin, but the method is obviously confined to those glucosides which yield a volatile, crystalline derivative, or sublime without decomposition on heating.

The method could not be applied to the residues obtained from the extractions of sap as they were not sufficiently pure and the quantity available was very small.

However, making use of the fact that glucosides hydrolyzable by emulsin are levo-rotatory and yield glucose on hydrolysis, Herissey (4) showed that a slight dextro change in rotation of a solution on treatment with emulsin may be taken as definite proof of the presence of glucosides. It was therefore decided to try the effect of emulsin on syrup concentrated in vacuo, in which the flavour was not developed.

300 c c. of the syrup was clarified with basic lead acetate and alumina cream ((16) p. 371), filtered, and the filtrate divided into nine parts of 25 c c. each in test tubes. (Two samples of emulsin were available in this work, and these will be designated Emulsin A and Emulsin B.) One tube was left blank; 0.1 g. emulsin A added to tubes II to V, and the same amount of emulsin B to tubes VI - IX. The tubes were then incubated in a water oven at 37°C. for 12 hours, after which they were cooled, filtered and polarized in 200 m.m. tubes. The results were as follows:

TABLE V

Tube		Rotation	Change in rotation.
I	Blank	18.48°	--
II	Emulsin A	18.74°	+ 0.26°
III	"	18.45°	- 0.03°
IV	"	18.65°	+ 0.17°
V	"	18.57°	+ 0.09°
VI	Emulsin B	18.92°	+ 0.44°
VII	"	19.00°	+ 0.52°
VIII	"	19.13°	+ 0.65°
IX	"	19.01°	+ 0.53°

Notes

These figures are the average of five readings.

With the exception of tube III, a dextro change in rotation was produced by treatment with emulsin, though evidently emulsin B was more active than A.

To check up these observations, fresh sap was treated with emulsin as follows: 100 c c. of sap was added to each of three flasks. 0.2 g. emulsin was added to two of these, and toluene was used as a preservative. The three flasks were kept in an incubator at 30-35°C. for two days, then portions of each were filtered and polariscopic readings were made on the filtrate. Reducing sugars were determined by the Munson and Walker copper reduction method ((16) p. 379), weighing the cuprous oxide direct.

Results follow:

Flask	1 Blank	2 0.2 g. Emulsin A	3 0.2 g. Emulsin B
Rotation	+4.61°	+4.64°	+4.59°
Change in Rotation		+0.03°	-0.02°
Reducing sugars (g. per 100 c.c.) (as invert)	0.0244	0.0406	not det'd.
Increase in reducing sugars		0.0162	

The experiment was repeated on another sample of sap using emulsin B.

Flask	1 Blank	2 0.2 g. emulsin B.
Rotation	+4.75°	+4.94°
Change in Rotation		+0.19°
Reducing sugars (g. per 100 c.c.)	0.022	0.0489
Increase in reducing sugars		0.0269

It can be seen that the very slight increases in reducing sugars, and dextro-changes in rotation are not sufficient evidence to enable us to say that glucosides are present, particularly as sources of error in the copper-reduction method for invert sugar may be numerous in the presence of such a relatively large amount of sucrose. The percentages of reducing sugars in the untreated saps agree closely with the values reported by Sy (22), and at any rate it should be noted that the increase after treat-

ment with emulsin is 66 2/3% in the first experiment and over 100% in the second. Again, the formation of such a small amount of a dextro-rotatory sugar (glucose, if glucosides are present) could not be expected to cause an appreciable change in rotation.

Bourquelot's method. It was therefore decided to apply Bourquelot's method for the detection of cane sugar and glucosides (18) to fresh sap, sap concentrated by freezing, and vacuum concentrated syrup. The method consists in extracting the plant material with 90 - 95% alcohol containing a small amount of calcium carbonate to neutralize any acid which might cause hydrolysis of the glucoside, evaporating the extract to dryness in vacuo taking up the residue with water, making up to 250 c.c. The aqueous solution is divided into two parts - one of 50 c.c. (A) which serves for comparison, and the other of 200 c.c. (B). Invertase is added to B and both solutions are kept in an incubator at 30 - 35°C. for two days, when portions of each are removed, basic lead acetate is added, the mixture filtered and the filtrate tested polarimetrically in a 200 m.m. tube. If sucrose is present, B will show a decrease in rotation. Reducing sugars are determined in each liquid and from the difference, the amount of reducing sugar produced by invertase.

