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Quantification of Major Sugars in Natural and Commercial Honey Varieties Using High-Performance Liquid Chromatography

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ABSTRACT

The carbohydrate composition of honey serves as a critical indicator of its quality, floral origin, and authenticity. This study was conducted to quantify and compare the major sugar profiles—glucose, fructose, and sucrose—in natural multi-floral honeys collected from four distinct apiary locations in Basra Province, southern Iraq (Al-Haritha, Abu Al-Khasib, Al-Maqal, and Mahijran), alongside imported commercial honey obtained from local markets. Sugar separation and quantification were performed using high-performance liquid chromatography (HPLC). Distinct differences in retention times and sugar concentrations were observed among samples. Fructose was the predominant sugar in all natural honey samples, with the highest concentration recorded in Mahijran honey (47.58%), whereas commercial honey exhibited the lowest fructose content (18.16%). Sucrose concentration ranged from 10.87% (Abu Al-Khasib) to 15.90% (Al-Haritha) in natural honeys, while commercial honey contained 28.84% sucrose, substantially exceeding the maximum limit established by international honey standards. The fructose-to-glucose (F/G) ratio, a key parameter for honey crystallization behavior and authenticity, varied considerably among samples: Al-Haritha (1.37), Abu Al-Khasib (1.26), Al-Maqal (1.54), Mahijran (1.36), and commercial honey (0.63). The F/G ratio of commercial honey fell below the typical range reported for authentic floral honey, suggesting possible adulteration with sugar syrups. These findings demonstrate significant compositional differences between natural Basra honeys and commercial products, highlighting the utility of HPLC-based sugar profiling for honey quality assessment, geographical differentiation, and authenticity verification. The results underscore the need for routine analytical monitoring to protect consumer interests and ensure compliance with national and international honey quality standards.

1-Introduction

Honey is the sweet substance produced by honeybees from flowering nectar or from secretions found on living parts of plants, so that the percentage of reducing sugars must not be less than 60%, and the humidity level must not exceed 21%. The chemical components of honey are greatly affected by the type of flowers that Bees feed on [1]. Honey differs from flower nectar, which is its source, and due to the process of transformation from nectar to honey, where the chemical composition changes. The most important of these changes is its content of disaccharides, sucrose, whose content in honey decreases compared to nectar due to the invertase enzyme, which breaks down sucrose into simple sugars, fructose and glucose, whose content in honey increases [2]. Natural honey typically consists of the following basic components: carbohydrates, which constitute 82-85%, primarily fructose (538%), glucose (231%), maltose (7.2%), and a small amount of sucrose (approximately 1.5%). In addition to water at a rate of 15-17%, and protein at a rate of 0.1-0.4%, it also contains many vitamins, most importantly vitamins K, E, D, A, minerals, and enzymes such as amylase, invertase, glucose oxidase, catalase, and others [3]. Recent analyses have shown that natural honey contains sucrose, which is usually less than 3% and a maximum 10% in nectar and honey, and often does not reach a measurable amount. Sucrose (cane sugar), by combining with a water molecule, gives two molecules, glucose + fructose, in a precisely equivalent ratio. However, it is rarely found in a similar ratio, and this is related on the one hand to the sugar structure of nectar, which often contains the reduced sugars in the normal state in varying quantities, and on the other hand to the complex transglycosylation phenomena that occur from complex sugars [4]. The relationship between fructose/glucose F/G is described as exceeding the value (1), meaning that these honeycombs contain similar amounts of the two types of simple hexose monosaccharides, with a slight predominance of fructose. In contrast, honey produced by bees that collect it from the same plant species often contains more fructose than glucose, and rarely more glucose than fructose [5]. Honey sugars are considered an important criterion for measuring honey quality. They are used to differentiate between various types of honey and determine the floral source. As well as to detect fraud in honey by identifying the percentage of fructose, glucose, and sucrose present in honey. The sucrose content is an important indicator of adulteration; moreover, sugars are important in differentiating between flower honey and honeydew honey. The relative amounts of the monosaccharides fructose and glucose are important in classifying single-source honeys [6]. One of the most important objectives of the research is to identify the effect of different types of honey and their sources on their content of fructose, glucose, and sucrose sugars, and to identify the ratio of fructose to glucose resulting from the difference in the source of honey and the nectar collected from it.

