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Sugar Content of Honey from Wild and Kept Honeybees

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ABSTRACT

The purpose of this study was to analyze major sugar components of honey by high performance liquid chromatography (HPLC) method. Results, compared with data obtained from similar studies in Poland, the United States (U.S.), and Canada, showed no obvious discrepancies.

Results indicated that the fructose content in honey collected from kept honeybees was 38%; in winter honey, 36% and from wild honeybees, 45%. The glucose content in winter honey was 27%; in kept honey, 33%; and wild honey, 31%. Thus, the fructose-to-glucose ratios are between 1.1-1.2 in kept honey and 1.4-1.6 in wild honey. Only a small amount of sucrose was detectable in some samples, while the content of maltose in all samples was around 4.2%. The total sugar content in winter honey was 67%; honey produced in other seasons contained 75%, with wild honey up to 80%.

Key words : Sugars, Honey, Honeybees, HPLC method.

INTRODUCTION

Honey is a sweet substance which is secreted by honeybees. The worker bees gather sweet liquid from flowers and store it in the honeysac of their bodies. The honeydew and nectar are hydrolyzed and inverted to sugars by invertase in the bees' saliva. Honeybees seldom gather honeydew instead of nectar unless the nectar is deficient[1].

The major components of honey are sugars, including fructose, glucose and a small amount of maltose and sucrose. Honey also contains trace vitamin B_1,B_2,B_6,B_{12} and acetylcholine[2]. Fructose and glucose are the only two monosaccharides found in honey, but many other di- or higher saccharides are generated from these two sugars.

Since ancient times, honey has often been taken for medical purposes or, as in China as a binder for formulation. It is also frequently used as a nutritious supplement by Chinese doctors to replenish sugar deficiency.

A brief survey of the literature shows the common methods reported for the analysis of sugar content[3] are by: (a) the reduction method [4-6]; (b) the saccharimetric or polarographic method[4]; (c) the densimetric method[2]; (d) the refractometric method[7]; and (e) the colorimetric method[8,9]. When gas chromatography[10,11] is applied to analysis of sugar in honey, practically, all major compounds must be converted to their volatile derivatives; inevitable errors occur from tedious manipulation. There is still another choice: using the high performance li-

quid chromatography (HPLC)[12-14] which provides a rapid, convenient and accurate method for analysis of the sugars in honey.

The composition of honey varies with different species of honeybees, with nectar sources of plants, and with seasonal and environmental changes. Traditionally, Taiwanese farmers have learned how to keep honeybees and gather their honey from their ancestors. Honey or royal jelly represents an agricultral product of high economic value which helps to improve the farmer's living standard. In addition, the wild honey collected from rock or wood chinks are used in the formulation of Chinese medicines[15]. Honeys collected from kept honeybees can be used only as a source of nutrition. The present study was conducted by analyzing by HPLC method the sugar components in honey collected from the Kaohsiung area in southern Taiwan. The results were compared with data obtained previously, reported in the literature appearing in the United States[16,17], Europe[18] and Taiwan[19,20].

MATERIALS AND METHODS

I .*Meterials*

All the sugar standards (including frucose, glucose, sucrose, and maltose) were standard grades of Chemical Service Company (U.S.A.). The HPLC grade of acetonitrile (CH_3CN) used was supplied by Lab-Scan company (England). Eleven honey samples were gathered in the Liowkuei, Santimen and Kangsan in Kaohsiung hsien from November 1992 to April 1993. Kept honeybee honey was offered free by the farmers. Honey was collected by rotary centrifuge from the bee house. Wild honeybee honey was gathered by adding pressure to the honeycomb, with bees expelled by smoke in advance. All honey samples were refrigerator stored before analysis.

II.*Apparatus*

An Alcott model 760 HPLC pump (Alcott chromatograph, U.S.A.), equipped with a μ-Bondapak/NH_2 column (300 mm × 3.9 mm i.d. Waters), an ERC-7512 model RI detector (ERMA CR., Japan) and a model 7725 Rheodyne injector were used. Peak height ratios were determined using a SIC chromatocorder 12 integrator (Alphatech, Japan).

III.*Methods*

(I).*Moisture content determination*

The vacuum drying method was used for moisture content determinations. About a 5 g honey sample was placed in a vial and accurately weighed; the vial was then placed under vacuum for one day. The sample was weighed and put back under vacuum for further drying. The process was repeated until the weight change was not more than 0.5%.

$$\text{Moisture content}(\%)=\frac{\text{The weight loss by drying}}{\text{Original sample weight}}\times 100$$

(II).*HPLC operating condition*

Column : μ-Bondapak/NH_2 (300 mm × 3.9 mm i.d.)

Flow rate : 1.0 ml/min

Injection volume : 20.0 μl

Attenuation : 64

Detector temperature : 40°C

Mobile phase : acetonitrile : water=85 : 15 (v/v)

(III).*Procedure for preparing calibration curve*

The fructose, glucose, sucrose and maltose were dissolved in HPLC grade water, and the solution was diluted to 0.4-4.0 mg/ml concentrations for fructose and glucose; to 0.6-6.0 mg ml for sucrose and maltose. Each time 20 μl solution was injected accurately. When the components were separated completely, a chromatogram was obtained as shown in Figure 1.