The corresponding amount of cane sugar can be calculated, and from this the change in rotation that should

be produced by the hydrolysis of the cane sugar, using the formula

$$\alpha = \frac{[\alpha].l.c}{100}$$

where α = change in rotation.

$[\alpha]$ = specific rotation of the sugar (sucrose or invert)

l = length of tube in decimetres

and c = amount of sugar involved in the change.

The value obtained by calculation should be equal to the observed rotation.

After all the sucrose has been inverted, the liquid B is heated for ten minutes at 100°C. on a boiling water bath, then cooled, and 0.5 g. of emulsin added for every 100 c c. of solution. The further investigation is carried out in the same way as described for cane sugar. One can conclude that a glucoside is present whenever an increase in reducing sugars is realized, accompanied by a dextro change in rotation.

Rosenthaler (18) proposes in 'Index of Enzymolytic Reduction' which distinguishes every glucoside hydrolyzable by emulsin. It is the ratio of the sugar produced in 100 c c., calculated as glucose in milligrams, to the change in rotation in degrees (observed in a 200 mm. tube). The reduction indices are given for a number of the commonest glucosides and the value for sucrose is 601.

In applying this method to sap and syrup, the alcoholic extraction is unnecessary, so the procedure was followed from the treatment with invertase as outlined except that no basic lead acetate was required to clarify the liquids before filtering for polarization. The invertase preparation used was a solution of 1 g. of invertase scales in 100 c c. of water, 5 c c. of this solution being added to invert the sucrose.

(a) Sap. Three 200 c.c. portions were treated, 2 c c. of a maltase extract being used instead of emulsin in C, so that if α-glucosides were present, they would be hydrolyzed, since maltase is the specific enzyme for this type. (The maltase was extracted at 15°C. from dried yeast, using disodium phosphate in the extraction medium according to Krieble, Skau, and Lovering (8)).

The results may be tabulated as follows:

Solution	Rotation	Change Obs.	Change Calc.	Invert sugar g. per 100 c.c.	Increase	Index of Reduction
A (blank)	+4.69°			0.268		
B After invertase	-1.81°	-6.50°	-6.00°	3.650	3.623	557
B After emulsin	-1.40°	+0.41°		3.720	0.07	171
C After invertase	-1.80°	-6.49°	---	---	---	
C After maltase	-1.67°	+0.13°		3.765	3.640	

Notes:

1. The treatment with emulsin resulted in a dextro

change in rotation of 0.41° with a corresponding increase in reducing sugars of 0.07 g. per 100 c.c. The reducing sugars are reported as invert sugar. The index of reduction has been defined as the ratio of the increase in reducing sugars, expressed as milligrams of glucose per 100 c.c., to the change in rotation in degrees, but the increase is naturally the same whether the reducing sugars are calculated as invert sugar or glucose.

2. The expected change in rotation after treatment with invertase as calculated by the use of the formula given above, and working it out for B as an example:

3.623 g. invert sugar per 100 c.c. were produced, and this is equivalent to 3.442 g. sucrose;

Decrease in rotation due to disappearance of sucrose	= 4.578°
Decrease in rotation due to formation of invert sugar	= 1.426°
Total change expected	= 6.004°

3. The maltase evidently had no appreciable effect on the sap, as the change in rotation (+0.13°) is not comparable to that produced by emulsin, so it is probable that no α-glucosides are present.

