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**THE COMPOSITION OF HONEY**

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There are many legends and tales of the virtues and values of honey, and a perusal of the current American beekeeping journals shows that not all the legends and fairy tales are inherited from the past. Some are being manufactured and circulated today.

Much of the information given in this review has been discovered during the seven years since an earlier review was published in *Bee World* on the composition and properties of honey (Pryce-Jones, 1950). Both before and since that date, honey has been studied far more intensively in Europe than in America.

### THE SUGARS OF HONEY

Honey is primarily a carbohydrate product, and sugars may make up as much as 99% of the solids.

Until recently, the sugars of honey were thought to consist of glucose, fructose and sucrose; an ill-defined material of higher complexity termed 'dextrin' completed the picture. This simple concept was consistent with the inversion of the sucrose in nectar by the invertase of the honeybee, to form its constituent sugars. It was essentially our knowledge of the sugars of honey until the introduction of paper chromatography about 10 years ago. Prior to that, the only addition to the list of sugars in honey was made by Elser in 1924 who identified maltose. V. Fellenberg and Ruffly (1933) criticized Elser's identification, but it was confirmed in 1941 by van Voorst (1941), who used a fermentation method for honey analysis and reported that honey always contained several per cent of maltose.

An early report on the use of paper chromatography for separating the sugars of honey was made by Mayloth (1951). She reported the presence of glucose, fructose, sucrose, maltose, melezitose and dextrans, basing her identifications on relative movement on the paper and reaction to spray reagents. The next year Vavruch (1952) 'identified' fructose, glucose, sucrose, maltose, and 'probably melezitose' by similar procedures.

The first detailed report on the use of paper chromatography to investigate the identity of the sugars in honey appeared early in 1952. A paper by Täufel and Reiss (1952) summarized a thesis by Reiss, submitted

\*Paper presented at the Tenth International Congress of Entomology, Montreal, Canada, August 1956.

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to the Technical University of Berlin in 1951. They concluded that at least five sugars were present in addition to those ordinarily known. Further, they said that sucrose was found in only 4 of the 12 samples they examined ; this conclusion was based on an analytical procedure (Täufel & Reiss, 1951), which they termed differentiating hydrolysis. They attributed the previously reported analytical occurrence of sucrose to partial hydrolysis of honey dextrins.

Täufel and Reiss also considered the cause of the variable fructose/glucose ratio of honeys. They postulated that either a polyglucose is formed during ripening of honey, or glucose is isomerized into fructose. They then analysed the products obtained by feeding sucrose and glucose to bees. In the 'honey' produced by sucrose-fed bees they found only sucrose, glucose and fructose ; fructose exceeded glucose by about 3%. In the 'honey' produced by bees fed only glucose they found 7% fructose in addition to glucose. They concluded that the five new sugars found (which they did not identify) originated in the nectar, and also demonstrated the presence of several saccharides in nectar samples. They believed that the variation in the fructose/glucose ratio was largely due to isomerization of glucose into fructose. Finally, they pointed out the effects of their work on the conventional methods of honey analysis.

In another paper, which appeared the following year, Täufel and Müller (1953) continued the discussion. Considering the possibility that the new sugars originated in pollen, they analysed 12 pollen samples, but found only glucose, fructose and sucrose. They suggested the possibility that the sugars originated in resynthetic enzyme reactions arising from transglucosidation by enzymes in acid media — which had just been discovered — but came to no definite conclusion.

At our laboratory we had been investigating the minor sugars of honey since 1949. We found that we could agree with the German workers on two points ; that the amounts of the sugars were so small that identification was extremely difficult, and that there were a number of sugars in honey which had not been previously reported. However our number was around ten in addition to glucose and fructose.