The linear equations for concentration versus peak height ratio were obtained by regression analysis; thus, the calibration curve and

function were obtained.

(IV).*Procedure for preparing the honey sample*

The honey sample was accurately weighed at ca. 1 g and dissolved with 5 ml HPLC grade water. The solution was transferred to a 10 ml volumetric flask and diluted to mark with mobile phase solution. One ml of this solution was transferred to a 100-ml volumetric flask and diluted to mark again. These sample solutions were filtered through a 0.45 μm filter before analysis.

(V).*Sample analysis*

To analyze the sample, 20.0 μl of sample solution was injected into HPLC under operating conditions. The chromatogram of a sample is shown in Figure 2. Then, each sugar content was calculated by placing the peak height ratio into the previous calibration curve function.

RESULTS AND DISCUSSION

Figures 1 and 2 , show good peak separation under the operating conditions, no matter what was used, whether a standard solution or a honey sample solution. Eleven samples were examined by analyzing their moisture and major sugar content. The results are compiled in Table 1. Obviously, the total sugar content of the two winter honeys had an average of 67%; the kept honeys, 75%; the wild honeys, 80%. These facts indicate that honey production by honeybees is subject to seasonal changes. In winter, flowers are much less available. Honeybees find it difficult to gather enough nectar or honeydew for inverting to sugars. As a result, the sugar content of winter honeys was lower by 8-13%. The wild honey contained an especially high percentage of total sugar, by 5%. The reason could be that wider nectar plant sources are covered by wild honeybees.

According to HPLC results, there are significant differences in comparisons of the fructose content among winter, kept, and wild honeys.

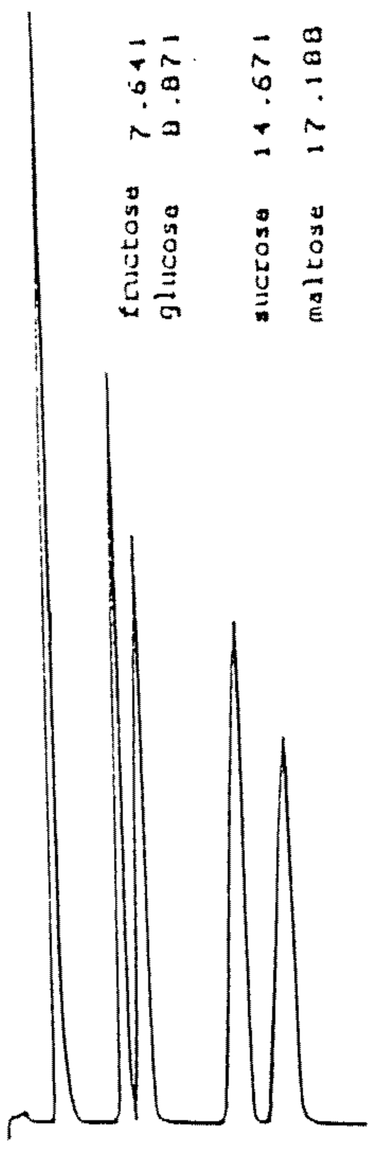

Figure 1. The HPLC chromatogram of sugar standards.

