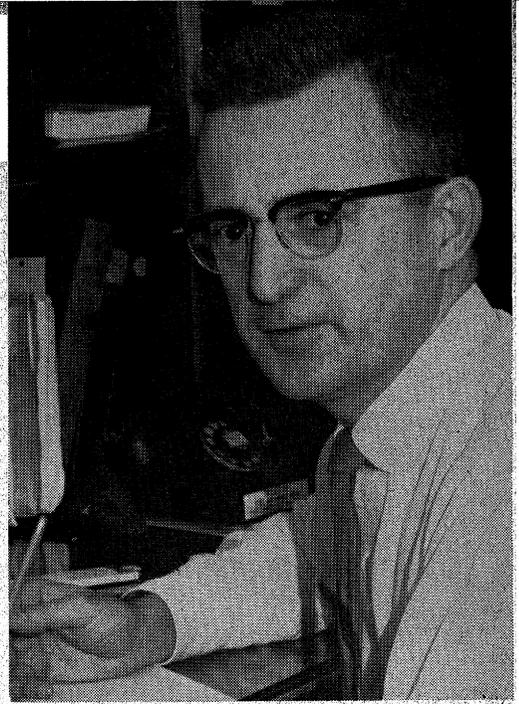


Analysis of Mixtures of Beeswax And Petroleum Waxes

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Beeswax is an important and valuable product of the honeybee. Its price and markets are relatively stable, compared to honey; there is a demand in this country for about twice the amount produced here.

Much of the domestic beeswax is used in the manufacture of comb foundation. With increasingly severe demands being placed on the performance of comb foundation, particularly in warm climates, some manufacturers of comb foundation had strengthened their product by adding other materials to beeswax for such use. Some of these additive waxes were more costly than beeswax, others were less expensive. Some manufacturers labeled the product and advertised that such additives were placed in the wax. Others made no mention of it. Foundation of greatly increased strength and higher melting point did fill a need in modern beekeeping practice.

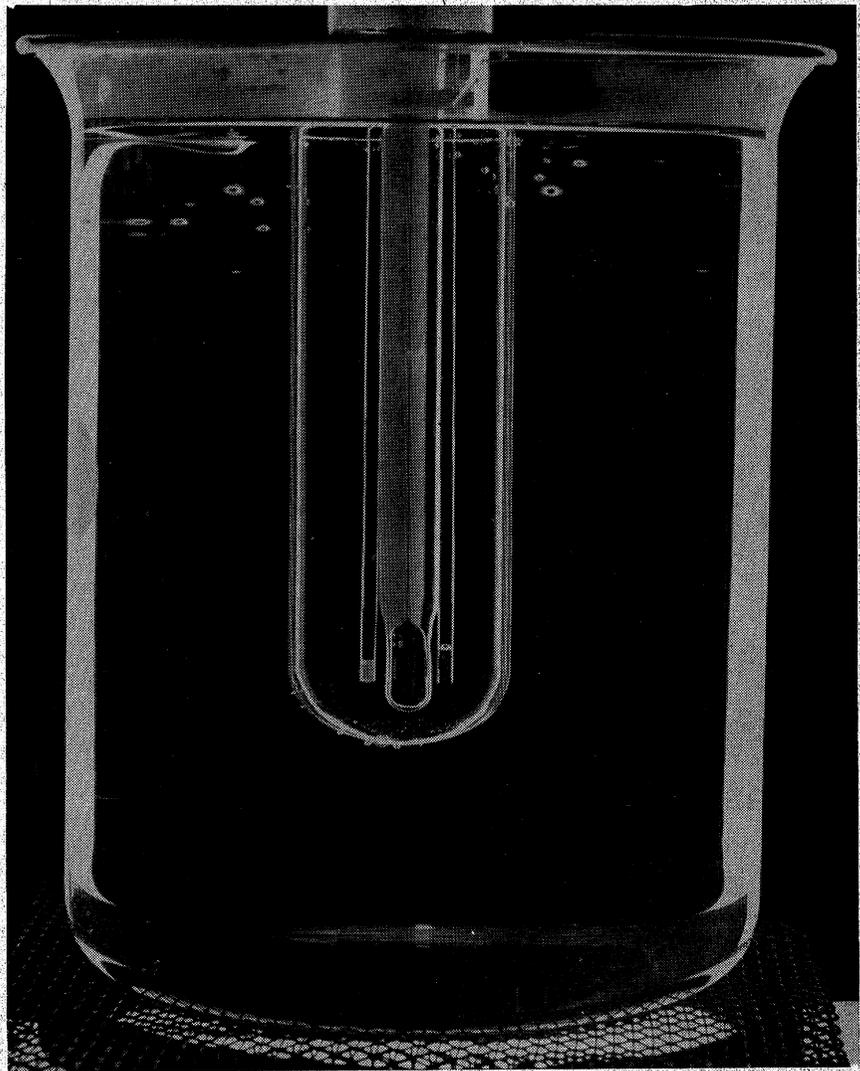
Since the greatest part of the combs remained in the beekeeper's possession and were not re-melted, a relatively small amount of additive wax at the beginning caused no great harm to the wax market. However, as time went on, the amount of other wax in some cases increased from a small percentage to a large amount and the product was marketed with no reference to its composition.