When the transglucosidation activity of sugar-splitting enzymes — specifically, yeast invertase — was discovered in England in 1950 (Bacon & Edelman, Blanchard & Albon), we considered the possibility that honey invertase would show a similar activity. It did indeed, with the production of at least 6 oligosaccharides from sucrose, in addition to glucose and fructose (White, 1952 ; White & Maher, 1953*a*). We isolated the principal sugar and determined its structure. It was  $\alpha$ -maltosyl-fructoside, a new non-reducing trisaccharide (White & Maher, 1953*b*). We called it 'erlose'. This sugar, together with other evidence, showed that honey invertase is a glucose-transferring enzyme, or gluco-invertase ; it differs in this respect from yeast invertase, which attacks sucrose from the fructose end, transfers fructose and is termed a fructo-invertase. Certain mould invertases, on the other hand, had been considered to be gluco-invertases, but have now been demonstrated to be fructo-invertases (Bealing, 1953). It is immediately apparent that we have here a possible mechanism for obtaining a fructose/glucose ratio of more than 1, since glucose is preferentially combined in these minor saccharides. It seems to be general that the plant invertases such as might exist in nectar are fructose-transferring, and lead to oligosaccharide intermediates different from those

produced by bee invertase. The transference of glucose to oligosaccharides was suggested as a means of increasing the fructose/glucose ratio, by Maurizio at the 15th International Beekeeping Congress (1954). In her opinion other mechanisms, such as the amount and activity of nectar invertase, also contribute. The predominantly animal origin of the honey invertase is indicated by the fact that its activity is glucose-transferring rather than fructose-transferring.

Meanwhile we were engaged in developing a new method of honey analysis. We agreed with Täufel and his co-workers that existing methods for honey analysis were inadequate, and we developed a procedure using selective adsorption as a pre-treatment for the honey solution. Application of the procedure (White & Maher, 1954a) to a number of honey samples showed reducing disaccharides to be general constituents of honey (White & Maher, 1954b). It was recognized that maltose was only one of several such sugars present.

Early in 1955 another publication from Germany by Goldschmidt and Burkert described work on the minor sugars of honey. The workers used paper chromatography with several solvent mixtures for the identification of the sugars, and also enzyme reactions on the paper. The identifications are not conclusive, since chromatographic evidence alone is not absolute; they are subject to confirmation by isolating the sugars and determining their physical properties. Goldschmidt and Burkert investigated honey, honeydew honey and sugar-fed honey. Most of their attention was given to honeydew honey; in this they stated that they found glucose, fructose, sucrose, maltose, isomaltose, erlose, kestose, melezitose, raffinose, dextrantriose, 4-glucosyldextrantriose and a higher carbohydrate. The same sugars were found in honey, but in lesser amounts, with the higher sugars absent from some. Honey from sugar feeding (contrary to the reports of Täufel) was found to contain maltose, isomaltose and erlose in addition to glucose, fructose and sucrose. It was concluded that sucrose, kestose, melezitose and raffinose are introduced with the nectar, and that maltose, isomaltose, erlose and dextrantriose are formed as secondary products by transglucosidation in the body of the bee. Reducing disaccharide material was reported by Austin (1956) as a general component of 40 samples of Canadian honey analysed by the selective adsorption method. He suggested that the 'maltose' content of any honey depends to some extent on methods used by the beekeeper in its production — on the time of extraction, the use of heat in processing, and the length of storage.

The only sugars of honey which have so far been adequately identified are glucose, fructose and maltose. With regard to the so-called 'honey dextrin', some writers confuse this alcohol-precipitable carbohydrate material of honey with starch dextrins, although there is no evidence that the two substances have anything in common. Extensive purification as reported by v. Fellenberg (1933) resulted in a material from flower honey and from honeydew honey which he considered identical. He described its properties, and showed that it differed from melezitose. Here again is an opportunity for the use of chromatography to extend our knowledge. Chromatography of the higher sugar fraction from our selective adsorption procedure for honey analysis has shown that there are several tri- and higher saccharides, which collectively might make up the so-called honey dextrin (White & Maher, 1954a).

