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Determination of Sugar content in Honey by HPLC Method Kalaburagi, Karnataka, India

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ABSTRACT: The nutritionally rich food produced by honeybees is honey which is processed under human control before it is marketed for a variety of uses. The physical properties and chemical composition in different honey samples indicated variations which are statistically significant and honey from varied regions have different honey which are collected from different regions and exhibit different chemical properties so different types of honey with unique characters which can been seen in Kalaburagi. The present investigation helps us to see the sugar constituents which are mandated for ensuring quality for human consumption. Hence, regular analysis of different types of honey is essential to avoid the contamination during harvesting, transportation and processing of honey by man in normal condition. It could be concluded from the above results that multifloral honey of *A. dorsata* and *A. florae* in terms of physico-chemical components are appropriate to the quality standards of international honey trade in Kalaburagi, Karnataka. Despite poor harvesting and storing practices, *A. dorsata* and *A. florea* honey possesses moderate physico-chemical components which are appropriate to the quality standards of CODEX. The study was carried our for reliability, suitability and accuracy of the method. The HPLC method was used to determine the amounts of sugar in samples of honey.

Keywords: Honey, Sugar, Hplc, Kalaburgi.

INTRODUCTION

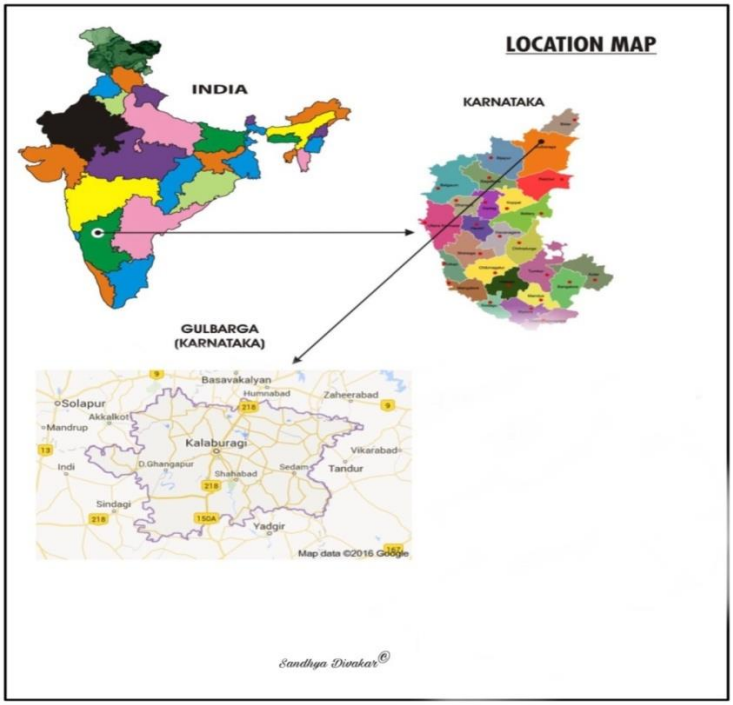
Natural sweeteners vary with respect to their chemical constituents. Honey is the oldest natural sweetener known is honey and is predominated in glucose/fructose in the ratio 1:1.2 and also contains disaccharides like sucrose, maltose etc in lower levels (Yilmaz *et al.*, 2014). Unlike honey, other natural sweeteners like sugarcane jaggery, palm jaggery, syrup made from sugarcane juice or sap of palmyrah palm or coconut palm or maple tree are dominated by disaccharides like sucrose. Determination of the composition of low molecular weight sugars is important for characterizing physiological and biochemical processes in plants (Glyad, 2002). HPLC with refractive index detector (RID) is widely used for determining sugars and there are several columns viz. amino column, lead carbohydrate column, etc. and mobile phases recommended for the purpose (Folkes and Jordan 1996; Pushparajah and Nicholas 2006). In case of amino columns, a chemically modified silica gel containing bonded aminopropyl group is used as a sorbent and aqueous CH_3CN is used as a solvent. The ratio between water and CH_3CN in the mobile phase depends on the nature of compounds under investigation. Researchers have reported that a mobile phase consisting of 75% CH_3CN is most suitable for oligosaccharide separation, while the systems with a higher CH_3CN content must be used for monosaccharide, using lower CH_3CN content the monosaccharides coincided in their retention time (Glyad, 2002). Method validation is a prerequisite when new matrices are studied for the analyte of interest (Rogers, 2013). Even though there are many reports on using aqueous CH_3CN and amino columns for sugar separation, there are no reports which focus on method validation of sugars in matrices rich in sucrose like that of palm sap, jaggery or syrup prepared from sugarcane juice or saps from *Cocus nucifera* L or *Borassus flabellifer* L. During the course of our study on syrups and jaggery, it was found that jaggery made from sugarcane juice showed a lower value for sucrose content (65%) when 85% CH_3CN was used as the mobile phase. This necessitated changing the ratio of CH_3CN to H_2O to arrive at the best suitable concentration of mobile phase that can be used for matrices rich in sucrose. In the present study, we are thus reporting a method validation study to determine the sugar content in honey, palm sap, palm syrup and Keywords Natural sweeteners Palm sap Palm syrup HPLC–RID Sugar-method sugarcane jaggery and palm jaggery using HPLC-RID which warrants the suitability of choosing the HPLC conditions for matrices extremely rich in mono or disaccharides. The method was validated in terms of their linearity, limit of detection and quantification, precision and accuracy.

