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Studies on Libyan Honeys

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ABSTRACT

The honey samples representing various floral types were obtained from different areas in Libya. Two other samples were obtained from hives where bees were fed on sugar syrup and date syrup respectively. The chemical composition and some characteristics of these samples were studied. The data obtained generally showed that the specifications of honeys produced in Libya conformed with those required for table honey. The applications of thin layer chromatography on cellulose-coated plates for the analysis of honey sugars yielded clear resolution and discrete spots. Some measures such as high level of calcium, low level of potassium and UV-absorption spectra of honeys were suggested to be used for identifying the sugar honeys and for ensuring the quality of blossom honeys. An attempt has been made to predict the granulation behaviour of honeys and a derived regression formula was suggested.

INTRODUCTION

Since honey is mainly a carbohydrate material, with 95–99% of the solids being sugars, the nature of sugars has been studied for many years. The major sugars found in honey are glucose, fructose, and sucrose. Various investigators have reported chromatographic evidence of the presence of about 10 disaccharides and 11 trisaccharides along with isomaltotetrose and isomaltopentose in honey (5,7,10,13,20,21,22,26). This shows that honey is a very complex mixture of sugars. On the other hand, the nature of the sugar complex of honey is such that results can be misleading unless either a suitable pre-analysis separation of sugar classes is made, or highly specific and sensitive new analytical methods are employed (5,16,26).

Honey derived from bees fed on sugar and date syrups, candy, etc., is not lawful in many countries. France, Germany, Spain, Switzerland and the Latin American codex expressly state that such honey would be illegal (5). In some of these countries any product stored by such bees may be sold as 'sugar honey' or 'artificial honey'. In some areas of Libya, bees are fed on sugar and/or date-syrups. In the absence of standard specifications or regulations regarding honey production and marketing, the honey produced from such bees is sold as natural honey. In the present study an attempt was made to find some convenient and practical methods for detecting such kinds of honey.

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Table 1 Honey samples.

Sample	Country	Locality	Dominant floral source	Harvest season
A	Libya	Al-Mordge	Windbreaks (Acacia spp., Pinus spp., Cupressus spp.)	June 1978
B	Libya	Tarhoona	Thyme (*Thymus vulgaris*)	July 1978
C	Libya	Tarhoona	Rosemary (*Rosemarinus officinalis*)	December 1978
D	Egypt	Upper Egypt	Cotton (*Gossypium hirsutum*)	September 1978
E	Libya	Tripoli	Orange, Grapefruit (Citrus spp.)	April 1978
F	Libya	Tripoli	Plant hedges (*Lantana camera, Hibiscus rosa-sinensis*)	June 1978
G	Egypt	Lower Egypt	Clover (*Trifolium alexandrmum*)	June 1978
H	Libya	Tripoli	Eucolyptus (*Eucolyptus cawaldulensis*)	July 1978
I	Libya	Ras-Helal	Wild plants	October 1978
K	Libya	Sebha	Alfalfa (*Medicago sativa*)	June 1978
L	Libya	El-Khoms	Derived from bees fed on sugar syrup[a]	March 1979
M	Libya	Tripoli	Derived from bees fed on date syrup[b]	May 1979

[a] Equal parts by volume of sugar and water.
[b] One part date syrup + two parts water (by volume).

Granulation (crystallization) of honey is one of the problems with which the packer is faced in the preparation of liquid-pack honey. In view of the variation in honey composition a means of predicting its granulation behaviour would be of considerable practical use. Several attempts have been made to explain the susceptibility of honey to granulation (5,7,10,11,23). It has been stated that crystallization took place rapidly in honeys with levulose/dextrose (L/D) ratio of 1.0–1.2 (5). A highly significant correlation was found between granulation tendency and dextrose/water (D/W) ratio (7). It has also been reported that the index (Brix-dextrose)/dextrose, (Br-D)/D was more useful than above-mentioned indices (23). In the present work, the correlation between granulation tendency and some previously proposed indices has been studied statistically.

The present investigation aimed at:

1. Evaluation of the main physico-chemical properties of multiflower honeys collected from different districts of Libya. Such analysis would furnish enough data to establish standards and general specifications of Libyan honey.
2. Looking for a convenient and practical method for detecting sugar honey obtained from bees fed on syrups. This will discourage the production of such honey, or adulteration of natural honey by direct addition of sucrose.
3. Attempting to find a method of predicting granulation behaviour of honey.

