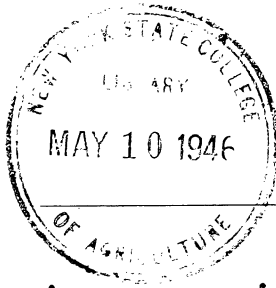


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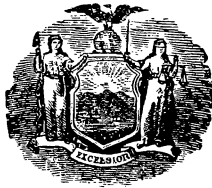


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SOME FACTORS CAUSING DARK-COLORED
MAPLE SIRUP

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ABSTRACT

THE chief factor in grading the quality of maple sirup is color. Light-colored sirup usually has a more delicate maple flavor than dark-colored sirup.

The color of maple sirup depends upon the alkalinity of the sap and the invert sugar content. The extent of color development is limited by concentration of either of these factors and is accompanied by decrease in alkalinity and destruction of reducing sugar.

Growth of bacteria causes increased alkalinity and inversion of sucrose, which, in turn, result in darker colored sirup. The bacteria grow even at temperatures slightly above the freezing point and cause significant deterioration in color.

Cleanliness of equipment and rapid handling of the sap, therefore, are very important factors in production of high quality, light-colored maple sirup.

SOME FACTORS CAUSING DARK-COLORED MAPLE SIRUP

F. W. HAYWARD AND C. S. PEDERSON

INTRODUCTION

THE color of maple sirup is one of the chief factors in grading for quality. For many years a premium price has been paid for sirups of lighter color provided other quality factors were met. Therefore, any information which would help to produce more light-colored sirup would be of value to maple producers.

It has long been known among producers that the sooner maple sap was evaporated after coming from the tree, the lighter would be the color of the sirup. It is also known that the later runs of sap make a darker colored sirup than the runs which came early in the season. Edson, Jones, and Carpenter¹ observed that bacterial growth in maple sap often caused deterioration of color grade of the sirup made from it.

EXPERIMENTAL

EFFECTS OF ACID AND ALKALI ON COLOR OF MAPLE SIRUP

While working on the evaporation of maple sap in the laboratory, it was observed that the color of the sirup was related to the hydrogen-ion concentration of the sap used; the higher the pH, the darker the color of the sirup. Therefore, varying amounts of acid and alkali were added to samples of the same lot of sap to observe the effect upon the color of the resulting sirup.

The sap employed in these experiments was collected in aluminum buckets, placed in glass carboys, and kept frozen in a cold room at 5°F. It was thawed and divided into liter samples. Measured volumes of standardized acid or alkali were added to various samples, and they were then evaporated in glass under controlled conditions to sirup. The intensity of the color of this sirup, diluted 10-fold, was measured in a spectrophotometer at 400 m μ .²

¹Edson, H. A., Jones, C. H., and Carpenter, C. W. Microorganisms of maple sap. *Vermont Agr. Exp. Sta. Bul. No. 167. 1912.*

²Color measurements are recorded as $\log I_0/I$ at 400 m μ . I_0 represents the amount of light passing thru water; I , the amount passing thru the maple sirup sample. The ratio of I_0/I is known as the light absorption of the colored sample and the $\log I_0/I$ is a measure which is directly proportional to the color intensity. 400 m μ (millimicrons) is a convenient choice of light wavelength for sensitive measure of the brown color of maple sirup.

It was found that the color of the sirup produced depends upon the amount of acid alkali which is added to the sap (Fig. 1). As more alkali is added to the sap, the sirup produced is darker, while the addition of acid produces a lighter colored sirup.

INFLUENCE OF INVERT SUGAR ON COLOR DEVELOPMENT

It is well known that sucrose is the principal sugar of maple sap. However, a small, but variable, amount of invert sugar is always present. In order to observe the comparative effect of the different sugars upon color development, various maple sap and alkaline sugar solutions were boiled in a glass flask which was equipped with a reflux condenser to prevent volume changes during the course of the experiment. At frequent intervals during boiling, samples were withdrawn and the pH and color of the solution measured.

