

Canadian Beeswax: Analytical Values and Composition of Hydrocarbons, Free Acids and Long Chain Esters¹

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ABSTRACT

Physical and chemical properties of 80 samples of Canadian yellow unrefined beeswax have been determined. Mean values were: melting point, 64.3 C; acid value, 18.7; ester value, 72.6; ratio number 3.89; saponification cloud point 62.5 C; and hydrocarbon content, 15.3%. There was no significant variation due to geography or climate. Values were very similar to those of U.S. beeswax and were within the specifications of National Formulary XIII, except that 21 samples had ester values in the range 70-72. A more accurate specification for ester value would be 70-77 instead of 72-77. Hydrocarbons, free fatty acids and long chain esters were analyzed by gas liquid chromatography, and the limiting values found make possible improved detection and estimation of adulterants. The upper limits for C₁₆ and C₁₈ acids were 5.8 and 3.3%, respectively, of the total free acids.

INTRODUCTION

There have been two extensive investigations of U.S. beeswax (1,2) but only one relatively short study (13 samples) of Canadian beeswax (3). To establish that there were no appreciable variations in the chemical and physical properties of Canadian yellow, unrefined beeswaxes from different parts of the country, 80 samples were collected from most of the commercial honey producing areas and analyzed. Methods used in beeswax analysis have been discussed frequently (2), and we wished to make sure that methods presently recommended would be applicable to all samples. It was possible that wax from areas with different bee pasture might give unusual results. In addition, so that paraffin, stearic acid and other adulterants could be detected more readily, we wished to establish the range of content and composition of the natural beeswax paraffins, free fatty acids and long chain esters.

EXPERIMENTAL PROCEDURES

Most of the samples were collected by the provincial apiculturists; 70 were pure or almost pure cappings wax, but numbers 22, 27, 36, 39, 40, 58, 59, 70 and 75 (see Table I) were from old combs. Portions of 100-200 g were melted at 100 C and filtered through Whatman no. 1 filter paper into shallow dishes made from aluminum foil (2). Wax was generally not heated for more than 5 hr in this procedure, and it has been shown that such heat treatment scarcely affects the acid and ester values (A.P. Tulloch, unpublished work).

Color was estimated by visual comparison with Munsell color chips (4).

Melting points were measured by the open capillary method (5).

Acid and ester values were determined by the U.S.P. method for carnauba wax (6), which uses isopropanol-toluene as solvent. Flasks were of Corning no. 7280 alkali resistant glass. Ratio number is the ratio of ester value to acid value.

Saponification cloud point was determined by the U.S.P. method (7). Printing in 24 point extra-bold type was placed under the flask, and the cloud point was taken as the temperature at which the print could not be read through the solution.

The above test methods are practically the same as those specified for yellow beeswax by American Wax Importers and Refiners Association Inc., 225 West 34th Street, New York, N. Y. 10001.

To determine hydrocarbon percentage, a hexane solution of 1 g beeswax was applied to a silicic acid column (100 g Biosil A, Bio Rad Laboratories, Richmond, Calif., activated by heating at 95 C for 18 hr [a higher activation temperature can cause poor recovery of the rest of the wax]), and hydrocarbons were eluted with 1500 ml hexane. The remainder of the wax was recovered by elution with 2000 ml chloroform containing 10% ethanol. Gas liquid chromatography was carried out using a Hewlett-Packard model 402 gas chromatograph with flame ionization detectors. The column was 3 ft x 1/8 in. stainless steel packed with 80-100 mesh, silanized, acid-washed Chromosorb W coated with 2% silicone SE-30. Injector temperature was 225 C, and flow rate was 50 ml He per min. For analysis of hydrocarbons temperature was programed from 125 to 350 C at 3 C/min.

For analysis of free acids and long chain esters, 25 mg of material recovered from the silicic acid column was dissolved in chloroform and treated with diazomethane in methylene chloride to convert free acids to methyl esters. Temperature was programed from 125 to 375 C at 3 C/min. The components were identified as described previously (8). For the present purpose, saturated and unsaturated components of the same chain length were not estimated separately.