MATERIALS AND METHODS

The Gulbarga district was previously a part of Hyderabad State and later on integrated with the new Mysore State (Now called as Karnataka), which come into existence on 1st November 1956. Under as state reorganization Act 1956, the district along with its existing seven talukas, *viz*., Afzalpur, Aland, Chincholli, Chittapur, Gulbarga, Jewargi, Sedam, formed a part of Mysore State. The name Gulbarga means a leaf with flowers as it is derived from Persian language-Gul means flower and Burg means leaf. The district is also known as 'Kalburgi' which means a stony land or stone proofing or a heap of stones in Kannada language. The three districts *viz*., Gulbarga, Raichur, formerly belonging to Hyderabad State and then added to the Karnataka State. Therefore, which is known as Hyderabad- Karnataka a most backward area in the state? Now the district established as an administrative division including the four districts *viz.*, Bellary, Bidar, Gulbarga and Raichur.

However, when we try to turn the pages of history we came to know that GULBARGA was known as 'KALABURAGI' in ancient days which mean stony land in Kannada. Gulbarga district is situated in the northern part of Karnataka State. In the earlier days, Gulbarga was a district of Hyderabad Karnataka area and became a part of Karnataka State after re-organization of states. Recorded history of this district dates back to the 6th Century A.D. The Rashtrakutas gained control over the area but the Chalukyas regained their domain within a short period and regained supreme for over two hundred years. The Kalahari's who succeeded them ruled in and around the close of the 12th century. The Yadavas of Devagiri and the Hoysalas of Dwarasamadra destroyed the supremacy of the Chalukyas and Kalachuris. About the same period the Kakatiya kings of Wrangle came into prominence and the present Gulbarga and Raichur districts formed part of their domain. The Kakatiya power was subdued in 1321 AD and the entire Deccan including the district of Gulbarga passed under the control of the Muslim Emperors of Delhi.

The revolt of the Muslim officers appointed from Delhi resulted in founding of the Bahmani kingdom in 1347 AD, by Hassan Gangu who chose Gulbarga to be his capital. When the Bahmani dynasty came to an end, the kingdom broke up into five independent Sultanates and the present Gulbarga district came partly under Bidar and partly under Bijapur with the conquest of the Deccan by Aurangezeb in the 17th century, Gulbarga passed back to the Mughal Empire. In the early part of the 18th century when Mughal Empire was declining Asaf Jha a general of Aurangzeb became independent and formed the Hyderabad State in which a major part of Gulbarga area was also included. In 1948 Hyderabad state became a part of Indian Union and in 1956, excluding two talukas which were annexed to Andhra Pradesh the remaining talukus of Gulbarga district became part of New Mysore State.