Finally, some foundation was marketed that appeared to have no beeswax at all and be composed of relatively inexpensive microcrystalline wax that could be purchased for a few cents a pound. Combs drawn from foundation containing a large percentage of microcrystalline wax when rendered resulted in a beeswax that carried variable and unknown amounts of impurities. Thus there was a possibility of accelerated trend towards lower quality beeswax. This could, in turn, lead users of beeswax to question the quality of our domestic wax with possible loss of markets. It is recorded that early in the century the German domestic beeswax market collapsed because of in-

roduction there of artificial comb materials.

Fortunately, the possibility of this occurring here has been forestalled by recent cooperative actions among the producers of comb foundation. They have agreed that all comb foundation will contain no inseparable materials other than pure beeswax.

The detection and determination of mineral waxes in beeswax has been a problem for many years. A general approach has been to ascertain the analytical constants for pure beeswax



Small samples of hydrocarbon material obtained in the determination of hydrocarbon content of the waxes are placed in capillary tubes, attached to a thermometer in a water bath. The water is heated until the samples melt, then let cool and the temperature noted at which they solidify.

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and then note how questionable samples differ from these values. This is useful for detecting gross admixture of other materials with beeswax, but it has long been known that mixtures of various materials can be put together that will respond properly to these analyses, thus simulating beeswax when in fact none may be present. Thus the analysis of questionable samples may be quite a complex matter, though the true nature of such mixtures can be shown.

These analytical constants for beeswax are generally used in government and commercial specifications and also in the various pharmacopoeias. It can easily be seen in Table 1, which lists some of these values, that there is a wide range of values for beeswax. This means that it is most difficult to show the presence of relatively small amounts of nonbeeswax materials in a beeswax

Pharmacopoeias

	Acid No.	Sapon. No.	Ester No.	M. P. °C.	Ratio No.
U. S. (XV)	18-24	-	72-77	62-65	
German	16.8-22.1	-	65.9-82.1	62-66.5	3.0-4.3
French	16.8-22.4	92-102	72-80	62-66	-
British	17-23	-	70-80	62-64	3.3-4.2
U. S. S. R.	17-20.5	-	66-76	63-65	3.42-3.9

Specifications

U. S. Federal	16.5-21.0	86-96	-	60.5-64.0	3.5-4.3
New Zealand	17-21	87-103	70-80	62-64	3.3-4.2
Toilet Goods Assn.	17-24	89-103	72-79	62-65	3.3-4.0

sample, by determination of these constants.

The materials most likely to be mixed with beeswax in quantity are petroleum waxes, particularly microcrystalline wax. This material may superficially resemble beeswax but is quite different, being a very inert and nonreactive material. Its presence