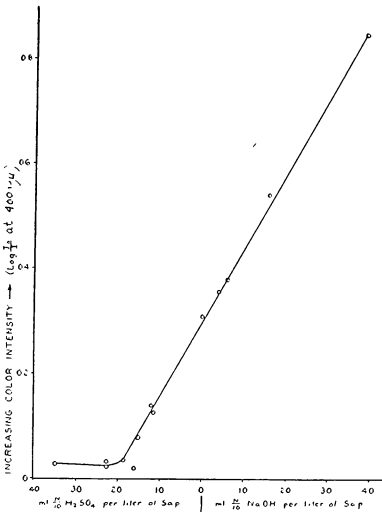


FIG. 1.—THE EFFECT OF ADDITION OF ACID OR ALKALI TO MAPLE SAP ON THE COLOR OF THE SIRUP PRODUCED.

Samples of treated sap were evaporated to sirup in glass under controlled conditions.

It was found that very little color develops in alkaline sucrose solution during heating up to 3 hours (Fig. 2). However, the presence of a very small amount, 0.1 per cent, of invert sugar in the sap, causes pronounced darkening during evaporation. The rate of color formation in untreated

maple sap is very similar to that in a dextrose-sodium bicarbonate solution, but the rate of development is not the same as in the case of the sucrose invert sugar mixture. It may be noted in Fig. 2 and in some of the succeeding graphs that considerable chemical change may occur before the solution reaches the boiling point.

CHANGES IN pH DURING BOILING OF ALKALINE SUGAR SOLUTIONS

During the heating of sugar solutions, various chemical changes are known to occur, and since pH affects the color development, the

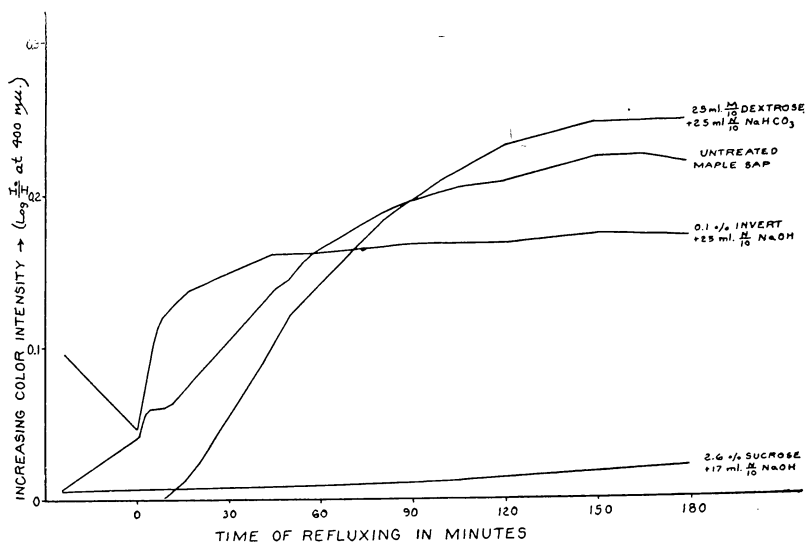


FIG. 2.—COLOR DEVELOPED DURING THE BOILING OF MAPLE SAP AND ALKALINE SUGAR SOLUTIONS.

Test solutions were refluxed to prevent volume changes. Samples removed for color measurements at intervals shown. Note that some color change occurs during the period required to produce boiling.

effect of heating upon relative acidity and pH was studied. During heating it is evident that the formation of color is always accompanied by a drop in pH and that the rate of formation of color is greatest when the most rapid drop of pH is observed (Figs. 2 and 3). The hydrogen-ion concentration (pH) of the alkaline sucrose solution changed slightly during 3 hours of refluxing (Fig. 3), but the pH of the mixture containing 0.1 per cent invert sugar dropped from approximately pH 10.9 to 6.4. Most of this change in pH and color occurred within the first 15 minutes.

