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Seventy-Five Years of Government Honey Research

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Early Federal Work on Honey

Although our chemical research program on honey has been underway for thirteen years, the history of chemical work on honey in this Department goes back at least seventy-five years. Probably the first sustained chemical work with honey in Government laboratories was that of Dr. Harvey W. Wiley, of Pure Food fame. Dr. Wiley, as Chief of the Bureau of Chemistry, directed a comprehensive program of analysis of common foods, the results of which were published as the celebrated Bulletin 13, *Foods and Food Adulterants*, beginning in 1887. The shocking condition of U. S. foods thus revealed played a major part in the passage of the Food and Drug Act of 1906. In 1890 Part 6 of this bulletin appeared, subtitled *Sugar, Molasses, Sirups, Confectionery, Honey and Beeswax*. The analyses of 500 samples of open-market honey, by ten State Chemists, were given in detail and it was found that at least 37% of them were grossly adulterated. As pointed out in an *American Bee Journal* article last year, the virtual impossibility of obtaining pure honey in the latter part of the nineteenth century greatly retarded its use. Thus honey was one of the foods that was used to bring about passage of Pure Food laws in this country.

Many samples of honey of various floral types from over the country were collected for the St. Louis Exhibition in 1903 by the National Beekeepers Association. These were later donated to the Bureau of Entomology and 85 of them, plus other samples, were analyzed by Dr. C. A. Browne, Chief of the Sugar Laboratory of the Bureau of Chemistry. This work was published as Bureau of Chemistry Bulletin 110, "Chemical Analyses and Composition of American Honeys," in 1908. It was a definitive work for its time and has remained a primary source of information about the composition of honey.

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Contributions of Lothrop and Walton

During the next 20 years there was no sustained program on honey in Federal laboratories; in the early 1920's William Seaman of the Carbohydrate Division investigated the color and colloid content of a large number of honey samples, but none of this work was published. E. K. Nelson and H. H. Mottern of the Food Laboratories studied the acids of honey at this time, and Dr. Nelson published a note on the flavor of orange honey. In 1928 Dr. R. E. Lothrop began chemical and technological work on honey in the Carbohydrate Division and continued for ten years, when he left honey research to become associated with the new Eastern Regional Research Laboratory near Philadelphia. Dr. Lothrop published many papers on the chemistry of honey, its analysis, the relation of its chemical and physical properties to its composition, and developed commercial methods for honey filtration that are in use today. He was succeeded by Mr. G. P. Walton who was concerned with honey analysis, especially for dextrins, developed a process making possible the use of honey in carbonated beverages at the beginning of the Second World War and devised ways to use honey in candied and glace fruit. The project was discontinued during the war. In 1948, after being away from honey research for several years, Mr. Walton initiated the present honey program at this Laboratory. After Mr. Walton's retirement in 1949 the writer has been responsible for the program.

Basic Research Program at the Eastern Laboratory

The honey research program has as a general objective the increasing use of honey. We try to do this by finding new uses, improving old ones, improving processing and by obtaining as much fundamental information about honey as possible to serve as a foundation for future technological work.

In this article I will describe our work on the analysis and composition of honey. During these thirteen years we have found many new components of honey, learned something of its enzymes, and made considerable progress in development of accurate methods for honey analyses.

The standard book of analytical procedures in the agricultural and food field is *Methods of Analyses of the Association of Official Agricultural Chemists*. It is revised every five years; the ninth edition has just appeared. At the time our current program began, the section on honey analysis carried the same procedures used by Wiley and Browne so many years ago.

In 1950 we began our cooperation with the Kansas Agricultural Experiment Station, who agreed to investigate the potentialities of honey in commercial baking. It became necessary for us to analyze fifteen honey samples of different floral types which had been selected for this work.