(b) Sap concentrated by freezing. Three 100 c c. portions of sap concentrated by the freezing method to 23%

total solids were next treated by Bourquelot's method. The following results were obtained:

Solution	Rotation	Change Obs.	Change Calc.	Invert sugar g. per 100 cc.	Increase	Index of Reduction
A (blank)	+24.80°			0.40		
B After invertase	- 9.10°	-33.90°	-33.97°	21.08	20.68	610
B After emulsin	- 8.98°	+ 0.12°		21.32	0.24	2000
C After invertase	- 8.74°	-33.54°	-32.94°	20.44	20.04	591.5
C After emulsin	- 8.56°	+ 0.18°		20.92	0.48	2666

Notes:

1. The dextro change in rotation on treatment with emulsin is very slight, though the increase in reducing sugars is appreciable.

2. The index of reduction calculated for sucrose in B and C agrees closely with the theoretical value of 601.

(c) The method was then applied to a syrup concentrated in vacuo. 100 cc. of syrup B (Table III) was diluted to 250 cc. and treated with invertase and emulsin as in (a) and (b).

Solution	Rotation	Change Obs.	Change Calc.	Invert sugar g. per 100 cc.	Increase	Index of Reduction
BA (blank)	+37.36°			0.16		
BB After invertase	-13.73°	-51.09°	-47.20°	29.00	28.84	564
BB After emulsin	-13.50°	+0.23°		29.72	0.72	3130

Notes:

Again the dextro change in rotation is very slight, though this may have been affected by sucrose which had not been inverted as the calculated change in rotation for inversion of the sucrose does not agree very closely with the observed value.

(d) Since there has been some slight evidence of the presence of glucosides in the sap of the maple and in syrup prepared from it under special conditions, it was considered possible that glucosides exist in the tree itself. Therefore, Bourquelot's method was applied to two portions of maple wood: I, From a branch, II, a large chip cut from the trunk about 2½ inches deep and at the height at which the tree was tapped. These were obtained during the sap season from a hard, or rock maple (Acer saccharum).

I. 250 g. of the wood, including the bark, was shaved off with a knife.

II. The bark was removed, and 250 g. of the sap wood shaved off as with I.

These were extracted as follows: 95% ethyl alcohol, sufficient to cover the shavings and to which a few grams of calcium carbonate had been added, was brought to boiling on a water bath in a 3 litre flask. The shavings were added slowly without interrupting boiling and the boiling was continued for 30 minutes under a reflux

condenser. After the contents had cooled, the alcohol was decanted, and the extraction was repeated exactly as before. The combined alcoholic extracts were filtered -- that from I was brown in color, due to tannins and coloring matter from the bark, while II gave a golden yellow colored solution -- and evaporated to dryness in vacuo on a waterbath. A considerable amount of brown, resinous, residue was obtained from each extract and this was taken up with water, a large portion remaining undissolved. However the mixtures were made up to 250 cc., filtered and treated with invertase and emulsin by Bourquelot's method as described above. The results obtained were:

Solution	Rotation	Change Obs.	Change Calc.	Invert sugar g. per 100 cc.	Increase	Index of Reduction
IA (blank)	+0.35°			0.076		
IB After invertase	-0.04°	-0.39°	-0.51°	0.378	0.302	774
IB After emulsin	+0.02°	+0.06°		0.424	0.046	766
IIA (blank	+ 1.11°			0.259		
IIB After invertase	-0.16°	-1.27°	-1.44°	1.115	0.856	640
IIB After emulsin	+0.12°	+0.28		1.122	0.007	25

Notes:

1. A very small amount of sucrose is extracted from the wood by the alcohol, but it is apparently present in greater quantity in the trunk of the tree than in the branches, judged from this examination of only one portion of each. Evidence for the presence of sucrose is given by the fact that the reduction indices are reasonably close to the theoretical value.

2. A dextro change on treatment with emulsin, together with an increase in reducing sugars is to be noted in both cases, which though slight, is appreciable when compared to the initial rotation. Nothing can be deduced from the reduction indices.

Discussion of results.

It may be contended that the observations on the changes of rotation and reducing sugar content are in many cases within the range of experimental error, but the mere fact that dextro changes on treatment with emulsin, accompanied by increases in reducing sugars, are consistent is at least an indication that glucosides are present in the sap of the sugar maple. All polariscopic measurements were made under the most similar conditions possible, care being taken that the solutions were perfectly clear, and the rotation recorded was in all cases the average of five readings.

