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DEGREE OF PIGMENTATION AND ITS PROBABLE RELATIONSHIP TO THE MINERAL CONSTITUENTS OF HONEY

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Although honey's chief characteristic does not appear to be the quantity of mineral matter which it contains, yet two reasons suggest themselves why there is need for more information on its inorganic constituents than is now available. One centers around the newer emphasis placed by students of nutrition on the value of manganese and copper in the diet, the other around the present practice in merchandizing this saccharine food which reaches the consumer in its natural state, that is not refined by any manufacturing processes.

It is not required that honey be sold in interstate commerce on the basis of floral source. This is a matter which is left to the discretion of the several states. Rather it must bear a grade in which color, appearance and freedom from generally recognized disagreeable flavors are given predominant consideration. To that end there have been adopted such color designations as *white*, modified when necessary to indicate lighter tints by the words *water* or *extra; amber*, likewise qualified to differentiate the lesser pigmented members of this group by the terms *extra light* or *light;* whereas the adjective *dark* is reserved for the most heavily pigmented honeys.

Although it is perhaps suspected by those conversant with the chemistry of honey that, as between a deeply pigmented product and one less so, the mineral content of the former will in general exceed that of the latter, yet no published reference to this situation has apparently been made heretofore. Be that as it may, however, color and quality often bear an inverse relationship to each other in the lay mind. For this reason it seemed worth while to seek quantitative data in support or contradiction of this circumstance for it would follow, on *a priori* grounds at least, that a deeply pigmented honey ought to be deemed superior to one of light color from the standpoint of nutritive value if the mineral content of the former should prove to be higher. On making an analysis of the ash of twenty-two floral nectar and honey-dew honeys which had been selected at random from widely separated localities in this country in order to reduce any suggestion of accidental results, such was actually found to be the case. This observation in itself is not presented as novel, rather the fact that iron, copper and manganese appear to predominate in the mineral matter of dark honeys.[1]

[1] A line of demarcation has been arbitrarily drawn between the *white* honeys, typified by those from clover or alfalfa and probably indicative of the layman's conception of a light colored product, and the *light amber* honeys as representing the dark

A search of the literature of the past three decades reveals that, although data on the total mineral content of honey are plentiful, yet comparatively few are available with respect to the aforementioned elements.[2-8] Gottfried[2] has already called attention to the probable existence of a relationship between the manganese content and the albuminoids of honey. Lindow and his colleagues[6] in this country and Svoboda in Czechoslovakia are probably among the first to report the presence of copper in this food though unfortunately their records do not reveal a description of the material under examination. Fehlmann[3] found silica in several Swiss honeys and advanced the opinion that its presence there was purely adventitious. Sundberg and Lundgren[7] also reported it as present in the honeys of Swedish origin which they examined. An inspection of their data shows that there is apparently a progression in the silica content of their samples with an increasing depth of color. Existing data for iron[3,4,7,8] with the probable exception of those of Elser[4] and his Swedish contemporaries,[7] do not lend themselves very well to a similar analysis.

Experimental

Material.—With four exceptions (marked by an asterisk in Table I) all samples were obtained in the comb and extracted in the laboratory. As for the others, there was no apparent reason for assuming that they had been extracted in the apiary under conditions leading to the introduction of extraneous metal or mineral matter.

Ashing of Samples.—Weighed amounts of honey in approximately 75-g. portions were first evaporated in large platinum dishes on a hot-plate until substantially all of the water had been driven off. The resulting honey solids were then carbonized over an open flame, after which they were reduced to an ash in an electrically heated muffle furnace whose temperature was kept at a dull redness. The top and sides of the furnace were lined with sheet platinum for the purpose of preventing contamination of the contents of the dishes.

Inasmuch as the determinations of silica were to be made macrochemically it was, therefore, desirable to obtain fairly large mineral residues, and this was only possible by ashing more material than is the usual practice. To that end three to eighteen portions of each honey, depending upon the material available, were separately incinerated, the weight of the respective residues being noted. It was found that the average deviations in the determination of the ash by this mode of procedure lay between ± 0.0008 and $\pm 0.006\%$ for the entire series.

Determination of Silica.—Silica was determined in the usual way, that is, treatment of the ash with 10 cc. of hydrochloric acid (1 + 1), evaporation of the whole to dryness and the addition to the residue of 5 cc. of 1 *N* hydrochloric acid and a like volume of

ones, this group herein being headed by those in which Spanish needle is the predominating floral source.

[2] Gottfried, *Pharm. Zentr.*, **52**, 787 (1911); **53**, 4040 (1912).

[3] Fehlmann, *Schweiz. Bienenztg.*, **48**, 129 (1912).

[4] Elser, *Archiv. Bienenkunde*, **7**, 276 (1926).