MATERIALS AND METHODS

Eight honey samples (2 kg each) representing various floral types were obtained from different tracts in Libya during the period from April, 1978 to May, 1979. For comparison, 2 other samples were obtained from 2 different localities in Egypt. Two more samples were obtained from hives, where bees were separately fed on sugar-syrup and date-syrup.

The samples of honey were labelled alphabetically as shown in Table 1.

Methods of analysis: The following determinations were run in triplicate and the results were reported as a mean of three runs. Viscosity–viscosimeter 'Rheomat 15 T' (8,18); refractive index–'Abbe Refractometer' (8); pH-meter–'Beckman Chem-Mate' (17); Polarization–polarimeter 'Karl Kolb Model 14.220' (8,17); and colour–spectrophotometer 'Double beam coleman 124 (8). The UV-absorption spectra were obtained for aqueous solution of the honeys. Total soluble solids–'Abbe Refractometer'; acidity, total ash (carbonated), potassium, sodium and calcium content of the honeys–flame photometer 'Carl Zeiss pF 5'–were also determined according to the methods described in (8,17,18).

Honey samples were analyzed for the total sugars and reducing sugars employing the 'Lane-Eynon' volumetric method (2,17,18). The sucrose content of honeys was determined by 3 different techniques, namely, polarimetric, Lane-Eynon volumetric method before and after inversion, and thin layer chromatography (2,17,18).

The individual sugars arabinose, glucose, fructose, maltose (isomaltose), and raffinose were qualitatively and quantitatively determined using a combination of chromatographic and micro-colorimetric procedures (2,9,14) with some modifications to suit the material analysed. The technique is summarized as follows: thin layer chromatography (T.L.C.) was applied in order to separate the sugars in honey samples. The separation was performed on plates containing 'Cellulose DF' as adsorbent. Honey sample consisting of 800 micrograms in aqueous solution was spotted. The plate was then placed in a developing tank containing the solvent system 7:1:2 isopropanol: ethyl acetate:water. After the solvent reached the front, the plate was dried and placed once more in the same solvent system and rechromatographed in the same direction (multiple development). Pure sugars were chromatographed, in the same manner, along with the honey sample in order to identify the sugars present in honeys.

Statistical analysis: The results obtained were statistically analysed for standard error, coefficient of variability (CV), simple correlation and simple regression. The statistical analysis was performed according to the methods described by Clarke (4).

RESULTS AND DISCUSSION

It is generally known that composition and characteristics of a particular honey depends upon two factors: composition of the nectar whence it originated and some external factors such as climatic conditions and beekeeping practices (3,5,10,19). Nectars from different plants vary widely in nature and concentration of their constituents (3,5,11,16,19).

Tables 2 and 3 show the physico-chemical characteristics and gross composition of honey samples under study. The considerable variation in composition encountered among samples could be attributed to the fact that they represented different floral types. From these tables it could be generalized that the specifications of honeys produced in Libya conform with those required for table honey in the majority of other honey-producing countries (3,5,11,16,19). However, Libyan honeys showed somewhat higher values in pH, and ash content and lower values in calcium content than the corresponding values recorded for honeys produced in other countries (5,7,23,27). A significant variation could easily be noticed in the viscosity values obtained among honey samples. Lothrop, cited in (5), and others (24), believed that viscosity variations in honey were due to non-sugar materials, particularly dextrins

Table 2. Physico-Chemical Characteristics of Honeys.

Sample	Viscosity at 20°C P	Refractive index at 20°C	Direct immediate polarization at 20°C	Direct constant polarization at 20°C	Invert polarization at 20°C	Color[a]	pH
A	80.10	1.4967	−9.23	−9.03	−5.51	3.73	6.2
B	205.80	1.5017	−10.53	−10.41	−9.69	1.49	6.2
C	166.00	1.5007	−10.33	−10.18	−9.52	1.28	6.1
E	154.03	1.4980	−10.01	−3.50	−3.13	1.17	5.6
F	110.30	1.4993	−10.02	−3.84	−3.15	1.87	5.9
H	103.20	1.4935	−8.55	−9.89	−9.15	2.97	5.3
I	49.51	1.4960	−11.49	−10.39	−8.83	2.71	6.5
K	472.00	1.5065	−6.27	−6.06	−3.66	1.33	5.5
Mean and standard error	167.62 ±46.93	1.4991 ±0.0013	−9.55 ±0.56	−7.91 ±0.98	−6.58 ±1.06	2.07 ±0.34	5.91 ±0.41
D	[b]9.38	1.4886	−13.72	−7.03	−6.34	2.43	5.9
G	78.14	1.4940	−8.74	−8.54	−7.99	1.21	5.0
L	35.90	1.4985	−0.21	+0.28	−3.5	1.96	5.0
M	16.60	1.4977	−0.97	−0.90	−2.56	3.33	4.9