The index of reduction proposed by Rosenthaler (18) seems to be supported in these experiments in the inversion of sucrose, but in the treatments with emulsin, it was found that there was no conformity whatsoever. Naturally, it is open to great variation when applied to such small changes as are observed here, and is quite probably influenced by the presence of a comparatively large amount of sugar. It would be of practical value when the glucoside was present in appreciable quantities.

In conclusion, it can be said that a glucoside, (or glucosides), hydrolyzable by emulsin, possibly occurs in the sap of the maple tree. Since evidence has been found of its presence in syrup prepared by concentrating sap under reduced pressure, which has no true maple flavour, it is logical to assume that it is hydrolyzed by heating to 100°C., setting free the flavouring material, or part of it, as the non-sugar part of the molecule.

(d) Analysis of the Flavouring Principle.

It has been established by Nelson (13), and Skazin (23), that the flavouring material in genuine maple syrup is present only in minute amounts, and that the most satisfactory method of isolating it is by extracting the syrup with an immiscible solvent in which the substances are more soluble than in water. Nelson used ether as the

extracting agent, but Skazin found that chloroform dissolved a larger amount of material than ether, and used it in his investigations. The extraction of about ten gallons of syrup was carried out by the latter, and the analysis of the extract performed, Nelson's procedure being followed. However, the quantities of material obtained were insufficient for a thorough investigation, so it was decided to use a larger amount of syrup.

Accordingly, at a later date, twenty gallons of pure maple syrup were extracted in a drum by adding about ten litres of chloroform and shaking up the mixture from time to time. The experiment was interrupted, however, and when it was resumed as part of the present investigation, the syrup and chloroform had not been disturbed for over a year. The chloroform layer was siphoned off, 9 litres of the clear yellow extract being obtained.

(1) Analytical data on the extracted syrup were determined:

Total solids by refractometer	66.5%
Conductivity value	155
Canadian lead number	3.540

Thus the syrup was quite average, but after a portion had been diluted with water and heated on a water bath to drive off the chloroform, it was found that it did not have the

characteristic maple flavour. As had been expected, the flavouring material apparently had been extracted by the chloroform.

(2) The chloroform extract was first washed with several portions of distilled water to remove dissolved syrup, and was then concentrated on a water bath to a volume of about 500 cc. The concentrated extract, reddish-brown in color, and possessing a resinous odor, was treated as follows:

I. Removal of aldehydes.. The solution was shaken for twenty minutes with 675 cc. of half-saturated sodium bisulphite solution and allowed to stand. The chloroform layer was separated and extracted again with 350 cc. of the sodium bisulphite solution, then reserved for II. The combined sodium bisulphite extracts were washed four times with chloroform, and acidified with dilute sulphuric acid (3 vols. H_2SO_4 5 vols. water). The solution was freed from sulphur dioxide by allowing a stream of carbon dioxide to bubble through it for some time. It was then extracted five times with 250 cc. portions of chloroform, and the combined chloroform solutions evaporated carefully. The residue weighed 0.05 g., and was dark brown in color but not crystalline. It had a maple odor, sweet and suggesting vanillin.

Now since both Nelson and Skazin had found

that a vanillin-like substance was obtained from the sodium bisulphite treatment of maple extract, but that petroleum ether dissolved some of the resinous residue along with the pure substance, it was decided to try another solvent. Now vanillin was found to crystallize well from carbon tetrachloride, so the residue was boiled out with a small portion of this solvent. After careful evaporation of the carbon tetrachloride, a residue was obtained which was dark brown in color and not crystalline, so it was redissolved and returned to the original residue. The carbon tetrachloride was evaporated, and the residue was boiled out several times with small portions of petroleum ether. When the petroleum ether extract was evaporated, a residue was obtained which became partly crystalline. The crystals were needle-shaped and had a faint odor of vanillin but were not pure. The yield was very small, -- 0.0098 g., so that no tests could be applied. The residue insoluble in petroleum ether was considerable, but was not crystalline, and had a resinous odor.