Changes during the boiling of maple sap are similar to those in the dextrose-sodium bicarbonate solution. Fresh maple sap is known to contain dissolved carbon dioxide,³ and, from the results of this experiment, it seems probable that sap is buffered with bicarbonate. It would be expected, then, that when the sap is heated the bicarbonate would be decomposed and the carbon dioxide escape, leaving the solution alkaline. This possibility is indicated by the increase of pH

³Bois, E., and Dugal, L. C. La Seve d'erable et son pH. *Naturaliste Canadien*, 67:137-141. 1940.

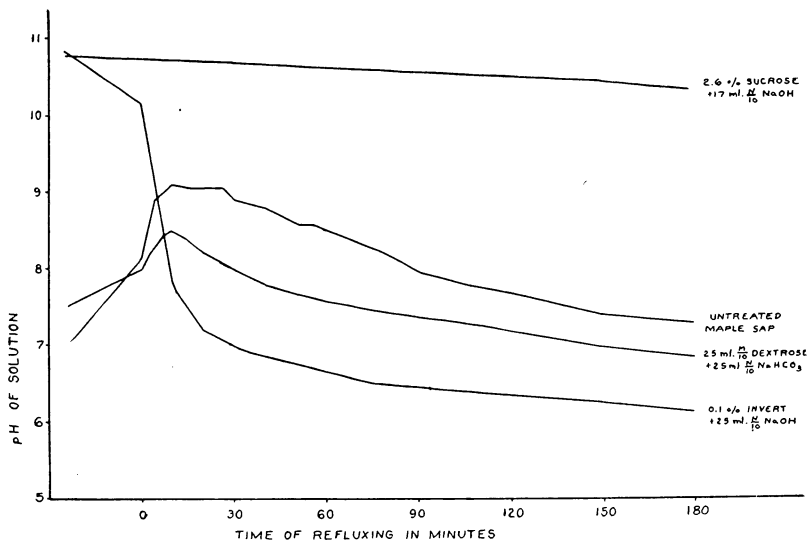


FIG. 3.—CHANGE OF pH WHICH OCCURS DURING THE BOILING OF MAPLE SAP AND ALKALINE SUGAR SOLUTIONS.

Test solutions were refluxed to prevent volume changes. Samples removed for pH measurements at intervals shown. Note that considerable pH change occurs during the period required to produce boiling.

during the early stages. As the boiling is continued, the formation of colored substances is accompanied by a drop in pH value similar to the change occurring in the sucrose-invert sugar mixture.

EFFECTS OF ALKALI AND DEXTROSE CONCENTRATIONS ON COLOR

Since both dextrose and alkali affect the color, one might expect that color would increase as these constituents are increased. Solutions of 25 milliliters of 0.1 molar dextrose with varying amounts of 0.1 normal sodium hydroxide were made up to 1 liter with distilled water. These solutions were boiled under a reflux condenser for 3 hours and, after cooling, the color intensity was measured. A similar series of experiments was made using 25 milliliters of 0.1 normal sodium hydroxide and varying the volume of 0.1 molar dextrose.

It was found (Figs. 4 and 5) that when the amount of one constituent is held constant, the color developed depends on the concentration of the other up to the point at which the first component is exhausted. Beyond this point no increase in color is observed when more of the variable constituent is used. It is thus apparent that both

components must be present for color formation to take place and that the final color of the solution may be limited by restricting the amount of either one, even tho a large excess of the other may be present.

DESTRUCTION OF DEXTROSE AND ALKALI DURING COLOR FORMATION

These results indicate that the alkali as well as the dextrose take part in a chemical reaction, and that their lessened amount limits further reaction. A solution of 50 milliliters of 0.1 normal sodium hydroxide and 25 milliliters of 0.1 molar dextrose, made up to 1 liter with distilled water, was boiled

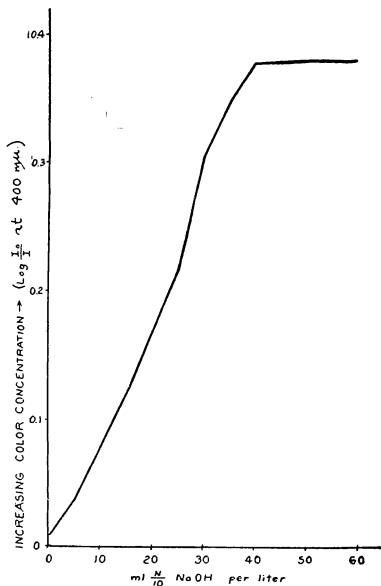


FIG. 4.—THE EFFECT OF ALKALI CONCENTRATION ON THE COLOR OF A HEATED DEXTROSE SOLUTION.