We will next consider the acid group of constituents. Formic acid had for many years been considered to be the principal acid of honey. Several papers from Germany before the First World War contradicted this view ; it was shown by Farnsteiner in 1908 and confirmed by Merl (1908), Heiduschka and Kaufmann (1911) and Fincke (1912), that only about 10% of the acidity of honey was due to this acid. Farnsteiner believed the acidity was due to malic acid ; Heiduschka and his co-workers reported the presence of the volatile acids butyric, valeric, caproic and capric, and also lactic, malic, tartaric, oxalic and succinic acids. The acids were not isolated and identified. Nearly twenty years later, Nelson and Mottern (1931) identified citric acid, malic acid, and succinic acid in honey. These compounds were isolated and identified by derivative formation ; acetic acid was also reported. Shortly afterwards Angeletti (1932) reported *d*-gluconic acid in a sample of 'musty' honey ; it was separated and identified as the calcium salt. Its presence was ascribed to mould activity.

As the advent of chromatography has opened the way to the separation of minute amounts of sugars, and led to recognition of the complexity of the carbohydrate composition of honey, it has become apparent that our knowledge of the acids can also be greatly increased by this valuable laboratory technique. Vavruch (1952) recently reported the paper chromatography of the acids of honey ; he found malic, tartaric, citric, and traces of succinic and lactic acids. Here again it is necessary to note that unequivocal identification cannot be made by chromatographic means alone ; physical characterization of the isolated compound or derivative is required.

In our laboratory we have been engaged in the further identification of the acids of honey. Using ion-exchange and column and paper partition chromatography for isolation, and X-ray diffraction for identification, sufficient work has been done to show the complexity of the problem. Some fifteen acids are evident from partition column chromatography, of which four have been identified ; no addition to the list of known acids in honey has however yet been made. This work is continuing.

#### VITAMINS IN HONEY

We now enter a field which has seen some controversy in the past. Early determinations of vitamin content by feeding tests were rather generally negative (Dutcher, 1918; Hawk *et al.*, 1921; Scheunert *et al.*, 1923; Kifer *et al.*, 1929). Later examinations using chemical and microbiological methods (Haydak *et al.*, 1942; Kitzes *et al.*, 1943 ; Vivino *et al.*, 1943) gave small but definite values for the various vitamins in honey. There is no doubt that honey contains variable amounts of thiamine, riboflavin, pantothenic acid, nicotinic acid, pyridoxine, ascorbic acid, vitamin K, folic acid and biotin. There is also no doubt that the amounts of these factors commonly found in honey are nutritionally insignificant to humans.

A recent controversy regarding the amount of vitamin C in honey has been summarized by Hansson (1949). Early animal assays for vitamin C were negative. Ulrich reported to the 7th International Congress of Entomology in 1938 that something like vitamin C occurred in honey, and several investigators (Kask, 1938; Griebel & Hess, 1939) reported

rather impressive amounts of so-called vitamin C in honey as shown by chemical methods — 20 to 300 mg. per 100g. However, Becker and Kardos (1939) declared, on the basis of animal feeding tests, that the reducing substance present was not vitamin C.

Haydak has published a series of vitamin C values, determined chemically ; these vary between 0.5 and 6.5 mg. per 100 g. honey. These values are very low — and Haydak himself (Haydak *et al.*, 1944) developed symptoms of vitamin C deficiency while subsisting on a diet of milk and honey (350 g. honey a day). The report of Gontarski (1948) that honey contains an enzyme which destroys vitamin C seems to confirm the practical absence of this vitamin from honey.

#### HONEY ENZYMES

The enzymes of honey are constituents of great interest and importance. Present only in the merest traces, they have a profound effect on the nature and characteristics of honey. They may arise from the nectar, or be added by the bee. Some enzyme activity might also be traced to the pollen content, and some might come from micro-organisms present in nectar or honey.