2-Materials and working methods

Five samples of honey, which are of multi-source floral honey, were brought from four apiaries in Basra Governorate: Al-Haritha, Abu Al-Khasib, Al-Maqal, and Mahijran, and a sample of imported honey obtained from the commercial market, which was filtered using a fine strainer for the purpose of ridding it of impurities such as wax and honey bee remains. It was then placed in a one-liter glass bottle, tightly closed, and kept at room temperature away from light until use. All information related to the samples was collected, including the time of collection and the type of honey. Solutions of honey at a concentration of 50% were extracted, and the solutions were filtered to get rid of impurities for the purpose of injecting these solutions into the HPLC device. For standard curves: A mixture of fructose, glucose, sucrose, and standard sugars was prepared and then injected into an HPLC apparatus.

Sugar determination:

Glucose, fructose, and sucrose were determined using High-Performance Liquid Chromatography (HPLC) with a (250mm × 4.5mm, 5 µm) (NH_2) column, UV-Visible detector, flow rate 2 ml/min, retention time 30 minutes, wavelength 190 nm, and oven temperature 32°C. A set of special chromatographic curves was obtained for each sugar, as each sugar is characterized by a retention time RT, defined as the time required for the compound to exit the separation column and reach the reagent. Where each compound has a retention time that distinguishes it from

others, from which the qualitative determination is made. The standard area A is the peak area resulting from the compound, which is determined by the reagent, and from this, the quantitative determination of sugars is made [7].

3-Results and discussion

Honey content of sugars

The results in Figures (1, 2, 3, 4, 5) and Table (1) attached to them show the group of separated sugars identified by HPLC technology, which are: fructose, glucose, and sucrose in honey taken from the regions of: Al-Haritha, Abu Al-Khasib, Al-Maqal, and Mahijran, in addition to the sample of commercial honey, respectively, as the results showed differences between the sugar ratios of the samples above. Figure (1) shows the sugar content of the Al-Haritha honey sample. As shown in Table (1), the results showed the highest concentration of fructose at 46.10 µg/gm, followed by glucose at 33.98 µg/gm, and then sucrose at 15.90 µg/gm, respectively.

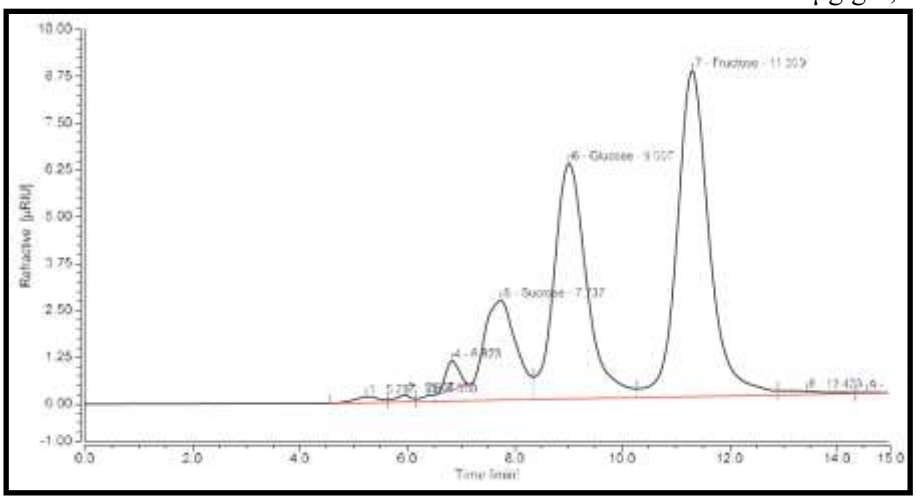

Figure (1) Sugars separated from Al-Haritha honey by HPLC technology.