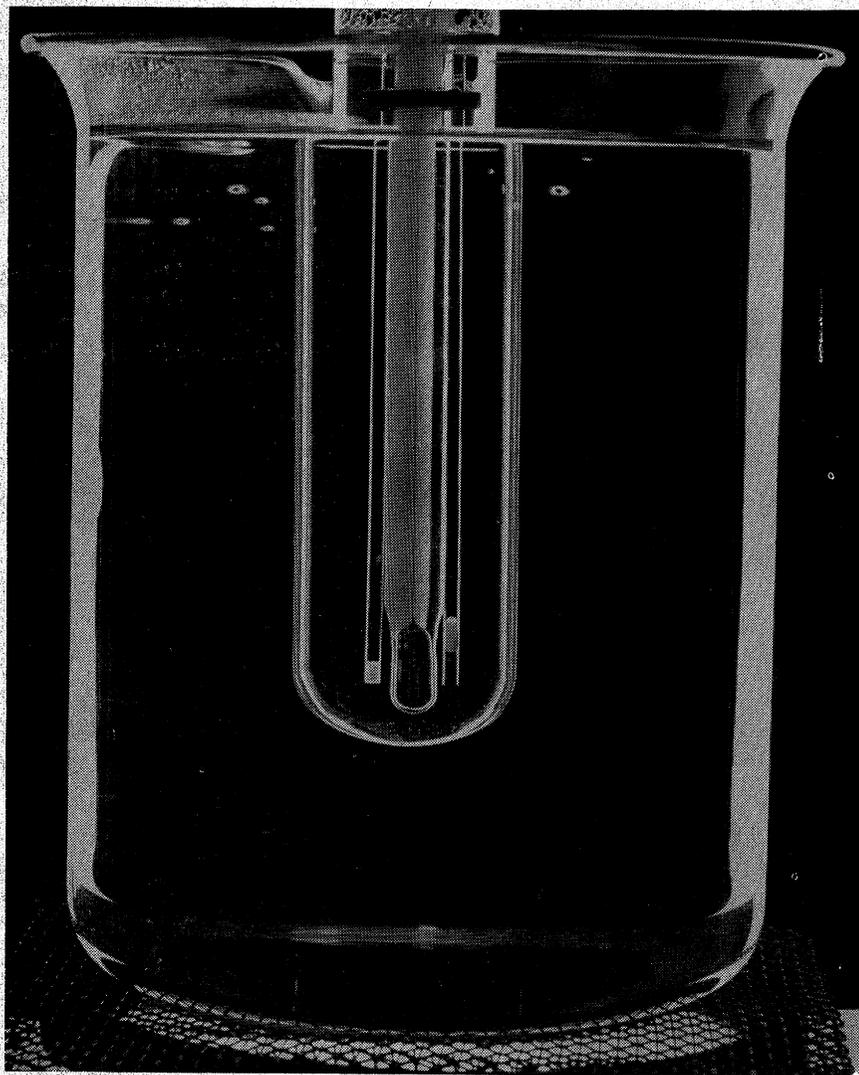
causes the analytical values such as shown in Table 1 to drop, except melting point, which will generally be higher. The problem with the use of these values to show the presence of mineral wax is the decision as to where the line separating the pure from the impure can properly be drawn.

Since petroleum waxes are largely hydrocarbon material, another approach to this problem is to determine the amount of hydrocarbon material in a beeswax. This has been done for many years by using cumbersome and lengthy chemical procedures. A problem, similar to that described above, immediately arises. This is the naturally occurring hydrocarbon content of beeswax. If it varies among different samples of wax, a degree of uncertainty as to the amount of petroleum wax present is introduced into the picture.

Some hydrocarbon values have been recorded in the literature for beeswax. The values of eight workers who have reported them in the past 70 years range from 10.4% to 17.3%, with most ranging from 12.5 to 14.5%. If a narrow natural range for this value could be substantiated, a reasonably sensitive method for microcrystalline wax in beeswax would result.

Of the eight investigators noted above, only two dealt with United States beeswax, and one of these gave only one value. Thus it was necessary to establish the range of values for hydrocarbon content of U. S. beeswax, in order to find if this procedure could be used for the determination of microcrystalline wax in beeswax. This we have done.

With the invaluable cooperation of the Bee Industries Association, wax renderers and beekeepers, 73 samples of beeswax were collected for this work. In addition to 34 samples of cappings wax, 25 old comb waxes were obtained, all from foundation known to be all beeswax. These samples, together with four special



In the first picture (at 65 degrees C) the sample at the left, from a mixed wax, has solidified while that on the right, from pure beeswax, is still liquid. The picture above (at 55 degrees) shows both solidified.

samples described later were analyzed for hydrocarbon.² We used a new, relatively simple method of analysis which we had developed for the purpose.

This method uses the principle of chromatography. A glass tube is partly filled with activated alumina, a sample of beeswax is dissolved in petroleum ether and allowed to run slowly through the vertical tube. All of the constituents of beeswax except the hydrocarbons are tightly held by the powdered alumina in the tube. The solvent is then removed from the solution that passed through the tube and the amount of residue weighed. This material is the natural hydrocarbon material from the beeswax. As an additional test we determined the freezing point of the hydrocarbon thus isolated from the beeswax.

In order to be sure that the wax

²Marilyn K. Reader and Mary L. Riethof carried out these analyses.