Enzymes which have been reported in honey include invertase, diastase or amylase, an oxidase, catalase (Auzinger, 1910 ; Gillette, 1931), a phosphatase hydrolysing glycerophosphate (Giri, 1938), and an acid-producing enzyme (Cocker, 1951). The early literature on honey enzymes was reviewed by Auzinger (1910). The statement has been made (Grüss, 1932) that the best honey should show all enzyme reactions of the living cell.

Of all these enzymes, the amylase has probably had the greatest amount of attention, because of the importance attached to the diastatic activity of honey in Europe, which will not be reviewed here. It is sufficient to state that it has been thought that since the diastatic activity is decreased by heat, it can serve as an indicator of the degree of heating to which a honey has been subjected. Only unheated or mildly heated honey is acceptable in many European countries. Since some honeys appear to be naturally low in diastatic activity, there has been some controversy, and difficulty has been encountered in exporting honey to these countries from the United States. As I understand the problem, it is not felt that the diastase as such is valuable, but that important physiological or nutritive properties of honey are damaged or destroyed by heat, and that diastase can be used as an indicator of heat treatment.

A recent publication by Kiermeier and Köberlein (1954) describes their work on connecting enzyme activity of honey with its history of heat treatment. They studied the effect of heating on the activity of diastase, invertase, and catalase, and the enzyme which destroys ascorbic acid in honey. Catalase activity was found in only one sample out of ten, and in that sample it was much too small for use as an indicator. When the authors examined the enzyme which oxidizes the ascorbic acid, they were unable to check the results which Gontarski had obtained regarding this enzyme. The destruction of ascorbic acid by honey solutions was verified, but the reaction took place as well with boiled honey solutions as with unheated honey, and it was not inhibited by cyanide. They believe it to be non-enzymatic — and of course not useful for detecting any previous heating of honey.

Kiermeier and Köberlein concluded that amylase and sucrase activity in honey varied exceedingly; that the same heating damaged the enzymes to different extents in different honeys; that detection of heating by diastatic activity alone is not possible, but that both diastase and sucrase activity must be considered. General agreement in this has been expressed by Schade, Marsh and Eckert (1956).

Before finishing this brief account of the enzymes of honey, one other should be mentioned, the acid-producing enzyme reported by Cocker in 1951. He investigated an occurrence which is familiar to anyone who has determined the acidity of honey by titration. The end-point is difficult, and the solution shows a marked tendency to drift back to the acid side. This was noted by Walton (1944), who thought it might be due to alkaline oxidation of sugars. He noted it with both honey solutions and honey syrups. Cocker ascribed the action to the activity of an acid-producing enzyme, and stated that the effect was not found with boiled honey, and only to a smaller degree with honey produced by bees from sugar fed to them. Cocker was unable to identify the acid formed, but stated that there was some indication of keto-acids. He had overlooked the paper by Gauhe (1941), published ten years earlier, in which she reported a glucose-oxidizing enzyme in the pharyngeal gland of the honeybee. Gauhe stated that the enzyme acted specifically on glucose, producing gluconic acid and peroxide. In this respect it is very similar to a so-called glucose oxidase—more properly a dehydrogenase—which is produced from moulds and has found some commercial application. She did not report its presence in honey.

#### PROTEINS AND AMINO ACIDS

There are other groups of constituents of honey, which have been investigated to a greater or smaller extent in the past, but on which no work has been reported for the past fifteen years. It was early recognized that honey had a definite small protein content. Lund (1909, 1910) set the nitrogen content for pure honeys at about 0.4%, and that of artificial honey much lower, and he devised a precipitation test to distinguish between them. Moreau (1911) reported the presence in honey of globulins, albumins, proteoses and peptones. Langer (1910) devised a biological test to distinguish between genuine and artificial honey. The albuminoids of the honey were precipitated, and the honey then mixed with a rabbit antiserum; a precipitate was formed only with natural honey. Several other immunological tests were developed about this time (Carl, 1910; Thöni, 1913). That of Thöni, involving an anti-bee serum, was claimed to be quantitative and to show the amount of admixture of artificial honey. It was used in Switzerland for this purpose at about the time of the First World War (Kreis, 1915). The last publication available to me which mentioned its use is a dissertation by Laske, dated 1915 and abstracted in 1921.