Samples containing a fixed amount of dextrose but with variable amounts of alkali as indicated were refluxed for 3 hours.

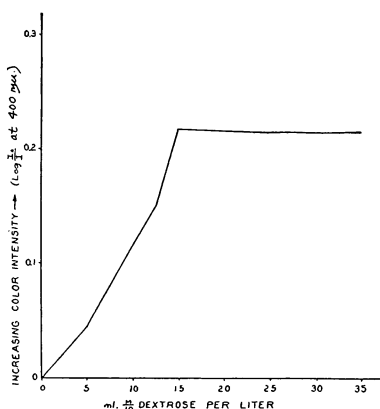


FIG. 5.—THE EFFECT OF DEXTROSE CONCENTRATION ON THE COLOR OF A HEATED ALKALINE DEXTROSE SOLUTION.

Samples containing a fixed amount of alkali but with variable amounts of dextrose as indicated were refluxed for 3 hours.

in a flask equipped with a reflux condenser. The samples, which were taken at intervals, were analyzed for color, reducing sugar, and titrable alkalinity. Darkening of the solution is accompanied by the destruction of the reducing sugar, dextrose, and of alkali during boiling (Figs. 6 and 7). It seems most probable that the dextrose reacts under these conditions to produce an acidic substance which neutralizes the alkali. Either this compound, or some other decomposition prod-

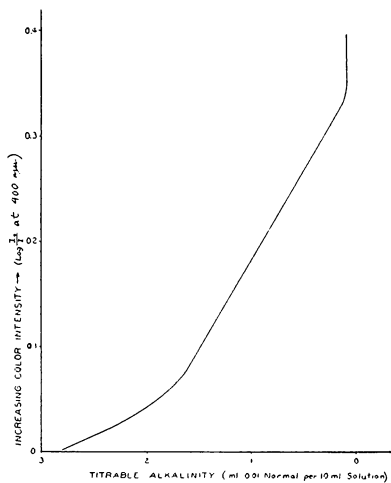


FIG. 6.—THE CHANGE IN TITRABLE ALKALINITY DURING COLOR FORMATION IN A DEXTROSE-ALKALI MIXTURE.

A sample of dextrose and alkali was refluxed and samples removed at intervals for analysis of color and titrable alkalinity.

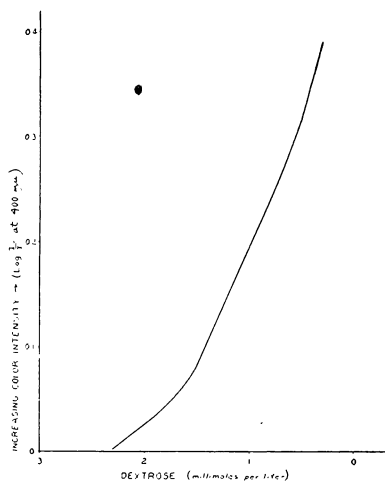


FIG. 7.—THE CHANGE IN DEXTROSE CONCENTRATION DURING COLOR FORMATION IN A DEXTROSE-ALKALI MIXTURE.

A sample of dextrose and alkali was refluxed and samples removed at intervals for analysis of color and dextrose content.

uct of the dextrose, reacts further under the influence of heat to form the colored substances which darken the solution. However, in a similar solution kept at room temperature over a period of 6 days instead of boiling, the reducing sugar and alkalinity showed great losses but no color was formed.

In studying the effect of the amounts of invert sugar and alkalinity, it was apparent that the color of a maple sirup is dependent on the invert sugar content and alkalinity of the sap from which it is prepared (Fig. 8). The color development increased both with increase in invert sugar and total alkalinity.