TABLE 2
ANALYTICAL VALUES FOR UNITED STATES YELLOW BEESWAX

Value	This Paper			Bisson et al.*
	Cappings	Old Comb	All	
M. P.	63.66	63.44	63.56	64.1°C.
Acid No.	18.33	18.33	18.33	18.6
Sapon. No.	91.08	90.72	90.94	93.9
Ester No.	72.75	72.39	72.61	75.3
Ratio No.	3.97	3.95	3.96	4.04
Hydrocarbon	14.36%	14.88%	14.59%	—
Hydrocarbon M. P.	55.1°C.	54.8°C.	54.9°C.	—
Sap. Cloud Test	62.5	62.5	62.5	—
Number Samples	34	25	59	56

* Their samples 43, 44, 45, 49 omitted.

samples were authentic beeswax, insofar as the regular analytical constants were concerned, we determined these constants for all samples. In Table 2 may be found the average values that were obtained for these, together with corresponding values for 56 of the 60 samples of western wax published by Bisson, Vansell and Dye.³ The agreement is considered satisfactory. Our slightly lower values

TABLE 3
Calculated Numbers of Beeswax Samples Having M. P. of Hydrocarbon Fraction within Indicated Interval

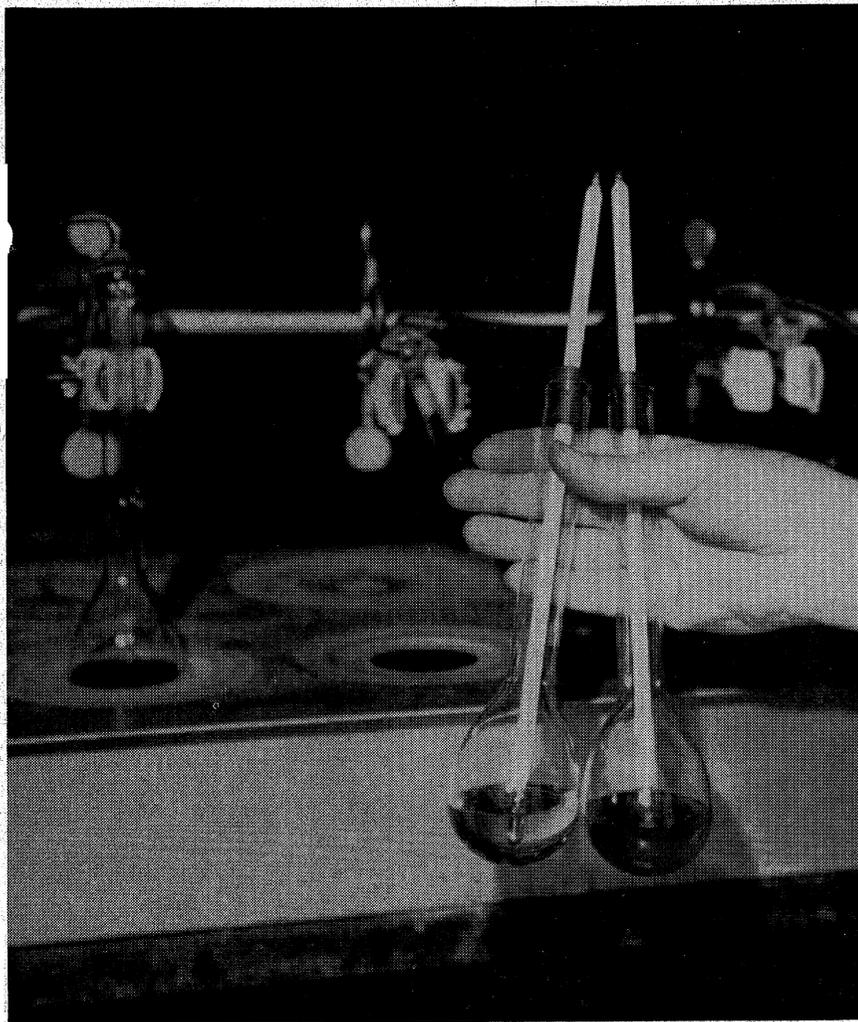
No. of Samples	Interval
2 of 3	54.4-55.4°C.
19 of 20	53.8-56.0
369 of 370	53.3-56.5
16666 of 16667	52.8-57.0

for saponification number are believed to be due to an improved analytical method. This is also reflected in the ester number, which is calculated with the saponification number.

The range we found for hydrocarbon values is quite wide, from 12.28% to 17.09%. Thus it might appear that a low-hydrocarbon wax could have an appreciable amount of microcrystalline wax added without exceeding the natural range of variation. However, this is not the case.