The free amino acids of honey have been determined by Lothrop and Gertler (1933), and by Schuette and Baldwin (1944). The latter authors found in 37 samples of light and dark honey an average content of 48 parts per million. The amino-acid content of honey has also been used as a measure of honey purity by means of the formol titration (Tillmans & Kiesgen, 1927). The lowest value found in 319 samples by de Boer in 1947 was still three times that of artificial honey. No identification of individual free amino acids in honey has been made. Vavruch (1952),

who used chromatographic procedures, reported the free amino-acid content to be negligible. In a trypsin hydrolysate of honey protein he demonstrated the presence of alanine, glycine, histidine, leucine or its isomer methionine, and probably serine, lysine and traces of glutamic acid and aspartic acid.

#### HONEY COLLOIDS

The colloidal material in honey, as a class, has also had little recent attention. It was shown some twenty years ago (Lothrop & Paine, 1931) that honey contained from 0.08 to 0.8% of colloidal material, of which on the average 54% was protein, and which had an isoelectric point at 4.3 and hence (most honey being more acid than this) was positively charged. The properties of the colloids — and their effects on the properties, processing and stability of honey — were rather extensively explored by Lothrop at that time. One presumes that the colloidal content of honey comprises the enzymes, some of the pigments, and some wax, but the identity of these materials with the colloids has not been shown. It has been shown recently that about two-thirds of colloid material in heather honey is protein (Mitchell *et al.*, 1955).

#### MINERALS

No discussion of honey is complete without including the mineral content. Here again, if we can judge from publications, about three-quarters of the work on the subject is in Europe. Haydak (1955) has recently reviewed the nutritional aspects of the mineral content of honey. He showed earlier (Haydak *et al.*, 1942) that sufficient iron and copper are present to maintain haemoglobin in rats on a milk and honey diet. Gorbach and Windhaber (1939) reported 8 elements in flower honey and about 21 to 25 in honeydew honey. These are largely present in merest traces and are detected spectrographically. The most plentiful ash constituents are Ca, Mg, Fe, Mn, Cu, P, Si, Ni, B, Al, and K. The average ash content of honey is about 0.2% (Browne, 1908).

#### OTHER SUBSTANCES IN HONEY

Nearing the end of our list, we come to materials which are largely unknown or are difficult to classify. The flavouring materials of honey are probably the most important single group of compounds in it; yet for good and obvious reason our ignorance of them is great. Beyond the identification of methyl anthranilate (Nelson, 1930) in orange honey, and diacetyl (Schmalfuss & Barthmeyer, 1929) in honey we have no further knowledge. New techniques and equipment for studying flavouring materials are coming into use, so perhaps the future will bring us more information in this field.

The next group of materials is the catch-all labelled 'miscellaneous', where we enter items not easily classified elsewhere. These include known materials such as acetylcholine (Marquardt & Vogg, 1952), and unknown materials characterized only by their biological activities. In this last group we find a plant-rooting hormone (Oliver, 1940), an oestrogenic factor (Dingemans, 1938), a guinea-pig anti-stiffness factor (Church, 1954), and antibacterial substances (Dold *et al.*, 1937).

Toxic materials are not normally components of honey, and they are not discussed here.

## CONCLUSION

It would appear that research on honey is still in the phase in which further work produces increasing complexity rather than simplification. Certainly one can hope that before too long simplification will result from the current and future research on the composition of honey, and that reasonable explanations will emerge of the relationships between the properties of honey and its composition, and between the composition of honey and its origin.

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