RESULTS AND DISCUSSION

Physical and Chemical Values

Table I lists the values found for the 80 samples and Table II the mean and range values. The results show that the values fall into narrow ranges and that there is little difference between the old comb waxes and the cappings, except perhaps in the range for hydrocarbon percentage which is larger for the old combs. Also there are no marked differences between samples from the different provinces, except that there were more reddish yellow samples from Vancouver Island and parts of Nova Scotia. This is probably because in these areas bees use more dandelion and other members of the *Compositae*, the pollen of which imparts a reddish yellow color to the wax (1). In the other provinces, particularly the prairie provinces, cultivated clover and alfalfa make up much of the bee pasture. We had been concerned that waxes from areas with distinctly different bee pastures might give unusual results in some of the tests, especially the saponification cloud point, but there was only one was that behaved slightly differently in this test. During the test this sample developed a light haze at 67 C, but since the printing under the flask could be read until 62.5 C the cloud point was considered satisfactory. No traces of petroleum paraffin could be found by gas liquid chromatographic analysis of the hydrocarbon fraction of this wax. None of the other waxes gave unusual results in

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TABLE I
Physical and Chemical Properties of Canadian Yellow Beeswax

Number	Origin	Color ^a	Melting point	Acid value	Ester value	Ratio number	Saponification cloud point	Hydrocarbon, %
1	Vernon, B.C.	5.0Y (8/8)	64.4	18.5	73.2	3.96	62.8	14.7
2	Fort St. John, B.C.	2.5Y (7/8)	64.3	18.7	72.6	3.88	62.5	15.1
3	Vancouver Island, B.C.	10.0YR(7/10)	64.2	17.9	73.4	4.10	62.2	14.9
4	Vancouver Island, B.C.	7.5YR(7/10)	64.0	17.9	73.8	4.12	62.2	16.1
5	Muir Creek, B.C.	5.0Y (8/6)	64.3	18.0	73.1	4.06	62.8	14.4
6	Kamloops, B.C.	2.5Y (8/8)	64.7	18.6	73.3	3.94	63.2	15.2
7	Dawson Creek, B.C.	5.0Y (8/6)	64.3	18.7	73.1	3.91	62.3	15.1
8	Campbell River, B.C.	10.0YR(7/10)	63.8	19.0	72.4	3.81	62.4	15.8
9	Fraser Valley, B.C.	2.5Y (8/8)	64.0	18.2	72.7	3.99	62.8	14.8
10	Kelowna, B.C.	2.5Y (7/6)	64.8	18.3	73.9	4.04	63.0	14.0
11	Nanaimo, B.C.	10.0YR(6/8)	64.6	18.1	72.2	3.99	62.7	14.5
12	Fraser Valley, B.C.	2.5Y (8/10)	64.2	18.2	72.3	3.97	62.7	14.7
13	Coaldale, Alta.	2.5Y (8/8)	64.3	20.0	75.4	3.77	62.5	14.8
14	Fairview, Alta.	2.5Y (8/4)	64.2	19.3	73.1	3.79	62.2	14.3
15	Edmonton, Alta.	2.5Y (8/4)	64.6	17.8	72.6	4.08	62.8	15.1
16	Brooks, Alta.	5.0Y (8/8)	64.6	18.6	74.5	4.01	62.5	14.4
17	Brooks, Alta.	2.5Y (8/10)	64.2	19.1	73.5	3.85	62.6	14.9
18	Girouxville, Alta.	5.0Y (8/4)	64.3	19.0	73.5	3.87	62.8	14.7
19	Wetaskiwin, Alta.	5.0Y (8/6)	64.6	18.2	72.8	4.00	61.9	15.3
20	Red Deer, Alta.	2.5Y (8/4)	64.1	18.0	74.0	4.11	62.6	14.8
21	Sangudo, Alta.	2.5Y (7/6)	63.9	18.4	72.5	3.94	62.1	15.7
22	Sangudo, Alta.	3.5Y (7/6)	63.8	18.7	72.8	3.89	62.5	15.6
23	Pincher Creek, Alta.	5.0Y (8/6)	64.3	19.3	75.1	3.89	62.2	15.5
24	Medicine Hat, Alta.	2.5Y (8/10)	64.4	18.9	74.8	3.96	62.9	15.4
25	Central Alberta	10.0YR(7/6)	64.3	19.4	73.9	3.81	62.6	14.8
26	Nipawin, Sask.	2.5Y (8/4)	64.3	19.1	73.5	3.85	62.6	14.7