Sugar Analysis of Honey

Beginning with no knowledge of honey analysis, we studied five procedures recommended in the literature for the determination of dextrose and levulose in honey, in order to adopt one as our standard procedure. The results were sufficiently startling that we subjected them to statistical evaluation, which confirmed that there was more variation among the different methods on the same sample than among the samples of different floral origin when analyzed by any one method. Furthermore, and worse, we had no basis on which to decide which method, if any (including the "official" one) gave us the true results (1). Such a state of affairs is a challenge to the chemist, so we set out to develop better methods of sugar analyses for honey.

It had long been recognized that the old optical (saccharimetric) methods had limitations and that newer chemical methods (such as one developed by Lothrop) were preferable. However, the latter were still deficient due to the complex nature of the sugars of honey. It was only recently, when the new methods of chromatography were applied to sugars, that the way to accurate sugar analyses of honey became open

J. W. White and Jeanne Petty discuss results of paper chromatographic separations of sugars during the work on the mechanism of honey invertase action.

The automatic titrator, attended by Irene Kushnir, was used for many of the sugar analyses during the survey of American honeys.

to us. Whistler and Durso of Purdue had noted in 1950 that if a solution of sugars of different degrees of complexity be poured on top of a column of finely-ground charcoal and washed with alcohol solutions of increasing strength, the various filtrates so obtained contained separated groups of sugars in increasing degrees of complexity. In other words, this could provide a pretreatment for a honey solution to separate the interfering materials from the dextrose and levulose and also perhaps permit the analysis of sucrose and other sugars.

Using this selective adsorption on Charcoal, we modified chemical sugar methods to measure the sugars in the filtrate, put it on a quantitative basis (2) and began to learn something of the actual sugar composition of honey. After analyzing about twenty samples it was apparent that honey had considerably less dextrose and somewhat less levulose than previously thought, and contained a whole new (to honey) group of sugars, the reducing disaccharides. These averaged about 7.5%, and ranged as high as 14%, of the weight of honey (3).

This analytical procedure has provided a new picture of the composition of honey. It has been accepted first action by the Association of Official Agricultural Chemists and is included in the ninth (1960) edition of the *Book of Methods*. It has been used for honey analyses in Canada, South Africa, and Chile.

Survey of American Honeys

One effect of the new analytical method was to render obselete the results of honey analyses by older methods. This meant that the work of Browne (Bulletin 110) was no longer authoritative and pointed up the need for an analytical survey of the various honeys produced in this country. Other factors also made this desirable, such as the changes in agriculture and beekeeping methods over the intervening fifty years, not to mention the need by the food industry for more accurate data on honey. Hence in 1956, using increased funds obtained by the efforts of the honey industry, we initiated a project to collect and analyze as completely as we could samples of honey from as many sources and localities as practicable.

This we have done, using honeys from the 1956 and 1957 crops. It was a considerable undertaking, with 504 samples from 47 of the 50 states being subjected to 17 physical and chemical analyses. The nearly ten thousand results were transferred to IBM cards, analyzed, classified, and printed by machine. A Department

(1) White, J. W. Jr., Ricciuti, C. and Maher, J. "Determination of Dextrose and Levulose in Honey. Comparison of Methods." J. Assoc. Offic. Agr. Chemists *35*, 859 (1952).

(2) White, J. W. Jr. and Maher, J. "Selective Adsorption Method for Determination of the Sugars of Honey." J. Assoc. Offic. Agr. Chemists *37*, 466 (1954).

(3) White, J. W. Jr. and Maher, J. "Sugar Analyses of Honey by a Selective Adsorption Method." J. Assoc. Offic. Agr. Chemists 37, 478 (1954).

Bulletin has been prepared giving all individual results, interpretations and analytical procedures. This should appear in print within a year.