[a] Expressed as attenuation index using $\lambda = 560$ nm (8,13).
[b] Heat was applied during processing.

and proteins. Some kind of honeys contained dextrins which cause an abnormal viscosity of honey i.e. dilatancy (5,24).

It has long been recognized that polarization largely depends on the sugars of honey, their types and relative proportions. Data reported in Table 2 indicated that all

Table 3 Gross chemical composition of honeys.

Sample	Total soluble solids %	Degree of acidity [a]	Ash %	Potassium mg/100 g	Sodium mg/100 g	Calcium mg/100 g	Total sugars as invert sugar % [b]	Reducing sugars %[b]
A	82.50	27.40	0.04	2.40	2.95	1.97	67.40	58.53
B	84.30	11.50	0.06	9.70	2.97	1.96	80.12	77.88
C	84.00	13.10	0.11	9.84	7.87	1.02	78.87	76.58
E	83.00	11.40	0.08	5.00	3.00	1.01	74.93	73.05
F	83.50	17.60	0.21	49.97	19.99	2.00	75.18	73.12
H	81.30	20.70	0.12	14.82	7.90	1.02	70.70	68.82
I	82.20	19.40	0.29	144.50	24.91	1.05	73.78	70.72
K	86.20	16.70	0.10	9.91	19.82	1.20	77.85	71.60
Mean and standard error	83.38 ±0.53	17.23 ±1.91	0.13 ±0.03	30.79 ±17.08	11.18 ±3.18	1.40 ±0.17	74.85 ±1.51	71.29 ±2.10
D	79.40	30.00	0.38	74.91	11.99	1.07	72.62	71.43
G	81.50	21.00	0.06	4.97	2.98	1.08	73.58	71.82
L	83.20	11.00	0.15	2.00	4.49	6.99	79.94	70.28
M	82.90	14.90	0.17	2.00	2.99	8.47	81.59	79.37

[a] Expressed as number of millilitres of 0.1 N NaOH required to neutralize 100 g of honey.
[b] Estimated by using of the Lane-Eynon Method.

flower-honey samples were levorotatory and exhibited a mutarotation which was quite characteristic of sugars present.

The colours of honey samples ranged from very pale-yellow (samples B, C, E, K), through amber (sample F, H, I) to a reddish-amber (sample A). It is agreed that the colour of honey is of great importance for consumer preference, and it has been associated with flavour and mineral content (5,10). There is no standard method throughout the world for classifying honey by colour. Each country has its own approach. In the present study, optical density was used for the direct determination of honey colour.

The acids contribute to honey-flavour complex. It has been found that gluconic acid, and not the citric and/or formic acids, was the dominating acid in honey (5,11,13,15,16). Moreover, there are about 16 amino-acids in honey (5,13,15). As shown in tables 2 and 3, pH values of the honey samples were not directly related to their free acidity (samples A, B and C; and samples F and D). This may be attributed to the buffering action of the various salts and minerals present.

Significant differences could be noticed between the characteristics of the honeys derived from bees fed on syrups (samples L and M) and those of the blossom-honeys (samples A, B, C, D, E, F, G, H, I, K). As shown in Tables 2 and 3, samples L and M had remarkable lower values in both viscosity and potassium content while they showed higher calcium content as compared to the corresponding values recorded for the flower-honey samples. These variations could be attributed to the differences in composition between the syrups on which bees were fed, and the floral nectar gathered by bees. Moreover, the polarization of the samples L and M was either dextrorotatory