BACTERIAL GROWTH AND CHEMICAL CHANGES IN MAPLE SAP IN THE SUGAR BUSH

Edson, Jones, and Carpenter⁴ observed that bacterial growth in sap caused a decrease in color grade. During the 1945 season, samples of maple sap were taken at intervals from certain buckets in the experimental sugar bush. Bacterial counts were made on these samples, and

⁴*Loc. cit.*

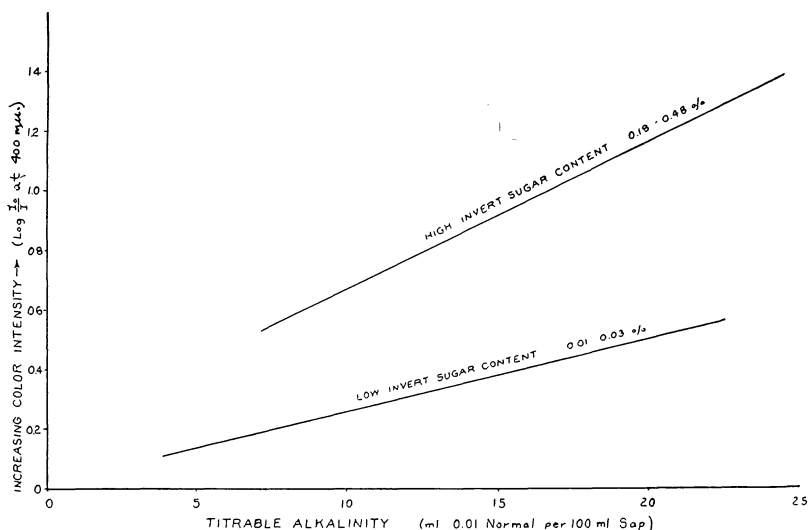


FIG. 8.—THE EFFECT OF ALKALI AND INVERT SUGAR CONCENTRATION OF MAPLE SAP ON THE COLOR OF THE SIRUP PRODUCED.

Various amounts of alkali were added to saps of different invert sugar contents and the samples evaporated to sirup under standard conditions.

they were analyzed for invert sugar and titrable alkalinity. A portion of each sample was evaporated to sirup, under controlled laboratory conditions, and the color of a 1 to 10 dilution of this sirup measured.

It was found that the color, invert sugar content, and alkalinity all increased as the bacterial count rose (Fig. 9).

GROWTH OF BACTERIA UNDER CONTROLLED CONDITIONS

In order to get a more complete picture of the changes which occur in maple sap due to the action of specific bacteria, a study was made of the growth of bacteria in maple sap and the chemical effects which result. During the study of the numbers of bacteria present in sap, regular isolations of colonies were made from plates. The pure cultures isolated represented several types of conditions in maple sap, such as slimy and greenish sap. The cultures were compared and identified. From these, seven cultures, representing the most common types present in sap, were selected to demonstrate their effect upon maple sap. Four of these cultures, Nos. C48, D17, M21, and B10, would grow slowly at 35°F, and three, Nos. D36, C43, and B17, could be grown at 44°F.

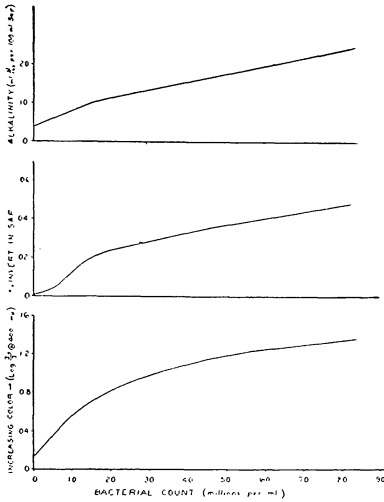


FIG. 9.—THE RELATION OF BACTERIAL GROWTH IN MAPLE SAP TO THE ALKALINITY AND INVERT SUGAR CONTENT OF THE SAP AND TO THE COLOR OF THE SIRUP PRODUCED.