This work would not have been possible without the cooperation of many beekeepers, extension apiarists, State bee inspectors, Professors of Apiculture and State and National organizations and their officers. The analytical work at this Laboratory was carried out by Mary H. Subers, Irene Kushnir, Mary L. Riethof, Robert B. Hager, Frederick W. Pairent, Laverne Scroggins, Allison Smith, Ronald Campbell, Dolores Boe, Oksana Panasiuk, and the writer. A summary of the results of the survey appears in a series of articles beginning in the November, 1960 *Gleanings in Bee Culture.*

Rare Sugars In Honey

With the knowledge that reducing disaccharides were present in honey in appreciable amounts, we undertook to isolate and identify these materials. With the help of such recent techniques as gradient elution carbon chromatography (4), preparative paper chromatography and infrared spectra of potassium chloride discs containing the sugars (5), we isolated and identified the following reducing disaccharide in honey (6):

Maltose:—This common diglucose sugar had been reported in honey some years ago by Elser and confirmed by van Voorst. It is the disaccharide sugar found in corn sirup.

Isomaltose:—This is an uncommon sugar, related to maltose. Its best source is from the hydrolysis of dextran, a fermentation polysaccharide used as a blood extender and industrial gum.

Nigerose:—This is a very rare dextrose-containing sugar. It had previously been isolated from "hydrol" which is the "molasses" remaining from dextrose manufacture from corn. It is also part of a polysaccharide "nigerose" produced by a mold.

Turanose:—This sugar, composed of dextrose and levulose, may be produced by splitting of the trisaccharide melezitose which is found in certain types of honeydew. Its identification in honey appears to be the first isolation of this sugar from a natural product; the same may be said of nigerose and maltulose.

Maltulose: — This sugar contains dextrose and levulose, and in structure resembles both maltose and turanose. It can be made from maltose by treatment with limewater.

Of these minor sugars, isomaltose is present in largest amount. We believe that they do not exist in nectar but are produced by the conditions that bring about the change of nectar to honey, including the presence of honey invertase, the high acidity, and the high solids content. We have not yet studied the more complex sugars which occur in honey.

The Acids of Honey-Measurement and Identity

Another of our more basic studies on the composition of honey has been of the acids present. Many years ago it was thought that formic acid was the principal acid of honey, having been added by the bee to preserve it. As more became known of the acids, the relative importance of formic acid diminished; recent belief has been that the principal acid of lemon juice, citric acid, is the predominating honey acid.

Those who have worked with honey in the laboratory or plant by neutralizing its acidity with alkali have encountered a puzzling phenomenon. Honey solutions, normally acid, show a definite tendency to revert to their acid condition when they are brought to neutrality. That this has long been recognized is shown by part of the directions for determining the acidity of honey by the A.O.A.C.—the end point is taken arbitrarily as the amount of alkali needed to maintain a pink indicator color *for ten seconds.* The rapid fading of the color shows a return of the solution to the acid state. This had been attributed in the past to production of acids from the sugars by oxidation by air or to the production of acid by an enzyme in honey.

As part of our program to learn more about the components of honey we began a study to identify the acids. During this work it was noted that while most of the sugar-free acid fractions were quite normal in their behavior during neutralization, several of them showed the same "fading end-point" that is characteristic of honey. This led us to propose that honey contained lactone. A lactone is a neutral compound that can slowly react with water (hydrolyze) to form an acid.

When we came to analyze the collected honey samples for our survey of American honeys, we developed a new method (7) for the determination of honey acidity which also provided a measure of the lactone content and a stable end-point. As we had analyzed the samples by this method, it became apparent that material of lactone nature is a general constituent of honey. It was present in all honey samples, sometimes half as much as the free acidity.

During this time, progress was also being made in identifying the acids in honey. It was found (8) that the principal acid in honey is not citric acid but rather gluconic acid. This is an acid derived from dextrose which can form a lactone. Thus undoubtedly all or most of the lactone material in honey is gluconolactone, in equilibrium with gluconic acid. Gluconic acid had some years ago (9) been found in a sample of "musty" honey, where its presence was ascribed to the presence of a mold known to produce gluconic acid. In our identification work the number of *positively* identified honey acids was raised from three (citric, succinic, malic) to nine (butyric, acetic, formic, lactic, pyroglutamic, and gluconic acids). Several others were not identified (8).