Sugar in honey by HPLC method. Three natural honey and three packed honey was collected. The natural honey was collected in tightly closed glass containers and stored in room temperature till analysis. The six samples are named as H1, H2, H3, H4, H5 and H6. Sugar content tests were performed using HPLC according to Bogdanov *et al*. (1997) on a high-pressure SHIMADZU liquid chromatograph equipped with LC-10ATVP liquid chromatograph pumps, DGU-14A degasser, CTO-10AVP column thermostat, RID-10A refractometric detector, POL-LAB CHROMA 2001 software and SUPELCOSIL LC-NH2), 25 cm × 4.6 mm, 5 µm chromatograph column. The amount of the sample injected onto the column was 20 µl. The separation was conducted at a temperature of 30°C with

the flow rate of 1.3 ml/min. The identification of sugars in honey was done by comparing retention times of individual sugars in the reference vs. tested solution (qualitative analysis). The contents of those compounds were assayed based on the comparing peak areas obtained in the examined samples with those from the reference solution (quantitative analysis). To make the presentation of the obtained results more comprehensive the following was calculated: weight of the sugar and percent of the sugar.

Statistical analysis. Calculation of weight of sugar and percent of sugar is done by applying the following formula.

$$\text{Sugar weight in honey (mg)} = \frac{\text{Peak of sugar in honey}}{\text{Area peak in standard}} \times \text{weight of standard (mg)}$$

$$\text{Sugar percentage in honey (\%)} = \frac{\text{(mg) sugar in honey}}{\text{Weight of honey sample}} \times 100$$

RESULT

Table 1 shows the HPLC test for H1 sample, where the percentage and weight of sugar in honey is calculated. The sugar weight in H1 sample is 3.4 mg and percent of sugar in H1 sample is 62.8%. Fig. 1 represent sugar peak in H1 sample.

Table 2 shows the HPLC test for H2 sample, where the percentage and weight of sugar in honey is calculated. The sugar weight in H2 sample is 3.43 mg and percent of sugar in H2 sample is 68.6%. Fig. 2 represent sugar peak in H2 sample.

Table 1: Showing HPLC test for H1 sample.

Peak	Retention time	Area	Relative retention time	Area %
1	2.290	110302	0.958	2.820
2	2.391	3799958	1.000	97.150
Total		3910260		100.00

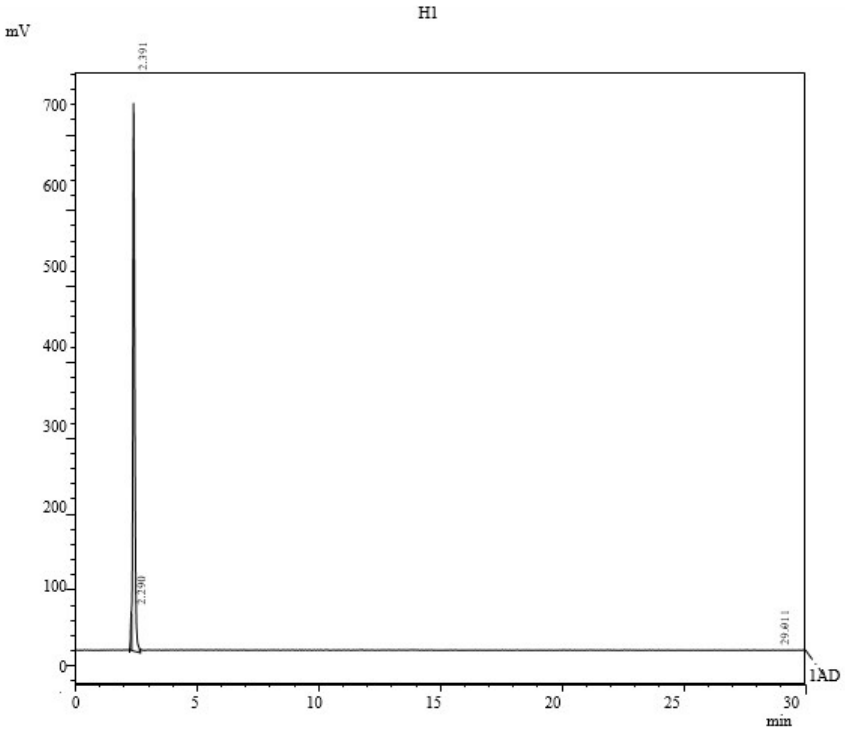


Fig. 1. Representing Sugar in H1.

Table 2: Showing HPLC test for H2 sample.