Figure (2) shows the sugar ratios of the Abu Al-Khasib honey sample. As shown in Table (1), the highest concentration of fructose sugar was 44.80 µg/gm, followed by glucose at 35.45 µg/gm, and then sucrose at 10.87 µg/gm, respectively.

The chemical composition of honey varies, and its types differ; these differences in composition and characteristics are due to differences in plant origin, geographical location, and the type of soil in which plants grow. The composition varies depending on the origin of the flowers or nectar that bees feed on. In addition to several environmental factors during production, such as weather conditions and humidity inside the cells and honey processing during extraction and storage [8]. There are some differences between the plants that provide nectar. Chestnut honey, linden honey, heather honey, orange blossom honey, and some species of Lamiaceae are rich in sucrose. Moreover, rapeseed honey, clover honey, and buckwheat honey are poor in sucrose [9].

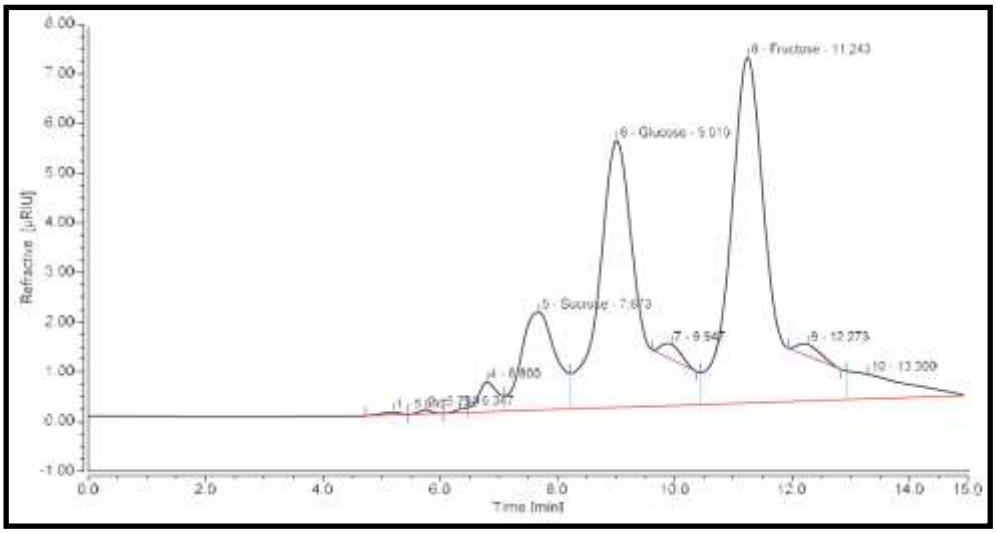

Figure (2) Sugars separated from Abu Al-Khasib honey by HPLC technology.

Figure (3) shows the sugar ratios of the Al-Maqal honey sample. Table (1) shows the highest concentration of fructose sugar at 45.85µg/gm, glucose at 29.70µg/gm, and then sucrose at 14.52µg/gm, respectively.