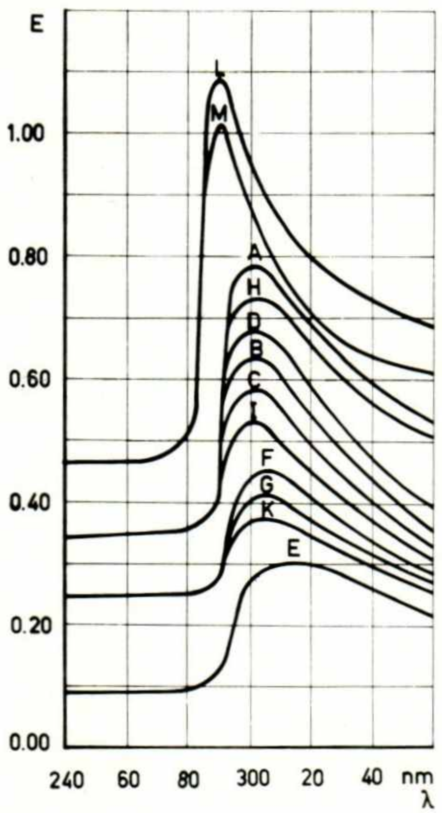

Fig. 1. UV-absorption spectra of honeys.

(sample L) or relatively quite small levarotatory value (sample M). This could be attributed to the types and relative proportions of the sugars present in honeys.

It is generally agreed that UV-spectroscopic examination provides some useful information on the principal functional groups of compounds (6,17). Figure 1 illustrates the UV-absorption spectra for aqueous solutions of the honey samples under study. The extinction curves of the blossom-honey samples were quite similar in their character and configuration. They had a maximum absorption at the wavelength of 300 nm (for samples A, B, C, D, M, I); and 305 nm (for samples F, G and K). The absorption spectrum of the citrus honey (sample E) showed an intense absorption in the wavelength range of 305–325 nm. This may be attributed to the existence of different groups of chromophores, which have intensive absorption in the preceding wavelength ranges of spectra. It was reported that five essential pigments were detected in honey: chlorophyl, carotene, xanthophyll, and two pigments of unknown composition, one

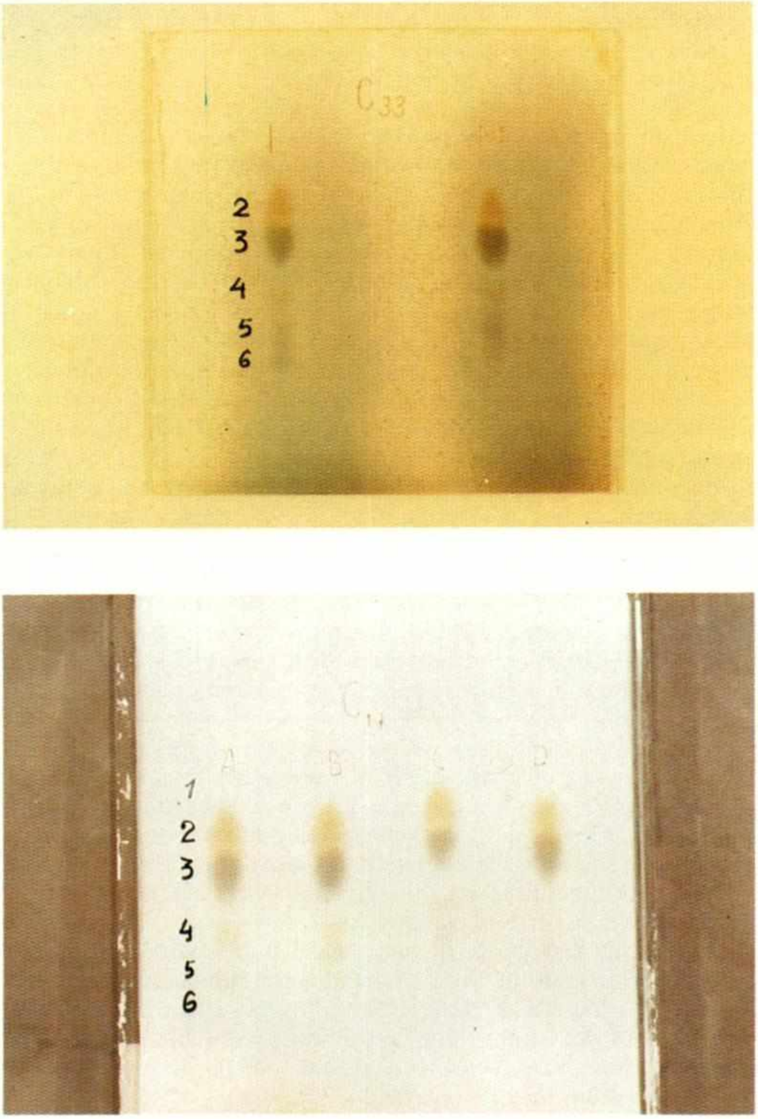

Fig. 2. Sugar chromatograms of honeys.