Sap samples were evaporated under standard conditions.

The most prevalent type of bacteria isolated from sap is represented by culture D17, a strain of *Pseudomonas fluorescens*. It clouds the sap and sometimes produces a greenish tint. The culture represents 93 of a total of 183 isolations. Next in frequency in occurrence was a creamy mucoid type of organism, M21, which produced a slimy milky-like sap. Except in flagellation, it appears to be identical with the organism described by Edson, Jones, and Carpenter⁵ as *Bacillus aceris* (*Achromobacter aceris*, Bergey, *et al.*). Culture D36 is apparently a variant of D17. The other four types occurred less frequently and at present seem to be strains of the genera *Achromobacter* and *Flavobacterium*.

Seven samples of maple sap were inoculated with the above cultures and incubated at 35° and 44°F, one sample was incubated without an inoculum, and to another was added 0.1 per cent phenol to inhibit growth. No attempt was made to sterilize the sap before inoculation, but rather it was planned that by mass inoculation the comparative effect of the few organisms present in the sap would be overcome by the inoculum.

At intervals during a period of 11 days, samples were taken for bacterial counts and chemical analyses consisting of the determination of alkalinity, invert sugar, and color development on heating.

It was found that, altho the bacteria grew slowly in the sap at 35°F, a marked growth was obtained during the 11 days (Fig. 10). Cultures grew more rapidly at 44°F, but at this temperature the presence and growth of green fluorescent organisms similar to D17 were apparent. This overgrowth of other types did not occur in inoculated flasks incubated at 35°F.

⁵*Loc. cit.*

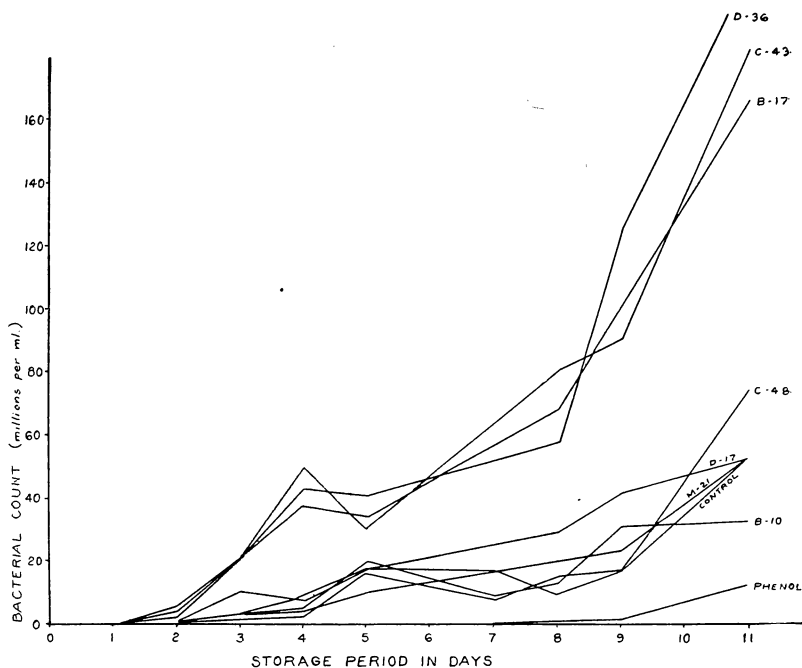


FIG. 10.—GROWTH OF VARIOUS TYPES OF BACTERIA IN MAPLE SAP.

Samples of sap were inoculated with the various types of bacteria and incubated at 35°F (cultures D17, M21, C48, and B10) and 44°F (cultures D36, C43, and B17) and counts made at intervals indicated.