Peak	Retention time	Area	Relative retention time	Area %
1	2.292	134668	0.959	3.726
2	2.391	3479586	1.000	96.274
Total		3614255		100.00

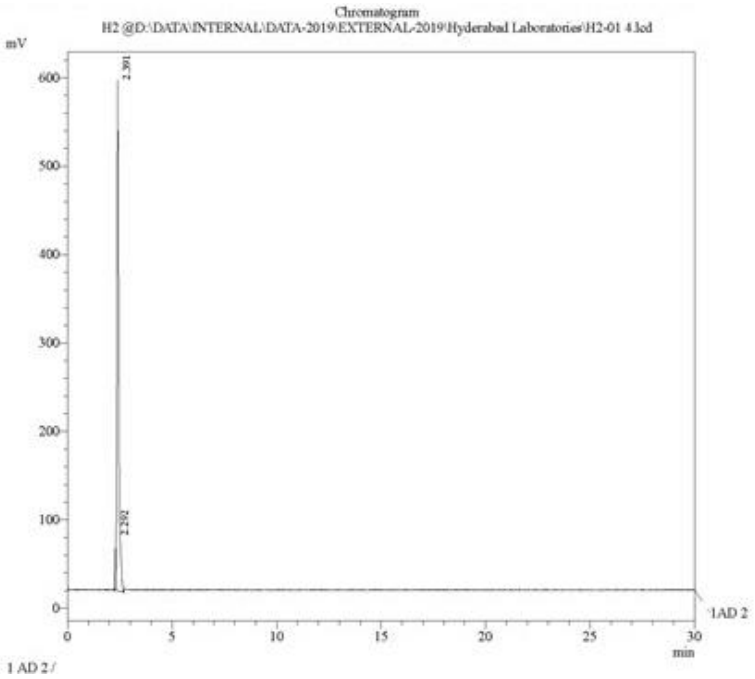


Fig. 2. Representing Sugar in H2.

Table 3: Showing HPLC test for H3 sample.

Peak	Retention time	Area	Relative retention time	Area %
1	2.308	262432	0.967	6.219
2	2.388	3957580	1.000	93.781
Total		4220012		100.00

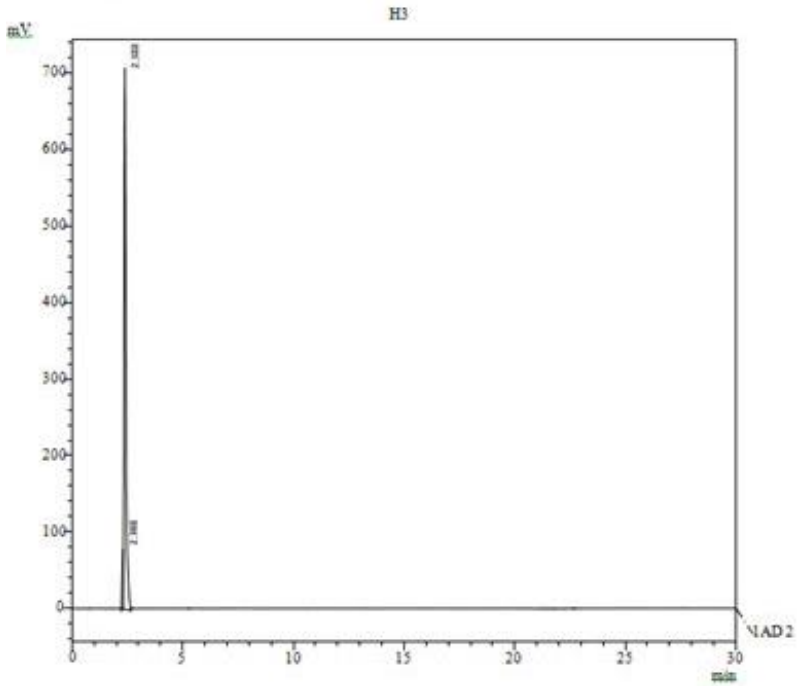


Fig. 3. Representing Sugar in H3.

Table 3 shows the HPLC test for H3 sample, where percentage and weight of sugar in honey is calculated. The sugar weight in H3 sample is 3.01 mg and percent of sugar in H3 sample is 60.2%. Fig. 3 represent sugar peak in H3 sample.