27	Nipawin, Sask.	2.5Y (7/8)	64.1	18.7	71.4	3.82	63.0	15.8
28	Saskatoon, Sask.	5.0Y (8/4)	64.4	19.1	73.1	3.83	63.0	14.0
29	Zenon Park, Sask.	2.5Y (8/6)	64.2	19.5	72.0	3.69	62.5	14.9
30	Big River, Sask.	5.0Y (8/6)	64.2	18.3	73.3	4.01	62.5	15.1
31	Preeceville, Sask.	2.5Y (7/8)	64.4	18.8	71.6	3.81	62.6	16.0
32	Fairlight, Sask.	2.5Y (8/6)	64.8	19.1	72.1	3.78	62.6	15.9
33	Nipawin, Sask.	5.0Y (8/6)	64.4	18.4	72.7	3.95	62.8	16.0
34	Norquay, Sask.	2.5Y (7/8)	64.0	19.2	72.2	3.76	62.2	16.7
35	Regina, Sask.	5.0Y (8/4)	63.5	19.0	73.3	3.86	62.3	14.4
36	Birch Hills, Sask.	10.0YR(7/6)	64.6	19.4	71.5	3.69	62.0	15.0
37	Moose Jaw, Sask.	5.0Y (8/4)	64.1	18.9	72.3	3.83	62.0	15.2
38	Saskatoon, Sask.	2.5Y (7/8)	64.5	18.2	72.2	3.97	62.2	15.3
39	Saskatoon, Sask.	2.5Y (7/10)	64.0	18.7	71.5	3.82	61.9	17.4
40	Saskatoon, Sask.	2.5Y (7/8)	63.8	18.1	71.0	3.92	62.0	17.7
41	Ridgedale, Sask.	5.0Y (8/6)	64.6	19.1	72.9	3.82	61.9	16.1
42	MacGregor, Man.	10.0YR(7/10)	64.7	18.6	71.1	3.82	63.4	16.0
43	Central Manitoba	2.5Y (7/8)	64.2	19.4	72.8	3.75	63.0	14.7
44	Sinclair, Man.	10.0YR(7/10)	63.9	18.8	71.7	3.81	62.8	16.6
45	Portage La Prairie, Man.	2.5Y (7/10)	63.9	18.6	73.6	3.96	62.7	15.2
46	Kleefield, Man.	2.5Y (7/8)	64.4	19.3	73.5	3.81	62.5	15.5
47	Roblin, Man.	2.5Y (7/6)	64.3	19.2	73.2	3.81	62.4	15.7
48	Bird's Hill, Man.	5.0Y (8/6)	63.9	20.2	71.7	3.55	61.6	15.3
49	Shelburne, Ont.	5.0Y (8/4)	64.6	17.8	71.8	4.03	63.2	14.5
50	Stirling, Ont.	10.0YR(7/10)	64.7	18.5	72.5	3.92	63.4	13.8
51	Vernon, Ont.	5.0Y (8/8)	64.2	19.5	71.7	3.68	63.3	15.4
52	Lombardy, Ont.	5.0Y (8/6)	64.3	19.3	72.4	3.75	62.8	16.0
53	Acton, Ont.	2.5Y (7/8)	65.0	18.3	73.8	4.03	63.0	15.9
54	Durham, Ont.	2.5Y (7/8)	65.0	18.5	73.7	3.98	62.6	15.7
55	Lindsay, Ont.	2.5Y (7/4)	64.8	18.7	72.1	3.86	62.3	14.8
56	Fergus, Ont.	2.5Y (7/6)	64.6	18.8	72.7	3.88	62.0	14.3
57	Napanee, Ont.	5.0Y (8/8)	64.3	18.7	73.3	3.92	62.6	15.1
58	Napanee, Ont.	10.0YR(3/2)	63.7	21.8	73.7	3.38	62.2	11.6
59	Arnprior, Ont.	10.0YR(4/4)	65.1	18.5	70.3	3.80	62.0	16.6
60	Rimouski, Que.	2.5Y (8/4)	64.7	18.9	74.0	3.92	61.6	14.8
61	Rimouski, Que.	5.0Y (8/6)	64.3	18.6	73.1	3.93	62.2	15.4
62	Levis, Que.	5.0Y (8/8)	64.7	17.4	71.5	4.11	62.6	15.9
63	Lotbiniere, Que.	5.0Y (8/10)	64.6	19.0	71.9	3.78	62.6	15.9
64	St-Jacques le Mineur, Que.	2.5Y (6/6)	64.5	18.4	72.8	3.96	62.3	15.6
65	Ste-Victoire, Que.	2.5Y (8/10)	64.0	19.6	71.7	3.66	62.7	16.1
66	Aylmer, Que.	2.5Y (7/8)	63.9	19.5	72.3	3.71	62.2	15.6
67	St-Guillaume, Que.	5.0Y (7/4)	64.9	17.9	72.2	4.03	63.3	14.1
68	Masson, Que.	5.0Y (7/8)	64.6	18.5	72.2	3.89	62.5	16.0
69	Cowansville, Que.	2.5Y (8/12)	65.0	17.9	72.0	4.02	63.2	15.9
70	Roxton Pono, Que.	5.0Y (7/8)	64.8	18.9	70.9	3.75	62.3	15.5
71	St-Wenceslas, Que.	2.5Y (7/10)	64.3	18.3	72.8	3.98	62.7	16.3
72	Ste-Clothilde, Que.	5.0Y (7/6)	64.3	18.3	71.0	3.88	62.6	16.5
73	Cambridge Station, N.S.	10.0YR(7/10)	64.0	17.4	71.5	4.11	62.8	16.4
74	Barrachois, N.S.	10.0YR(6/10)	64.1	18.5	71.3	3.85	63.3	15.7
75	Onslow, N.S.	7.5YR(5/8)	63.4	18.7	73.5	3.93	62.9	15.2
76	Truro, N.S.	10.0YR(6/8)	64.0	18.2	72.0	3.96	62.9	15.8
77	Cambridge Station, N.S.	2.5Y (7/10)	64.8	18.7	71.4	3.82	62.7	16.8
78	Kentville, N.S.	5.0Y (8/6)	64.2	18.7	72.5	3.88	62.6	14.1
79	Truro, N.S.	5.0Y (8/10)	64.0	18.1	71.9	3.97	62.2	16.4
80	Truro, N.S.	2.5Y (7/10)	64.1	18.7	71.6	3.83	62.5	15.4