The titrable alkalinity was determined by adding an excess of 0.01 normal sulfuric acid to a measured volume of sap, boiling the solution for 5 minutes to expel the carbon dioxide, and back-titrating with 0.01 normal sodium hydroxide, using phenolphthalein as an indicator. It may be observed (Fig. 11) that in samples kept at 45°F, the titrable alkalinity increased rapidly until it reached a peak on the eighth day, then dropped off sharply. This alkalinity peak was not reached in samples incubated at 35°F. Reversing of relative acid and alkali production among bacteria is common, and altho these bacteria are usually considered weak acid producers, there is little doubt that they first produce a slight alkalinity. If the cultures are grown at higher temperatures, this alkalinity production is usually not observed. At the end of 25 days, all of the samples except that containing phenol showed no titrable alkalinity but were distinctly acid.

The invert sugar content of the samples was determined colori-

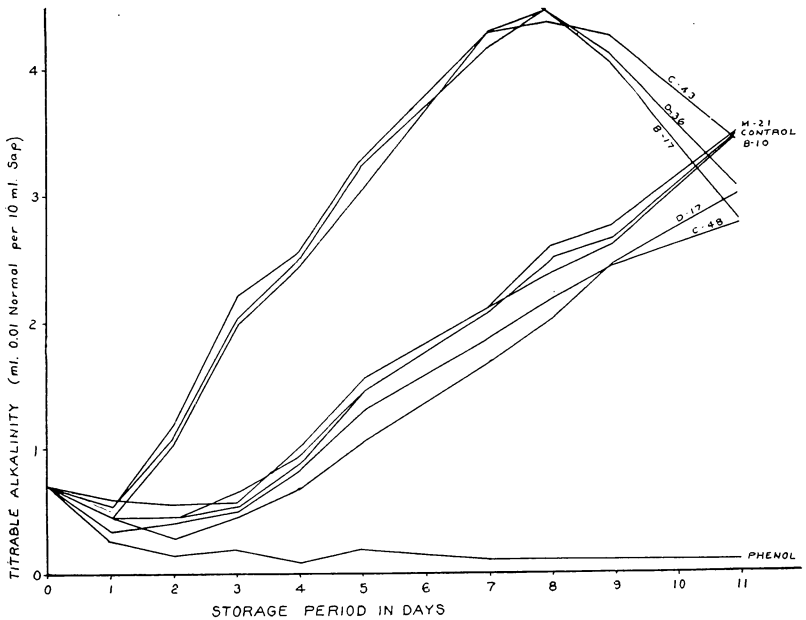


FIG. 11.—CHANGES IN ALKALINITY OF MAPLE SAP DURING GROWTH OF VARIOUS TYPES OF BACTERIA.

Samples removed at indicated intervals from various maple sap cultures incubated at 35°F and 44°F.

metrically by the Folin-Wu blood glucose method. The invert sugar content increased with increased growth of bacteria (Fig. 12). A peak in production of total invert sugar was not attained.

To develop color a 20-milliliter sample of sap in a loosely stoppered test tube was placed in a boiling water bath for 3 hours. The solution was then cooled, filtered, and the amount of color determined with a Coleman spectrophotometer set at 400 millimicrons.

The changes in bacterial growth, total alkalinity, and inversion of sucrose in the sap, was accompanied by a corresponding increase in color of the sap developed in heating (Fig. 13). In the control samples containing phenol, the growth of bacteria was inhibited with no increase in alkalinity and only a slight inversion of sugar. As a consequence, little, if any, change in color development was observed.

These results have clearly shown that the increased alkalinity and invert sugar of maple sap are due to the growth of bacteria with which the sap becomes contaminated under field conditions. The