yellow and the other green (10). Remarkable differences in the configurations and extreme points could be noticed between the absorption spectra of the blossom-honey, samples A, B, C, D, E, F, G, H, I and K, and these of honeys produced by bees fed on syrups, samples L and M in Fig. 1. The latter had a sharp maximum absorption at the wavelength of 290 nm, which is indicative of some coloring materials such as caramels (products of sugar decomposition) and melanoidins (products of amino-carbonyl reaction) (6,12). This may justify the using of UV-absorption spectra of honeys for the detection of the sugar-honeys (honeys derived from bees fed on syrups).

The nature and content of sugars in honey are known to have a decisive effect on its physical properties. The accurate analysis of honey for individual sugars was not simple, since the presence of the minor sugars introduced errors, unless sophisticated analytical procedures were employed. In the present study, thin layer chromatography was first applied to separate the individual sugars to identify them and then each sugar was separately determined using a microcolorimetric technique. Figure 2 illustrates the sugar chromatograms of honey samples. It could be noticed that honey-sugars developed on the cellulose plates were resolved into discrete spots, one for each sugar present. In all samples studied glucose, fructose, sucrose and maltose (isomaltose) were identified. Arabinose was found in one sample (A) which was obtained from a mountainous area. Raffinose and probably other higher sugars were found in most of the samples studied. The presence of raffinose in honey has been reported by many investigators (3,5,7,10,13,21). The action of enzymes originated from plants and bees was responsible for the production of these materials (5,7,16,19). Table 4 presents the individual sugars content of honeys. It could be noticed that glucose and fructose together accounted for about 84% of honey-sugars. With the exception of the samples E and I, more fructose than glucose was present. Much of the published work on sugar composition of honey showed that, as a rule, the fructose content of honey was greater than the glucose content, although it occasionally happened that the glucose was slightly more (22,25,26,27,28).

The sucrose content of honey samples was determined by the application of three different techniques namely Lane-Eynon method, polarimetric method, and thin layer chromatography. The variance among the three methods has proved to be insignificant ($F = 0.2569$). However, the coefficient of variability (CV) for the methods applied was found as 81, 70 and 72%, respectively. Thus, the polarimetric method appeared to be the most accurate technique for the determination of sucrose content of honeys. With reference to Table 4, it could be observed that honeys produced in Libya had a lower content of fructose and glucose and higher content of sucrose and raffinose (or higher sugars) than that reported for honeys from other countries (3,11,13,16,19,27). The relatively high maltose content recorded for the samples C, E and F may be attributed to the presence of proportions of honeydews in honey. The high sucrose content in both of the samples A and L was probably associated with the heavy sugar feeding (sample L), or may be with the subsequent adulteration of honey by the direct addition of sucrose (sample A). The relative low sucrose content of sample M may be explained by the fact that date-syrup, on which bees were fed, was composed mainly of the monosaccharides, glucose and fructose.

Figure 3 presents the histograms of sucrose, calcium, and potassium content of the honey samples. It is worth noting that all the blossom-honeys (except sample A, which could be suspected to be adulterated) showed lower content of sucrose and calcium and higher content of potassium than that found for the honeys collected from bees fed on syrups. Therefore, it can be concluded that the high level of calcium along with the low level of potassium may be regarded as an important criterion for the purpose of detection of heavy sugar-feeding in honey production. Setting a limit on the sucrose content, as usually provided in the present regulations, will not fulfil this purpose as bees could be fed on some sugary materials without raising the sucrose content of the honey obtained (sample M).

Table 5 presents the granulation behaviour of the honey samples and their relationship to some indices. The statistical analysis of these data indicated that granulation tendency was directly correlated to the D/W ratio with high significance ($r = +0.864$), while the correlation was highly significant on the negative side with (Br-D)/D ($r = -0.798$). The ratio of Millum, L/D was found to have insignificant effect ($r = -0.499$). In an attempt to find a means of predicting the granulation behaviour of honey, linear simple regression was carried out for the two indices having highly significant 'r' values, namely D/W and (Br-D)/D. The following linear equations were applied:

$$Y = a + b_1 X_1, \quad Y = a - b_2 X_2$$

Table 4 Analysis of honeys for individual sugars.