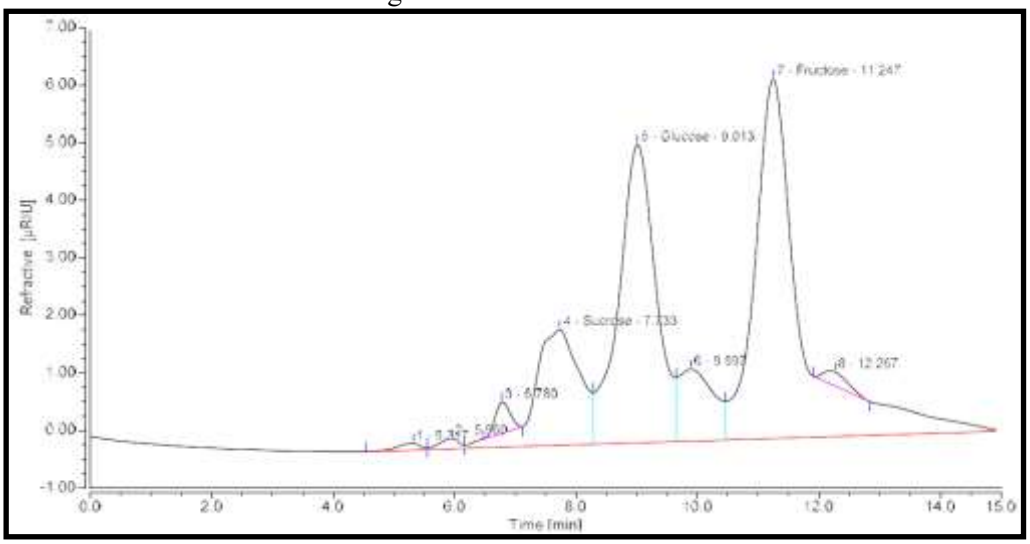

Figure (3) Sugars separated from Al-Maqal honey by HPLC technology.

Figure (4) shows the sugar content of the Mahijran honey sample. As shown in Table (1), fructose has the highest concentration at 47.58 µg/gm, followed by glucose at 35.09 µg/gm, and then sucrose at 13.61 µg/gm.

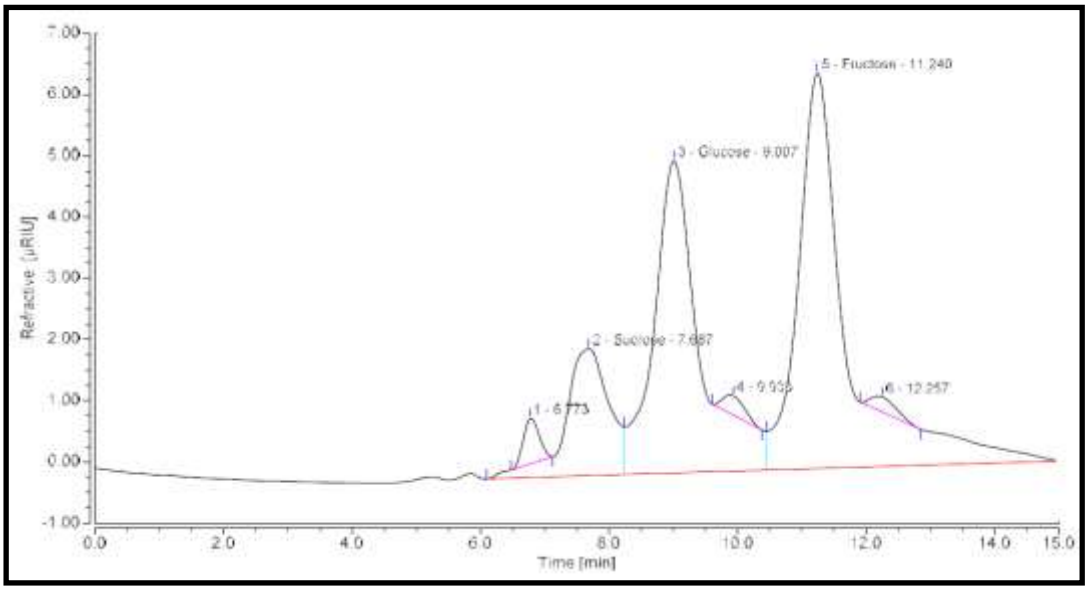

Figure (4) Sugars separated from Mahijran honey by HPLC technology.

From Figure (5), the sugars of the commercial honey sample shown in Table (1) reached the highest concentration of fructose sugar at 18.16µg/gm, glucose at 28.84µg/gm, and then sucrose at 12.62µg/gm, respectively.