II. Removal of phenols.. The chloroform solution after the treatment with sodium bisulphite was shaken out three times with 75 cc. portions of dilute ammonia (0.15 N). The combined ammonia extracts were acidified by pouring into 150 cc. of dilute sulphuric acid (0.5 N), and extracted twice with chloroform.

The chloroform extract, when evaporated, yielded a non-crystalline residue of a reddish brown color, which had a strong maple odor, somewhat similar to that of guaiacol. The weight of this residue was 0.55 g.

Reactions. (1) It dissolved readily in alcohol, but not in sodium carbonate solution so that it was not an acid.

(2) The alcoholic solution gave a green color with ferric chloride which disappeared on the addition of hydrochloric acid -- an indication of guaiacol and catechol,-(24)- but no violet-red color (given by catechol) was produced when sodium bicarbonate was added after the HCl. A comparative test with a solution of guaiacol gave identically the same results as the resin.

(3) The phthalein fusion test for guaiacol (12) was applied to the residue. This consists in mixing a small amount of the phenol with an equal bulk of phthalic anhydride in a test tube, moistening with one drop of concentrated sulphuric acid, and heating for 3 minutes on a sulphuric acid bath at 160°C. The mixture is then cooled, diluted, and neutralized with NaOH. Guaiacol gives a bluish violet color in the alkaline solution, but the resin gave a reddish brown solution after the same treatment.

III. Acids.. It was possible that the chloroform maple extract contained acids, though these would probably have been removed in the ammonia treatment in II. Nelson separated the acids before investigating the flavour. The chloroform solution from II was shaken up with 100 cc. of 0.5 N sulphuric acid, separated, and made alkaline by extracting with sodium carbonate solution. The sodium carbonate solution, yellow in color, was acidified with 0.5 N sulphuric and extracted with two 100 cc. portions of chloroform. On evaporating the chloroform extract, a trace of residue was obtained which contained a few thread-like crystals, hence acids if present in the extract, are negligible.

IV. After the aldehydes, phenols and acids had been separated from the chloroform extract, it was carefully evaporated to dryness. A considerable residue, of a brown color was obtained and part of it appeared to be crystalline. It was partially soluble in hot alcohol, so the alcohol extract was separated. Another portion was removed by petroleum ether and the residue was dissolved in chloroform. These extracts were evaporated to dryness;

(1) Alcohol extract	: reddish-brown resin, not crystalline.
(2) Petroleum ether extract	: light colored, crystalline.
(3) Chloroform extract	: brown, partly crystalline.

The crystals in (2) and (3) were similar in appearance so the two residues were combined. These were dissolved in the smallest possible quantity of boiling chloroform, and on cooling, crystals separated out. The crystalline substance was filtered off, and by further evaporation of the chloroform a second crop was obtained. The substance was recrystallized twice from chloroform and dried in a dessicator.

Properties: (1) The amount obtained was 0.1020 g. The crystals were pale yellow in color, crystallized in plates and had a slightly bitter odor, though very faint.

(2) The substance was found to be soluble in chloroform, ether and petroleum ether and very slightly soluble in water and alcohol.

(3) The melting point was 120°C. This is close to the melting point of absinthin, $C_{20}H_{28}O_4 + \frac{1}{2}H_2O$, which is 120 - 5°C. (12).

(4) An attempt was made to determine the molecular weight by the Rast camphor method, but it was found that during the fusion with camphor, the substance melting at first, later formed a yellow plastic lump, resembling a resin, which remained suspended in the liquid camphor. Apparently a polymerization takes place at temperatures near 176°C., the M. P. of camphor.

(5) Making a comparison of the properties

of thesubstance with those of absinthin (12); both are yellow in color, though absinthin forms microscopic crystals from alcohol, and the unknown was only slightly soluble in alcohol. Both are almost insoluble in cold water, absinthin gives a brown-red solution with NaOH, and a brown solution changing to greenish-blue with sulphuric acid. When tested on a microscope slide, the unknown substance showed very little tendency to dissolve in either sodium hydroxide solution or sulphuric acid, and though in the latter treatment a slight greenish tinge was observed, it was not sufficiently definite to compare with the reaction as reported for absinthin (12).