Sample	Arabinose %	Glucose %	Fructose %	Maltose (isomaltose) %	Higher sugar (Raffinose) %	Sucrose % 1	Sucrose % 2	Sucrose % 3
A	7.35	20.80	32.00	2.70	10.60	8.43	7.63	8.90
B	—	24.30	36.60	9.30	11.10	2.13	1.55	2.29
C	—	24.95	32.90	10.10	11.90	2.18	1.49	3.04
E	—	37.80	30.55	10.20	—	1.79	1.71	1.85
F	—	18.55	48.40	13.90	—	1.96	2.93	3.10
H	—	32.70	38.20	6.60	1.60	1.79	2.84	2.30
I	—	39.90	28.00	9.40	—	2.91	3.40	3.50
K	—	22.30	36.20	9.00	9.90	5.94	5.21	6.80
Mean and standard error	—	27.66 ±2.85	35.36 ±2.21	8.90 ±1.14	5.64 ±2.00	3.39 ±0.87	3.35 ±0.75	3.97 ±0.89
D	—	33.40	39.80	1.80	—	1.13	1.50	1.68
G	—	30.18	38.80	3.30	3.90	1.67	2.10	2.30
L	—	21.60	32.09	8.10	9.85	9.04	8.20	10.40
M	—	20.40	35.15	10.60	11.15	2.11	1.96	2.84

[1] Determined by the application of Lane-Eynon method, CV-81%
[2] Determined by the application of Polarimetric method, CV-70%
[3] Determined by the application of Thin Layer Chromatography, CV-72%

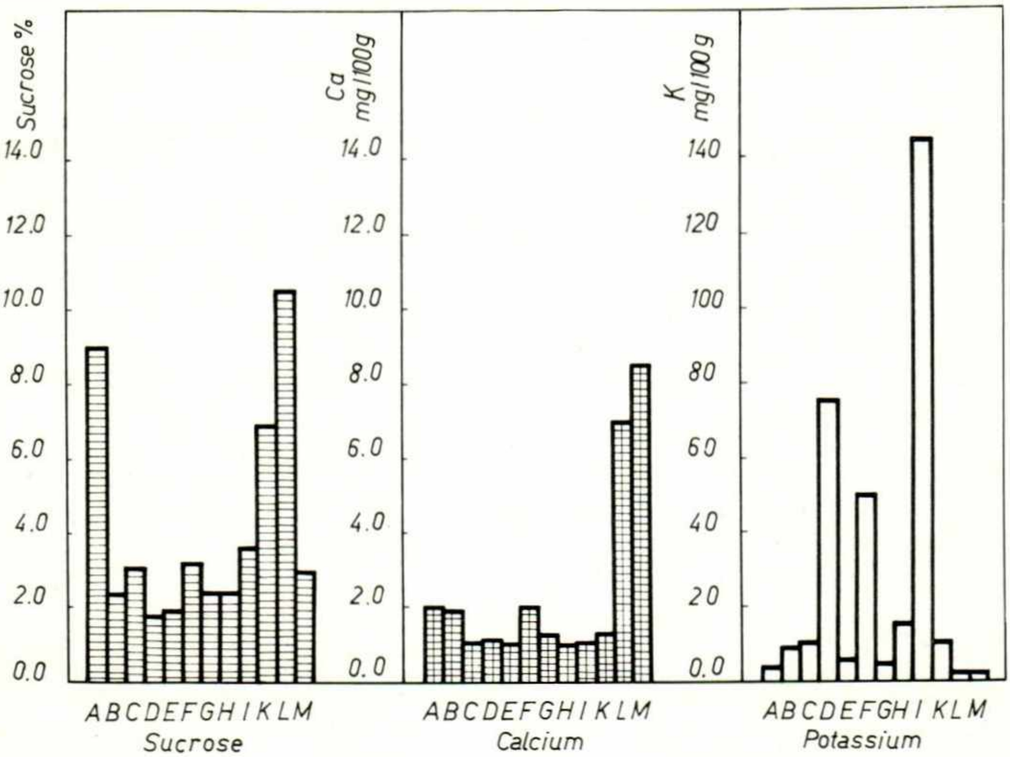

Fig. 3. Sucrose, Calcium, and Potassium content of honeys.

Table 5 Granulation characteristics of honeys and their relationship to some proposed indices[a].