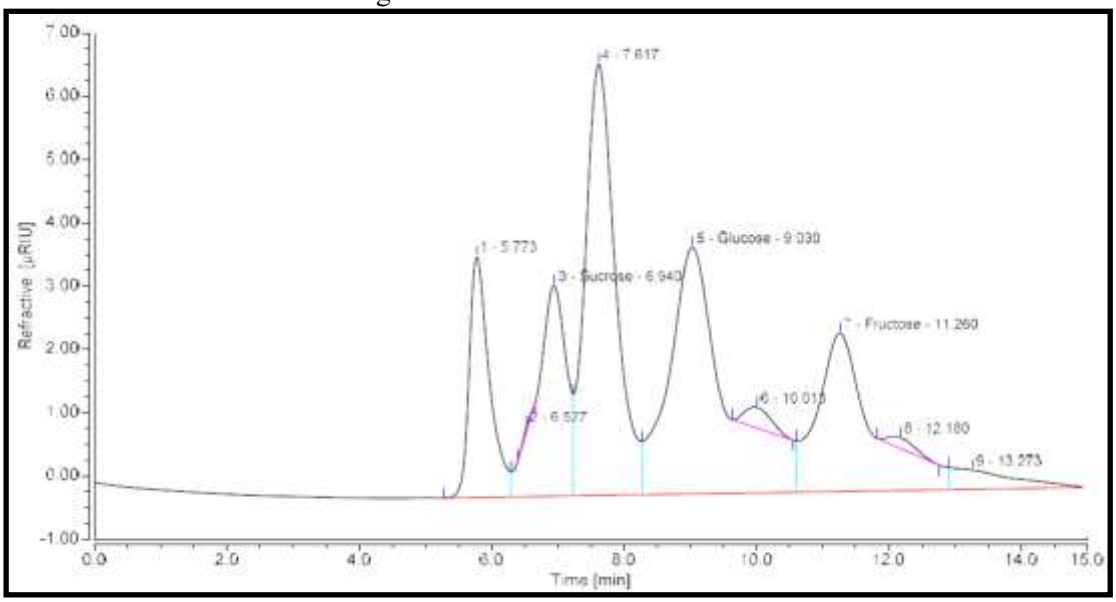

Figure (5) Sugars separated from Commercial honey by HPLC technology.

It was found that the sugars are simple, the most important of which are glucose and fructose, which alone represent only 90% of the total dry matter of honey, as the percentage of glucose reached 31%, while 38% was fructose. Fructose is considered the dominant sugar in almost all types of honey except for Taraxacum officinalis, Brassica napus, and Thrichostema laceolatum honey, in which the percentage of glucose was found to be higher than the percentage of fructose [10,11]. Besides [12] found that the highest concentration of monosaccharides, primarily fructose and glucose, was in samples of natural honey, while sucrose, a disaccharide, had the lowest concentration in samples of natural honey compared to honey samples taken from colonies fed on sugar syrup or invert sugar. The sucrose content is an important indicator of adulteration. In addition, sugars are important in differentiating between flower honey and honeydew honey, and the relative amount of monosaccharides fructose and glucose is important in classifying single-source honey types [6].

Table (1) Sugars separated from honey by HPLC technology.

No	Retention time (min)	Types of Sugars	Sugars from Al-Hartha Honey%	Sugars from Abu Al-Khaseeb Honey %	Sugars from AL-maeqil Honey%	Sugars from Muhijiran Honey %	Sugars from commercial honey%
1	7.737	Sucrose	15.90	10.87	14.52	13.61	12.62
2	9.007	Glucose	33.98	35.45	29.70	35.09	28.84
3	11.300	Fructose	46.10	44.80	45.85	47.58	18.16

Honey is a complex biochemical mixture containing several different substances that have not yet been discovered. There are relatively general differences in composition among all types of honey. These changes are a result of the source of nectar produced by the plant species visited by the bees, the nature of the soil on which the bee species lives, the environmental conditions of the environment, and the physiological state of the bee colony, etc [13,14]

Other important sources for bees to obtain nectar include honeydew, a sweet-tasting liquid secreted by aphids, apricot, and date palms. These substances are rich in sucrose, so most of the sugars in nectar are sucrose, glucose, and fructose [15].