Absinthin separates as a resin by boiling with dilute sulphuric acid, so this seems to be analogous to the separation of the unknown substance as a resin when fusing it with camphor.

D I S C U S S I O N O F R E S U L T S

This investigation of the flavouring principle was by no means thorough, as it has been restricted to some extent by the amount of time available for the work. The chloroform extract of pure syrup was separated qualitatively into fractions representing aldehydes, phenols, and an unidentified substance melting at 120°C. The amount of material separated in the aldehyde fraction was not large, (0.05 g.), and only a very small part of this was dissolved by petroleum ether, yielding a small quantity of a crystalline substance with a sweet, vanillin-like odor. Vanillin was not identified.

The phenolic residue seemed to represent the largest proportion of the flavouring material, and the maple odor was most intense in this fraction. This agrees with the observations of Nelson and Skazin. It is notable that at all stages in the analysis, resinous substances interfere with the isolation of pure crystals, so they must either be present as such in the syrup or be formed by the polymerization of certain compounds under the conditions existing during the treatment. The latter alternative is supported by the relationship shown in the compounds whose presence in the maple extract has been indicated. The color reaction for guaiacol, given by the resin obtained from the

ammonia-treatment, indicates that it probably contains a phenolic and a methoxy group ortho to one another. This is a characteristic grouping occurring in vanillin and coniferyl alcohol and also in methoxy derivatives of hydrophlorone. Now, as it has been pointed out before, (p. 27), the melting point and crystalline form of the 'vanillin-like' substance, isolated by Nelson, are closer to those of coniferyl alcohol than to those of vanillin. If the substance were coniferyl alcohol, it would be possible to correllate the data we have and apply them to the development of maple flavour.

First, evidence has been given of the presence of a glucoside in maple sap. Coniferin, the glucoside present in the bark of the fir-tree, yields glucose and coniferyl alcohol on hydrolysis. Then if this were the glucoside present in maple sap, it would be hydrolyzed by evaporating the sap at atmospheric pressure, producing coniferyl alcohol. (This is supported by the fact that the residues from the alcoholic extracts of maple wood, which were insoluble in water (p..54), gave a deep red color when tested with phloroglucinol and hydrochloric acid. According to Reinitzer (19) this is an indication of the presence of coniferyl alcohol.) However, coniferyl alcohol polymerizes easily, and since this involves the unsaturated side chain of the

molecule, the OH and OCH_3 groups would remain intact, and the polymerized compound would behave as a phenol, and give the color reaction for guaiacol. In the analysis of the chloroform extract, coniferyl alcohol would be removed by the sodium bisulphite treatment, as addition would take place to the double bond in the side chain, forming an addition compound. Thus the "crystalline aldehyde" of Nelson (13) may have been coniferyl alcohol. Also, the compound formed by the polymerization of the alcohol, would be extracted by the ammonia treatment, corresponding to the resin actually obtained in this study.

Therefore, on these considerations, the flavouring principle of maple syrup might be partly composed of a mixture of coniferyl alcohol and its resinous polymer, though other substances are no doubt present. The yellow, crystalline, substance isolated in this investigation, is probably not closely connected with the "aromatic" portion of the flavour, as the odor is very slight, though the taste may have considerably effect.

S U M M A R Y

1. A study was made of the conditions under which a genuine maple flavour is developed in products prepared from maple sap. It was found that:

(a) The process is a chemical change, since ethyl acetate extracts the precursor of the maple flavour, but not the flavouring substance after it has been developed.

(b) The development of the flavour is not an oxidation of the unknown constituents of sap but is brought about by heating at 100°C.

2. Bourquelot's method for the detection of cane sugar and glucosides was applied to fresh sap, sap concentrated by freezing, sap concentrated under reduced pressure, and maple wood. Evidence of the presence of a glucoside was obtained.

3. The analysis of a chloroform extract of pure maple syrup was carried out, and the results confirmed the findings of Nelson and Skazin. An unidentified substance, melting point 120°C., was isolated.

4. The development of maple flavour is probably due to the hydrolysis of a glucoside.

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