^aMunsell notation (4).

TABLE II
Mean and Range Values for Canadian and American Yellow Beeswax

		Melting point	Acid value	Ester value	Ratio number	Saponification cloud point	Hydrocarbon, %
Canadian							
70 Cappings	mean	64.3	18.7	72.7	3.90	62.5	15.2
	range	63.5-65.0	17.4-20.2	70.9-75.4	3.55-4.12	61.6-63.4	13.8-16.7
10 Old combs	mean	64.2	19.0	71.9	3.81	62.3	15.6
	range	63.4-65.1	18.7-21.8	70.3-73.7	3.38-3.97	61.9-63.0	11.6-17.7
80 Cappings and old combs	mean	64.3	18.7	72.6	3.89	62.5	15.3
	range	63.4-65.1	17.4-21.8	70.3-75.4	3.38-4.12	61.6-63.4	11.6-17.7
Baril and Lemay (3)	mean	64.2	17.7	71.7	4.05	---	---
	range	63.7-64.7	16.5-19.6	70.2-73.6	3.75-4.28	---	---
American							
White et al. (2)	mean	63.4	18.3	72.6	3.96	62.5	14.6
	range	62.7-64.4	16.7-20.1	70.8-75.3	3.64-4.31	61.8-64.6	12.3-17.1
Bisson et al. (1)	mean	64.1	18.6	75.3	4.04	---	---
	range	63.1-65.0	16.8-20.4	71.1-78.9	3.62-4.59	---	---