Honey Enzymes

Other components of honey which are of great importance in its formation and are of great interest to us are the enzymes. These have been called "biochemical catalysts." The existence of all living things depends on the action of their enzymes to bring about under very mild conditions of concentration, temperature, and acidity, processes that either require very stringent conditions to carry out in the test tube or cannot be done at all. Enzymes in honey

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(5) White, J. W. Jr., Eddy, C. R., Petty, J. and Hoban, N. "Infrared Identification of Disaccharides." Anal. Chem. *30,* 506 (1958).

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(7) White, J. W. Jr., Petty, J. and Hager, R. B. "The Composition of Honey. II. Lactone Content." J. Assoc. Offic. Agr. Chemists *41,* 194 (1958).

(8) Stinson, E. E., Subers, M. H., Petty, J. and White, J. W. Jr. "The Composition of Honey. V. Separation and Identification of the Organic Acids." Arch. Biochem. Biophys. *89,* 6 (1960).

(9) Angeletti, A. "The Presence of d-Gluconic Acid In Musty Honey." Giorn. farm. chim. *81,* 533 (1932).

may have been added to the nectar by the bee, may exist in the nectar itself, or may come from other sources such as pollen, yeasts, and so on. The most important of these enzymes is sucrose or invertase. This is added by the bee and brings about the change ("inversion") of sucrose in the nectar to simple sugars (dextrose and levulose) in honey. This is one of the most important changes during the conversion. Even after honey is ripened, extracted, and bottled, the invertase will remain active and can be demonstrated unless the honey has been heated in processing sufficiently to destroy it. Another important honey enzyme (for an entirely different reason) is the so-called diastase or amylase. This enzyme is one that converts starch to simpler sugars. Its relationship to honey ripening is unknown. Since it is easily measured and is of course destroyed by heating, the presence of a low level of diastase in honey has been used for many years in various European countries as evidence that it has been subjected to excessive heat, which in their opinion greatly reduces its value.

We are presently engaged in a study of the enzymes of honey, from both the basic point of view to learn more about them as enzymes and also from the practical side to learn the effect of storage and processing conditions on them. Some years ago we looked into the mechanism of the action of honey invertase on sucrose, finding (10) that it is quite different from yeast invertase. Honey invertase "attacks" the dextrose part of sucrose while yeast invertase has been shown by others to attack the levulose part. During its action(and no doubt during the ripening of honey) several more complex sugars are formed by transfer of dextrose molecules to sucrose or to other sugars present. We isolated one such sugar, a new trisaccharide, and proved its structure (11). We called it "erlose." Since then it has been found in honey and honeydew and in other products. In fact there appear to be (at least) two kinds of honeydew, depending on the particular insect that produces it: the more familiar melezitose type, and the erlose type (12). The former may crystallize in the honeycomb, the latter apparently not, though it is not known whether erlose survives collection and ripening by bees as does melezitose.

While on the subject of enzymes, we have used the excellent analytical method for honey diastase described by Schade, Marsh and Eckert at California, who did collaborative work on it with German authorities, and initiated its adoption as an A.O.A.S. method.

While at first glance the relationship of some of the fundamental chemical work as described in this article to the objective of increasing honey utilization may seem obscure, it has been amply demonstrated that practical advances in technology and industry must draw on such basic information. This has always been true. It means that any complete research program, even of the most practical nature, must if at all possible include a generous portion of basic work as insurance for future developments. We cannot indefinitely rely on others to do our fundamental work for us.

For example, we recently developed a method to detect the admixture of corn sirup with honey (13) based on the knowledge that certain higher sugars occur in corn sirup but not in honey. We have also developed a chemical test to detect toxic honey from laurel and rhododendron (14) based on fundamental work by Dr. E. C. Horning and his associates (15).

During the past thirteen years several chemists have been associated with the honey research program. I should like to acknowledge the competence, careful attention to detail, and willing cooperation of these individuals: Jeanne Maher Petty, Nancy Hoban, Mary L. Riethof, Mary H. Subers, Edwin E. Stinson, and Irene Kushnir.

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