[5] Nottbohm, *ibid.*, **8**, 207 (1927).

[6] Lindow, Elvehjem and Peterson, *J. Biol. Chem.*, **82**, 465 (1929).

[7] Sundberg and Lundgren, *Archiv. Bienenkunde*, **11**, 324 (1930).

[8] Svoboda, *Český Včelař*, **64**, 243 (1930).

water, and finally digestion of the mixture for thirty minutes on a water-bath, solution of the soluble constituents in hot water and filtration. The combined filtrates and washings from all the ash portions of each sample were evaporated to dryness, after which the residue was treated as before, the filtrate this time being made up exactly to 100 cc. The silica was volatilized as the tetrafluoride. After corrections were made for the blank on the reagents, a practice which was followed throughout this investigation, the silica content was calculated on the basis of mg. per kilo of honey and per cent. of the ash.

Determination of Iron.—Iron analyses were made colorimetrically by the ferric thiocyanate procedure[9] with application of the technique of Kennedy,[10] who suggested that the reaction product be extracted with amyl alcohol. Aliquots for analysis were chosen to fit the needs of the individual honeys, sample weights lying between 6 and 24 g. representing the limits employed for the whole series. The volume of standard solution which was used for comparison contained 0.04 mg. of iron. Data obtained in the determination of this and the succeeding elements are reported on the same basis as is the silica content.

Determination of Copper.—The Elvehjem and Lindow modification[11] of the potassium thiocyanate–pyridine procedure[12] was used for the determination of copper.

TABLE I

ASH, SILICA, IRON, COPPER AND MANGANESE CONTENT OF LIGHT AND DARK HONEYS

Predominating floral source	Origin	Ash, %	Silica mg. per kg.	Silica % of ash	Iron mg. per kg.	Iron % of ash	Copper mg. per kg.	Copper % of ash	Manganese mg. per kg.	Manganese % of ash
Alfalfa	Colorado	0.039	6.6	1.67	1.2	0.29	0.20	0.05	0.17	0.04
Alfalfa	Wyoming	.043	7.4	1.71	1.5	.36	.35	.08	.18	.04
Clover	Minnesota	.048	8.3	1.72	1.2	.26	.14	.03	.34	.10
Clover	New York	.167	9.7	0.58	3.9	.23	.70	.04	.22	.03
Clover	Wisconsin	.057	7.2	1.23	2.5	.43	.29	.05	.43	.07
Clover	Wisconsin	.040	8.8	2.17	2.1	.52	.30	.08	.38	.09
Clover	Wisconsin	.045	10.2	2.23	2.3	.51	.35	.08	.42	.09
Clover	Wisconsin	.046	9.9	2.12	3.2	.68	.21	.05	.29	.06
Clover	Wisconsin	.083	9.6	1.15	2.1	.25	.16	.02	.44	.05
Clover-alfalfa	Montana	.082	11.7	1.41	4.8	.58	.25	.03	.19	.02
Average of light honeys		.065	8.9	1.60	2.4	.41	.29	.05	.30	.06
Spanish needle	Illinois	.174	20.0	1.15	1.0	.06	.40	.02	.89	.05
Spanish needle	Illinois	.158	28.3	1.79	0.7	.04	.35	.02	.52	.03
Tulip poplar*	Georgia	.184	15.3	0.83	9.2	.50	1.04	.58	6.21	.38
Tulip poplar	Maryland	.521	9.3	0.17	..	..	..	..	1.48	.03
Mint	Florida	.143	5.4	0.37	..	..	..	..	1.14	.08
Buckwheat	Wisconsin	.129	14.1	1.09	33.5	2.59	0.70	.05	6.40	.49
Buckwheat	Wisconsin	.135	9.0	0.66	..	..	..	..	9.43	.71
Buckwheat	Wisconsin	.118	20.0	1.73	..	..	..	..	4.81	.41
Buckwheat*	Wisconsin	.074	10.3	1.37	8.9	1.12	.47	.06	0.46	.06
Buckwheat	New York	.095	8.2	0.85	3.1	0.33	.44	.05	9.53	1.00
Average of dark honeys		.173	14.0	1.00	9.4	.77	.56	.13	4.09	0.32
Honey-dew*	Wisconsin	.504	31.0	0.61	..	..	..	..	1.71	.03
Honey-dew*	Hawaii	.304	60.2	1.97	20.4	.67	.33	.01	0.85	.03
Average of honey-dew honeys		.404	45.6	1.29	..	..	..	..	1.28	.03

[9] American Public Health Association, "Standard Methods for the Examination of Water and Sewage," New York, 1925, 6th ed., p. 46.

[10] Kennedy, *J. Biol. Chem.*, **74**, 385 (1927).

[11] Elvehjem and Lindow, *J. Biol. Chem.*, **81**, 435 (1929).