Sample	Extent of granulation	Granulation in degrees[b]	Levulose / dextrose	Dextrose / Water	Brix-dextrose / dextrose
A	None	0	1.54	1.19	2.97
B	None	0	1.51	1.55	2.47
C	A few clumps of crystals	25	1.32	1.56	2.37
E	Complete hard granulation	100	0.81	2.22	1.20
F	None	0	2.61	1.12	3.50
H	3/4 of depth granulated	75	1.17	1.75	1.49
I	Complete soft granulation	100	0.70	2.24	1.06
K	None	0	1.62	1.62	2.87
D	1/4 of depth granulated	25	1.19	1.62	1.38
G	None	0	1.29	1.63	1.70
L	None	0	1.49	1.29	2.85
M	None	0	1.72	1.09	2.98
r-value			−0.499	+0.864[c]	−0.798[c]

[a]Granulation observed in heated honey after 6 months undisturbed storage at room temperature, honey in 1/4 L jars.
[b]Complete granulation = 100, the absence of granulation = 0.
[c]Highly significant.

in which Y = honey granulation, X_1 and X_2 are the ratios D/W and (Br-D)/D, respectively, a = constant, b_1 and b_2 = regression coefficients for X_1 and X_2, respectively.

The following formulae could be derived:

$$Y = -118.47 + \underset{29.3632^{**}}{92.71}\, X_1$$

$$Y = 114.44 - \underset{17.5853^{**}}{39.00}\, X_2$$

where numbers written under b_1 and b_2 represent F-values for the regression of X_1 and X_2.

This would mean that honey granulation increases by 92.71 units with an increase of one unit in D/W ratio, while an increase of one unit in (Br-D)/D ratio caused a decrease of 39.00 units in honey granulation. Such data may be of help in the preparation of liquid-pack honey, as it highlights the factors influencing granulation behaviour.

The results obtained lead to the following conclusions:

1. The specifications of honey produced in Libya generally conform with those required for table honey in the most of other honey-producing countries.
2. The high level of calcium along with the low level of potassium may be suggested as a significant criterion for the purpose of detection of heavy sugar-feeding in honey production.

3. The UV-absorption spectra of honeys are an important consideration for identifying of the sugar-honey (honey derived from the bees fed on syrups), and for ensuring the quality of blossom-honey.
4. By the application of thin layer chromatography on cellulose plates for the analysis of honey-sugars, clear resolution was attainable and discrete spots were obtained.
5. Among the indices used in predicting the granulation behaviour of honey, it was found that granulation tendency was directly correlated to the D/W ratio with high significance ($r = +0.864$), while the correlation was highly significant on the negative side with (Br-D)/D ($r = -0.798$). The ratio of Millum, L/D was found to have insignificant effect ($r = -0.499$). The following linear regression formulae have been derived:

$$Y = -118.4745 \pm 92.71\,X_1,$$

$$Y = 114.44 - 39.00\,X_2$$

where:

Y = honey granulation, X_1 and X_2 are the ratios D/W, and (Br-D)/D, respectively.

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دراسات على العسل المنتج في الجماهيرية

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المستخلص

تم دراسة الصفات الفيزيوكيميائيه لعينات من عسل النحل منتجه في المناطق المختلفه من الجماهيرية العربية الليبية الشعبية الاشتراكية في الفترة من ابريل ١٩٧٨ - مايو ١٩٧٩ . ولقد دلت نتائج الدراسة على أن خواص العسل المنتج في ليبيا تتفق بوجه عام مع المواصفات العالمية المطلوبه للعسل المعد للاستهلاك المباشر .

ولقد أمكن فصل السكريات الموجوده في العسل فصلا واضحا ودقيقا ثم تقديرها تقديرا كميا بواسطة استخدام طريقة التحليل الكروماتوجرافي على الواح رقيقه من السليلوز .

ولقد تم في هذا البحث آيضا دراسة صفات " العسل السكرى " الناتج من النحل المغذى صناعيا بمحاليل من رب التمر ومن الشراب السكرى . وقد أثبتت النتائج المتحصل عليها أن المستوى العالي من الكالسيوم والمستوى المنخفض من البوتاسيوم وأيضا منحنيات الامتصاص في المنطقة الفوق بنفسجيه من الطيف - كلها تعتبر وسائل عمليه وفعاله لاكتشاف العسل السكرى وتمييزه عن العسل الطبيعي المنتج بواسطة النحل من رحيق الأزهار .

ولقد أمكن بواسطة استخدام التحليل الاحصائي استنباط معادلات توضح الارتباط بين مكونات العسل السكريه وبين ميله الى التبلور أو التحبب .