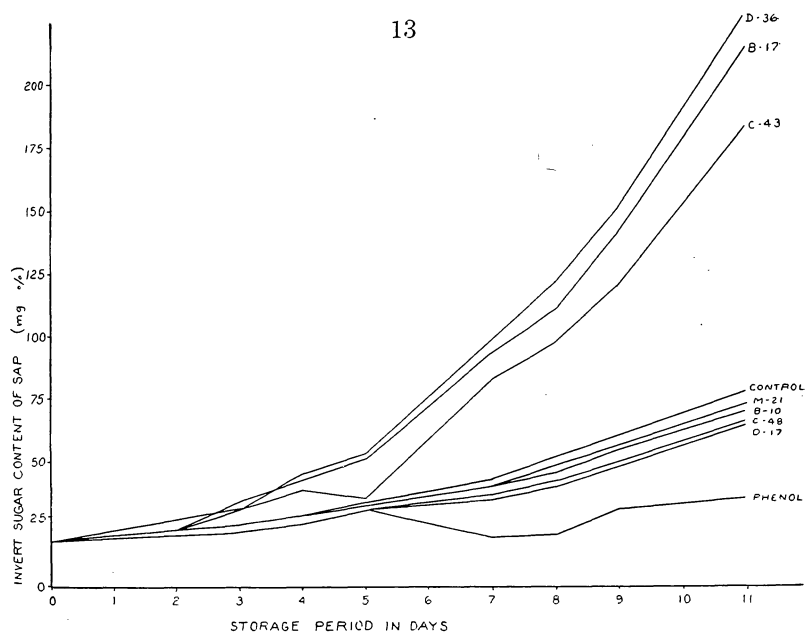


FIG. 12.—CHANGES IN INVERT SUGAR CONTENT OF MAPLE SAP DURING GROWTH OF VARIOUS TYPES OF BACTERIA.

Samples removed at indicated intervals from various maple sap cultures incubated at 35°F and 44°F.

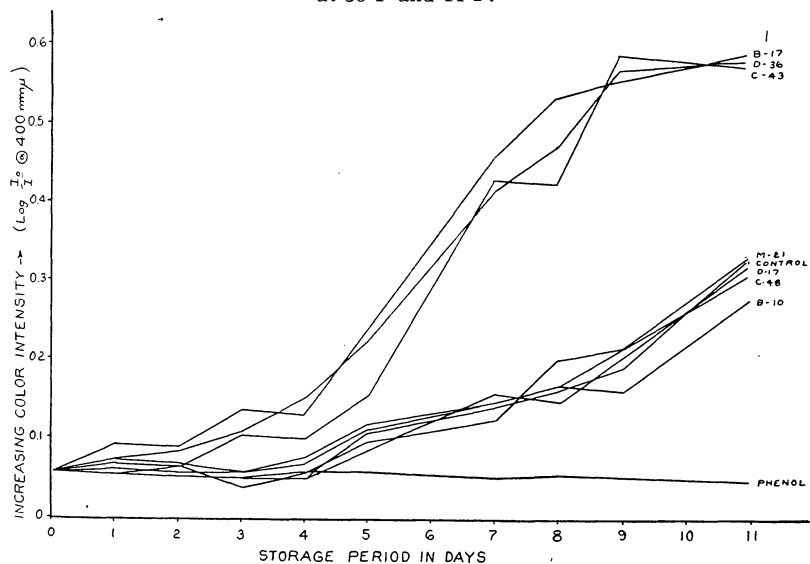


FIG. 13.—CHANGES IN THE COLOR OF THE SIRUP PRODUCED AS A RESULT OF BACTERIAL GROWTH IN THE SAP.

Samples of sap removed at indicated intervals from various maple sap cultures, evaporated to sirup under standard conditions, and the color measured.

color of the maple sirup produced depends upon the extent of these changes at the time of evaporation. For this reason, cleanliness of equipment and prompt evaporation of sap are important if a light-colored sirup is to be produced.

SUMMARY

The color of maple sirup depends to a great extent on the alkalinity of the sap and its invert sugar content. The extent of color development in maple sap is limited by the concentrations of alkali and invert sugar. Color formation is accompanied by a decrease in pH or alkalinity, and destruction of the reducing sugars.

The growth of bacteria, with which maple sap becomes contaminated under field conditions, causes increased alkalinity and inversion of sucrose. These changes result in a darker colored sirup. Bacteria found to occur in maple sap have been isolated and found to show appreciable growth at low temperatures.

Cleanliness of equipment, rapidity of handling maple sap, and storage at a low temperature are, therefore, very important factors in the production of high-quality, light-colored maple sirup. However, it should be emphasized that sap should be handled as quickly as possible since bacteria will grow even at temperatures very little above the freezing point of sap.