TABLE III
Official Specifications for Yellow Beeswax

Pharmacopeias		Melting point	Acid value	Ester value	Ratio number
France VII ^a	(9)	62-66	16.8-22.4	72-80	---
U.S.A. XV ^a	(10)	62-65	18.0-24.0	72-79	---
Brazil 2	(11)	62-66	17.0-24.0	72-79	---
Britain 1948 ^a	(12)	62-64	17.0-23.0	70-80	3.3-4.2
Argentina 4	(13)	62-65	17.0-23.0	70-80	---
Germany 7	(14)	61-66	17.0-22.0	66-82	3.0-4.3
U.S.S.R. 9 ^a	(15)	63-65	17.0-20.5	66-76	3.42-3.9
Other Specifications					
U.S. Government ^b	(16)	62-65	17.0-24.0	72-79	2.8-5.0
American Wax Importers and Refiners Association ^b		62-65	17.0-24.0	72-79	3.3-4.2
National Formulary XIII ^b	(17)	62-65	18.0-24.0	72-77	3.3-4.2
British Pharmaceutical Codex 1968	(18)	62-65	17.0-23.0	70-80	3.3-4.2

^aMore recent editions give specifications for white beeswax only.

^bAlso specify maximum cloud point 65 C.

any other test.

The results obtained in previous investigations (1-3) are also included in Table II. Our results are very similar to those of Baril and Lemay (3) and also to those of White et al. (2), who made the same determinations as we did. Bisson et al. (1) reported appreciably higher ester values, mean of 75.3 compared to 72.6 (2 and present work) and 71.3 (3). However their 60 samples are not representative of U.S. beeswax, since 30 came from California and 12 from one location in Utah; in addition 9 had unusually high ratio numbers. The samples of White et al. (2), however, are most probably representative of U.S. beeswax. It is interesting that we obtained the same mean ester value as White et al. (2) though these authors used a different procedure. At this stage we can conclude that the physical and

chemical properties of Canadian beeswax are extremely similar to those of U.S. beeswax.

It is useful to compare the present results with those given in official specifications of countries that produce or use large amounts of beeswax, and Table III lists a number of such specifications for yellow beeswax. The specified values differ only slightly from each other except for the ester value, for which the lowest permitted figure has a range of 66-72. Since in most cases the values were established 50 or more years ago (the U.S.P. values first appeared in U.S.P. IX, in 1916 [19]) and the results on which they were based do not seem to have been published, it is difficult to assess their reliability.

Our results and the results of White et al. (2) strongly suggest that the lower limit of 72 for ester value is too high. It is most unsatisfactory to have the lower limit so close to the mean value of 72.6; also 21 out of 80 Canadian samples and 23 out of 59 U.S. samples (2) had ester values below 72. As long as the specified minimum ester value is 72, it is possible for a perfectly pure wax with ester value in the range 70-72 to be regarded as adulterated. A specified minimum ester value of 70 would be much more accurate for use with U.S. and Canadian beeswax. Also the British, German and Russian pharmacopeias allow ester values of 70 or lower. In addition Titschak (20) has recently analyzed a large number of beeswaxes from all over the world (excluding Canada and the U.S.) and, excluding values clearly obtained from Asiatic beeswax, 103 out of 218 had values below 72.