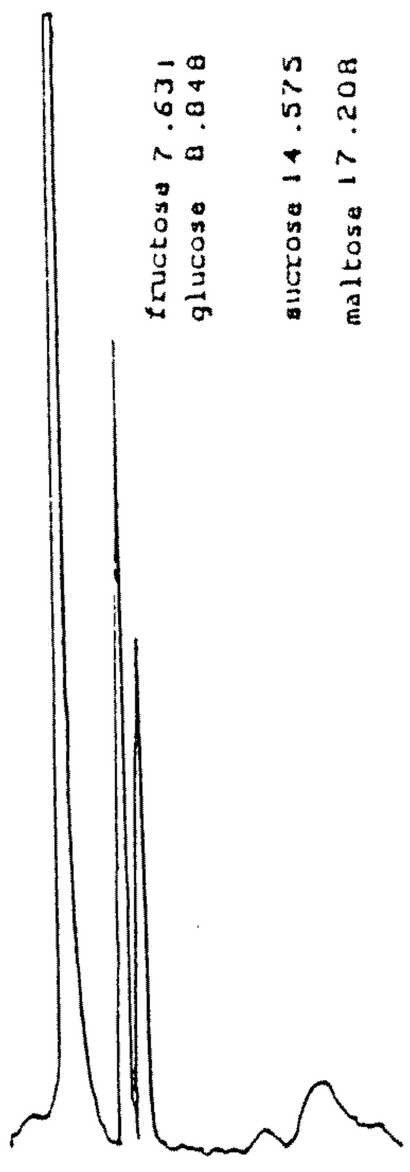

Figure 2. A typical HPLC chromatogram of a honey sample

Winter honeys contain ca. 36.1% fructose; other kept honey, 37.9±1.0%; wild honey, has notably higher fructose content of 44.8±2.2%. When the glucose contents are compared, kept honeys are the highest with 32.6±0.5% on the average; wild honey, 30.8±1.1%; winter honey, ca. 26.7%. These differences make the fructose-to-glucose ratios[20] for kept honeys at 1.17±0.02; wild honey, 1.45±0.10; winter honey, ca. 1.36. From the above analyses, honey from wild honeybees stands out with respect to the highest total sugar content, fructose content and fructose-to-glucose ratios. The results seem to indicate that wild honeys have a "higher nutritional value", as recognized by Chinese tradition. Thus, wild honeys can be sold at higher prices. The fructose-to-glucose ratios also provide a means to distinguish wild honey from kept honey, or "imitation" honeys. In additon, the differences in maltose content are not obvious among the samples, averaging 4.2%. Sucrose content is less than 1% in the four samples, the rest has only a trace amount.

By further examination of Table 1, a comparison can be made between present results and similar studies reported in the literature[16-18] worldwide. As to the moisture content other categories of sugars, except maltose, Taiwanese kept honeys showed no significant differences from Polish, American or Canadian honeys.

For a long time, monosaccharides have been thought to be the essential sugars most easily absorbed by humans. Thus, the real content of monosaccharides has become an important indicator for judging the quality of honey. By using the HPLC method to analyze the relative content of every component of the sugars in honey, there is a reliable way to distinguish

Table 1. The values of moisture and major sugars from honey samples

Honey	Honey source	Moisture%	Fructose%	Glucose%	Sucrose%	Maltose%	Total sugar%	Fructose Glucose ratio
Winter honey*	Not specified	15.8	35.6	25.8	—	4.1	65.5	1.38
Winter honey*	Not specified	19.0	36.6	27.6	—	4.1	68.3	1.33
Average		17.4	36.1	26.7	—	4.1	66.9	1.36
Kept honey	Longan flower	19.5	38.7	32.5	0.8	4.4	76.4	1.19
Kept honey	Longan flower	17.0	38.3	33.4	0.7	4.3	76.7	1.15
Kept honey	Longan flower	18.0	38.6	32.8	—	4.6	76.0	1.18
Kept honey	Litchi flower	17.3	37.9	32.1	0.9	4.3	75.2	1.18
Kept honey	Litchi flower	15.8	36.1	32.0		3.9	72.0	1.13
Average		17.5±1.22	37.9±0.95	32.6±0.51		4.3±0.23	75.3±1.71	1.17±0.022
Wild honey	Longan flower	17.5	47.6	29.1		3.6	79.9	1.62
Wild honey	Longan flower	17.4	46.6	31.9	—	3.9	82.4	1.46
Wild honey	Longan flower	16.6	42.1	30.5		4.2	76.8	1.38
Wild honey	Longan flower	16.5	43.3	31.7	0.6	4.8	80.4	1.37
Average		17.0±0.45	44.8±2.15	30.8±1.12	—	4.1±0.44	79.9±2.01	1.45±0.100
Polish honey		19.22	38.25	33.06	0.89	6.44	78.64	1.16
U.S. honey		17.20	38.19	31.28	1.31	7.31	78.09	1.22
Canadian honey		17.9	37.1	33.7	—	7.4	78.2	1.10

—Trace amount.

*Winter honey is a kept honeybee honey, gathered in winter from unspecified honey sources.

"real" honey from "imitated" honey (which has cheaper sweet syrup added). The truth is that if cane sugar or another sweetened liquid is added to honey, the chromatogram would show an intensified peak for sucrose. Sugars added externally will never increase, or convert to, the monosaccharide portion of honey.

The mystery of how wild honey can play a role as formulated in Chinese medicines remains unclear. The reason could not be solely contributed to sugars, but perhaps to other components such as acetylcholine or the minor minerals in honey. This area needs to be further explored.

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Journal of Food and Drug Analysis. 1994. 2(2)

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野生蜂與養殖蜂所產蜂蜜的糖類含量分析

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摘　要

本研究之目的在於以高效液相層析法就蜂蜜成分中最主要的糖類作分析以比較野生與養殖所得之蜂蜜中之糖類含量，並將結果與波蘭、美國及加拿大等類似研究之分析結果相比較，發現一般養殖蜂蜜並沒有明顯的差異。

本研究結果顯示出一般養殖的蜂蜜中約含38%果糖，冬蜜約含36%，兩者十分相近，而野生蜂的蜂蜜則在45%左右。葡萄糖之含量除冬蜜約爲27%外，其餘一般養殖蜂約爲33%，野生蜂之蜂蜜約在31%上下。因此，在果糖與葡萄糖之比值上一般養殖蜂蜜均介於1.1與1.2之間，而野生蜂蜜則約1.4—1.6。只有少數蜂蜜能檢測少量蔗糖出來，至於麥芽糖含量約在4.2%上下。在總糖分含量上冬蜜檢品約爲67%，而其他季節之一般養殖蜂蜜檢品約有75%左右，而野生蜂之蜂蜜則高達80%。

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