The value in Table 2 for which the range is least is the acid number. Next lowest is the freezing point of the hydrocarbon isolated from the beeswax. This has other interesting properties: the value is considerably lower than that for the "hydrocarbon" similarly isolated from microcrystalline waxes, and it is raised appreciably by relatively small additions of microcrystalline wax to the original beeswax.

It can be calculated that the freezing point values of the isolated hydrocarbon for samples of U. S. yellow beeswax will be distributed as shown in Table 3, assuming a normal distribution. This means that for example, this value for a pure wax would be expected to fall between 53.3 and 56.5° Centigrade in 369 of any 370 samples tested. From the last line in the table we can draw the statement that if the melting point of the hydrocarbon isolated from a beeswax is above 57° C. the odds are 99,994 in 100,000 that it is not a genuine



The saponification cloud test will indicate the presence of a few per cent of microcrystalline wax. When pure beeswax (left) is tested the solution is clear (note thermometer bulb) above 65 degrees C; when mineral wax is present it is cloudy (right) at or above this temperature. (All photos from U.S.D.A. by M. C. Audsley)

³Bisson, C. S., Vansell, G. H. and Dye, W. B., Tech. Bull. 716, U.S.D.A. (1940).

domestic yellow beeswax.

This melting point could be used to give a good measure of the amount of microcrystalline wax in a beeswax mixture, except for one difficulty. Microcrystalline waxes themselves are quite variable in their characteristics, perhaps more so than beeswax. Thus the melting point of the hydrocarbon of some 20 microcrystalline waxes that we examined ranged from 67.1 to 82.0 degrees. This makes it impractical to try to measure exactly the composition of a mixture this way. We can, however, state that the presence of about 3 percent of such wax in beeswax will raise the melting point of the hydrocarbon above 57° and thus prove the admixture. With many waxes, a 1% mixture will do this.

Another way to approach this problem is to base the calculation on the amounts of hydrocarbon found in a wax sample. In this case we must know the amount of such hydrocarbon material found in the microcrystalline waxes themselves. In the 20 samples referred to above, the average was 84.74%, with the values ranging from 75.29 to 91.27%. Since we know the average value for beeswax (Table 1) is 14.59%, we can set up a calculation based on these two averages. This was done, and tested by analyzing 12 mixtures of beeswax and microcrystalline wax, paired at random, varying from 0.9% to 59% microcrystalline wax. The calculated results were quite close

TABLE 4
ANALYSES OF WAX FROM CAGED BEES

	Sample			
	1	2	3	4
Melting Point (°C.)	62.92	63.00	63.18	62.38
Acid No.	20.63	21.06	20.45	19.7
Sapon. No.	92.36	93.89	94.04	93.55
Ester No.	71.73	72.83	73.59	73.86
Ratio No.	3.48	3.46	3.60	3.75
Sapon. Cloud (°C.)	61.0	60.4	60.2	60.4
Hydrocarbon (%)	12.55	12.54	11.27	12.30
Hydrocarbon M. P. (°C.)	55.1	55.4	55.4	55.2

to the known composition, even though we were using average values for our standards. The average difference for the 12 samples was well under 1% microcrystalline wax.

During these investigations, 4 special beeswax samples were prepared by the Bee Culture Investigations Laboratory at Madison, under the direction of Dr. C. L. Farrar. They were waxes produced by caged bees of two strains, as follows (none had access to pollen). Table 4 shows the analysis of these waxes.

	Rossmann Hybrid	Starline Hybrid
Fed Sugar Sirup	1	3
Fed Clover Honey	2	4

Comparison of these values with the averages for regular beeswax shows the differences in composition caused by the availability of pollen and propolis to the bees. All 4 samples were white.

During this work several other proposed methods for detecting micro-

crystalline wax in beeswax were tried. Flash points of 10 beeswaxes were found to range from 490° to 525° F., while the addition of 30% of a petroleum wax of flash point 570° raised that of a beeswax only by 10° F. The saponification cloud test, a relatively simple chemical test for paraffin waxes, is included in the Federal Specification for beeswax. It was found to be quite useful in indicating the presence of microcrystalline wax in beeswax down to the 2% level.

In this test the wax sample is boiled with alcoholic caustic to saponify it and then cooled under controlled conditions. The temperature is noted at which the first cloudiness or turbidity appears in the solution. A maximum value of 65° C. is specified in the test.

The technical details of this work and the complete analytical data on the individual samples are published in the Journal of the Association of Official Agricultural Chemists.