[12] Spacu, *Bul. Soc. Stiinte Cluj.*, **1**, 284 (1922); *Chem. Abst.*, **17**, 1772 (1923); Biazzo, *Ann. chim. applicata*, **16**, 96 (1926); *Chem. Abst.*, **20**, 2029 (1926).

The range of sample weights used lay between 23 and 124 g. Their copper content was checked by means of a colorimeter against a reference solution containing 0.02 mg. of this element.

Determination of Manganese.—Manganese determinations were made by the periodate method of Willard and Greathouse[13] with certain modifications in procedure as suggested by Peterson and colleagues.[14,15] The method in question rests upon the comparison of color intensities of the permanganate ion. Aliquot portions of the several ash solutions, representing not less than 35 g. nor more than 228 g. of sample, were taken for comparison with a standard potassium permanganate solution, the volume of the latter being determined by the individual needs of the sample, but in every instance containing 0.05 mg. of manganese or a multiple thereof up to 0.2 mg.

Discussion

It is evident that the ash content and degree of pigmentation apparently bear some relationship to each other, for the dark honeys were found to contain more mineral matter than the light colored ones. Quantitative relationships with respect to these two major color differences cannot be set up, of course, because of the fact that honey is seldom derived exclusively from the nectar of one species of flower. But even with this limitation the general conclusion finds corroboration in the data of Browne[16] which, when subjected to a similar analysis, also reveal the fact that the light colored honeys which he examined contained less mineral matter than did the dark ones. This correlation of data cannot be extended beyond this point for analyses were not made to include manganese and copper since the clinical advantages of the presence of these elements in the diet were unknown at that time. A parallel is found, however, when more recent data, reported by foreign investigators, are reinterpreted. The data of Sundberg and Lundgren,[7] for example, although limited to seven samples, show the same trend in the silica, iron and manganese content of dark honeys in contrast to lesser pigmented ones. Similarly, this condition obtains in the iron and manganese data of Elser,[4] who examined honeys grown in Switzerland.

It is also significant that this preponderance of total mineral matter in the dark colored honeys is reflected both in the average and in the maximum quantities of the constituent elements thereof (Table II). The order of magnitude of the minima is also larger except for the silica content and probably, too, the iron, yet in the latter case comparisons appear hardly valid because of the unequal number of analyses in both groups.

It is indeed an interesting observation that buckwheat honey, which in the competition for consumer preference and price is often handicapped

[13] Willard and Greathouse, THIS JOURNAL, **39**, 2366 (1917).

[14] Lindow and Peterson, *J. Biol. Chem.*, **75**, 169 (1927).

[15] Skinner and Peterson, *ibid.*, **88**, 347 (1930).

[16] Browne, Bull. 110, Bur. Chem. U. S. Dept. Agric., 22–39 (1908).

TABLE II

VARIATION IN ASH, SILICA, IRON, COPPER AND MANGANESE CONTENT OF LIGHT AND DARK FLORAL HONEYS

	Floral Honey							
	Light				Dark			
Constituent	Samples	Min.	Max.	Av.	Samples	Min.	Max.	Av.
Ash[a]	10	0.04	0.16	0.06	10	0.07	0.52	0.17
Silica	10	7.20	11.70	8.90	10	5.40	28.30	14.00
Iron	10	1.20	4.80	2.40	6	0.70	33.50	9.40
Copper	10	0.14	0.70	0.29	6	.35	1.04	0.56
Manganese	10	.17	.44	.30	10	.52	9.53	4.09

[a] Per cent., other constituents in terms of mgs. per kg.

by its dark color and a not so delicate flavor, should contain, in comparison with clover and alfalfa honeys, strikingly more manganese and copper.

An explanation for the conditions which are noted above may conceivably be found in the suggestion that the characteristics and flavor of honey are influenced to a marked degree by nectar and pollen. They, in turn, may very well vary in composition and quality according as the plant which produced them is affected by such growth factors as the meteorological conditions prevailing in its habitat and the nature and fertility of the soil, as it possesses some peculiar ability to utilize nutrient materials or thrives because of some unique mineral requirements.

These studies on honey pigmentation will be continued in the hope of ascertaining whether or not any relationship exists between pollen and mineral content, particularly manganese. The observation of Famintzin and Przybytik[17] that the ash of the pollen of the pine, *Pinus sylvestris*, contains this element will be made the point of departure in extending this search to the pollens of those plant species which serve as sources of honey.

Summary

Data have been presented in support of the hypothesis that there apparently exists in honey a relationship between the degree of pigmentation and the quantity of mineral matter, notably manganese and copper, which it contains.

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[17] Famintzin and Przybytik, *J. Russ. Chem. Soc.*, 371 (1885); *J. Chem. Soc.*, **50**, 172 (1886).