The sucrose content may decrease during storage due to the presence of the invertase enzyme in honey. High sucrose levels in honey can be attributed to early harvesting, which prevents the breakdown of sucrose into glucose and fructose, to excessive feeding of bees with sugar syrup, or as a result of adulteration or adulteration of the honey [16,17] . The results of the study conducted by Anupama and Sapna [18], in which they studied the sucrose content in eleven samples of Indian honey, ranged from 1.5% to 2.1%.

The ratio of Fructose to Glucose F/G in honey:

Figure 6 illustrates the (F/G) ratios for the analyzed honey samples. The ratios were 1.371 for Al-Haritha honey, 1.263 for Abu Al-Khasib honey, 1.543 for Al-Maqal honey, 1.355 for Mahijran honey, and 0.629 for the commercial honey sample. Natural honey contains monosaccharides, comprising 85-95 % of its total sugars [19]. Honey is described as a concentrated aqueous solution of two types of sugars, fructose and glucose, with very small amounts of other sugars. The sugars in honey are affected by geographical location and climatic conditions [10].

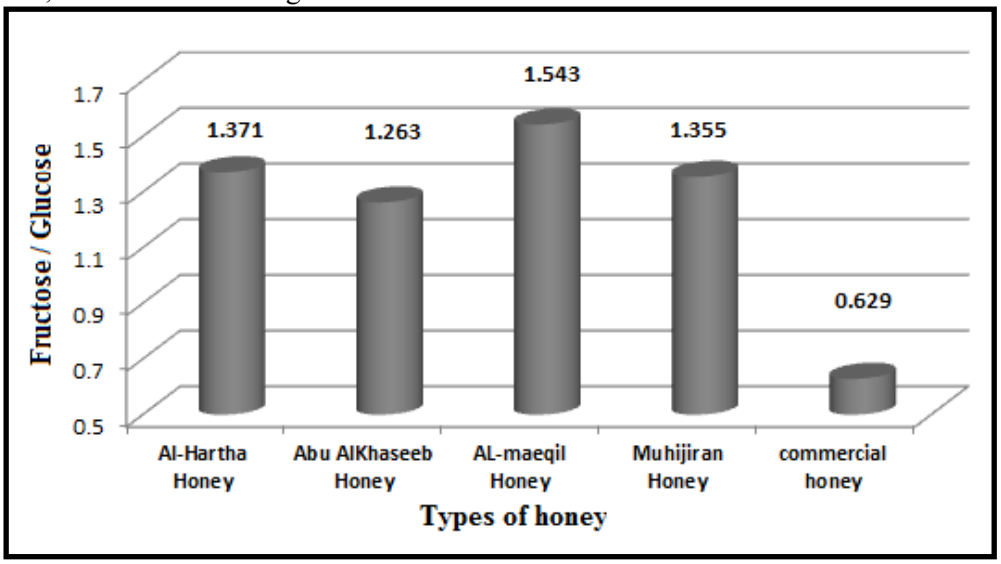

Figure (6) The fructose/glucose in types of honey.

The F/G ratio reaches 1.7-1.5 in some types of Robinier honey, Sauge honey, and Châtaignier honey, in addition to some types of Miellat honey. Types of honey rich in fructose remain

in their liquid state for a long time and usually do not crystallize until after several years. On the contrary, when the (F/G) ratio is less than 1, which is rare, it crystallizes after the harvesting process and sometimes in honeycombs, such as Pissenlit honey and Colza honey [20]. Whereas the (F/G) ratio in 50 samples of Algerian honey ranged from 1.11 to 1.36 [21]. Furthermore [22], found that the (F/G) ratio in 24 samples of Romanian honey ranged from 0.8 to 1.57.

Recommendation

We recommend establishing a standard specification in Iraq for the properties of honey, and that each honey container be labeled with comprehensive information including its type, place of production, components, bee feeding practices, proper methods for preserving and storing samples, and regular honey analysis.

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Conflict of Interest

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Compliance with ethical standards

This article lacks research involving human or animal subjects

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