Gas Liquid Chromatographic Analysis

The compositions of the hydrocarbons (Table IV), free

TABLE IV
Hydrocarbon Composition: Mean and Range Values for 80 Canadian Yellow Beeswaxes

Chain length	Mean, %	Range, %
21	0.4	0-0.9
23	2.5	1.7-3.7
24	0.1	0-1.4
25	7.2	5.5-9.8
26	0.9	0-1.9
27	27.9	23.0-33.2
28	0.4	0-1.6
29	16.5	10.4-18.5
30	0.1	0-0.4
31	21.1	17.1-24.1
32	0.1	0-0.7
33	21.5	17.3-25.5
35	1.3	0.8-3.7

TABLE V

Composition of Free Fatty Acids: Mean and Range Values for 80 Canadian Yellow Beeswaxes

Chain length	Mean, %	Range, %
16	1.1	0-5.8
18	0.6	0-3.3
22	3.0	1.9-4.4
24	34.7	28.5-45.7
26	12.1	10.2-14.7
28	12.3	10.1-14.2
30	11.2	7.4-14.4
32	10.5	6.3-13.8
34	11.2	8.5-16.8
36	3.3	1.0-7.9

acids, as methyl esters (Table V) and long chain esters (Table VI) were determined by gas liquid chromatography (GLC) so that we could obtain mean and range values for the components of the 80 waxes and thus assess the possibility of detecting and estimating adulteration by GLC.

White et al. (2) pointed out that, since the hydrocarbon percentage of beeswax varies considerably (11.6-17.7% in the present investigation), this value could not be used to detect hydrocarbon adulteration at the 5% level. However isolation of the hydrocarbon fraction by chromatography results in a five- to six-fold concentration of hydrocarbon adulterants, so that detection of paraffin by GLC estimation of unusual amounts of components with an even number of carbon atoms or of components with more than 35 carbons might well be possible. The saponification cloud point and measurement of the freezing point of the hydrocarbon fraction can often detect paraffin adulteration at the 2-5% level (2); but these methods cannot give a reliable estimate of the amount of the adulterants. There were only relatively small differences in the composition of the hydrocarbons of the 80 samples. Major components were C₂₇, C₃₁, C₃₃ and C₂₉, in that order. Maximum values for C₂₄ and C₂₆ were 1.4% and 1.9%, respectively; values much in excess of these would most probably be due to adulteration by petroleum paraffin.

In a detailed analysis of one beeswax sample, no C₁₆ or C₁₈ acids were found (8); but small amounts occur in some of the 80 samples investigated. Table V shows that the mean and range values for C₁₆ were 1.1 and 0-5.8, and for C₁₈ were 0.6 and 0-3.3 (as percentage of total free fatty acids); C₁₆ was always larger than C₁₈. Thus it would be possible to detect and estimate adulteration by commercial stearic acid (which also contains C₁₆ acids) by GLC analysis. In each sample the longer chain acids all showed the same characteristic pattern: C₂₄ was the largest peak, then C₂₆ and C₂₈, then C₃₀, C₃₂ and C₃₄; finally, C₂₂ and C₃₆ were always the smallest peaks (excluding C₁₆ and C₁₈).

The results in Table VI show that, while absolute amounts of each long chain ester varies the relative amounts do not change much, that is the major peaks are always C₄₆ and C₄₈, then C₄₀ followed by C₄₂ and C₄₄ about equal, and lastly a much smaller C₅₀. Deviation from this pattern would most probably indicate adulteration. Tripalmitin appears just after the C₅₀ monoester and mixed palmitic,

TABLE VI

Composition of Long Chain Esters: Mean and Range Values for 80 Canadian Yellow Beeswaxes

Chain length	Mean, %	Range, %
40	15.2	11.8-19.4
42	12.2	9.5-14.9
44	12.5	8.9-15.4
46	25.3	19.5-32.1
48	25.0	20.8-36.9
50	9.2	5.2-15.3
52	0.4	0-1.7

stearic acid glycerides and tristearin after that. Since beeswax gives only very small peaks in this region, adulteration by tallow or hydrogenated tallow should be readily detected. As pointed out before, the long chain ester composition reflects the composition of the long chain alcohols of beeswax esters (8) so that Table VI also indicates the variations to be expected in the alcohol components of beeswax.

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