Table 4: Showing HPLC test for H4 sample.

Peak	Retention time	Area	Relative retention time	Area %
1	2.283	141204	0.956	3.170
2	2.389	4312944	1.000	96.830
Total		4454148		100.00

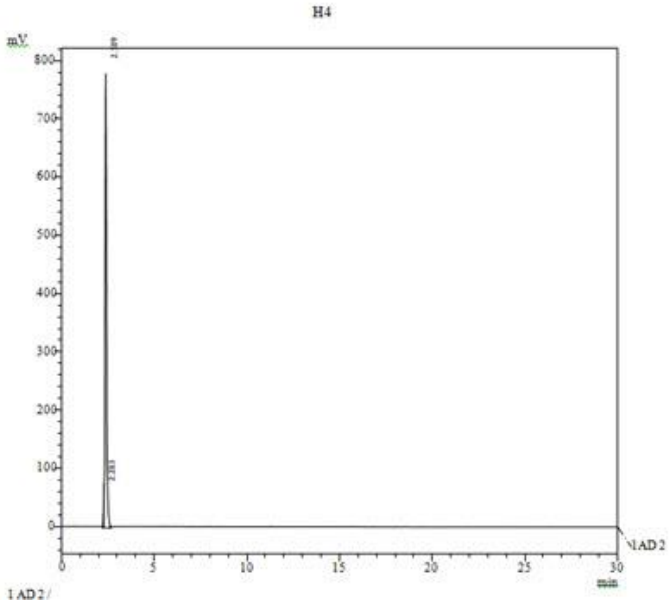


Fig. 4. Representing Sugar in H4.

Table 4 shows the HPLC test for H4 sample, where the percentage and weight of sugar in honey is calculated. The sugar weight in H4 sample is 2.76 mg and percent of sugar in H4 sample is 55.2%. Fig. 4 represent sugar peak in H3 sample.

Table 5: Showing HPLC test for H5 sample.

Peak	Retention time	Area	Relative retention time	Area %
1	2.308	267772	0.964	4.643
2	2.395	5499688	1.000	95.357
Total		5767460		100.00

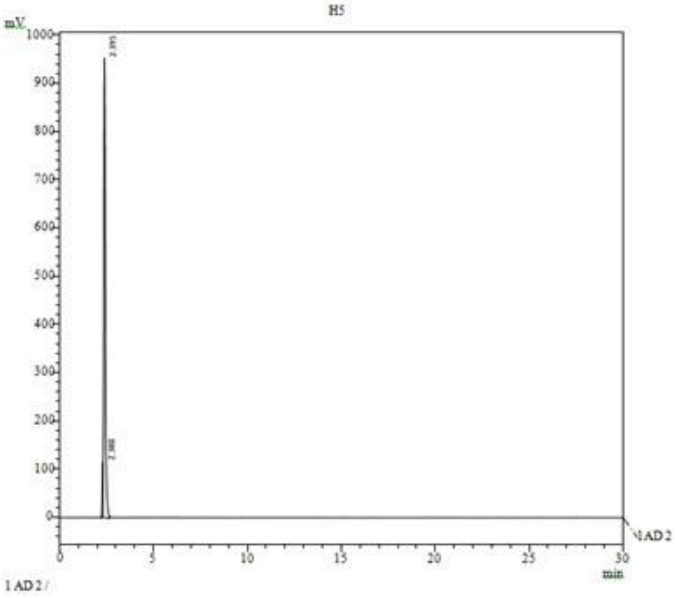


Fig. 5. Representing Sugar in H5.

Table 5 shows the HPLC test for H5 sample, where the percentage and weight of sugar in honey is calculated. The sugar weight in H5 sample is 2.17 mg and percent of sugar in H5 sample is 43.4%. Fig. 5 represent sugar peak in H5 sample.

Table 6 shows the HPLC test for H6 sample, where the percentage and weight of sugar in honey is calculated. The sugar weight in H6 sample is 2.12 mg and percent of sugar in H6 sample is 42.4%. Fig. 6 represent sugar peak in H6 sample.

Table 7 Shows the weight of the sugar and percent of the sugar in honey. Total six samples result is shown i.e. H1 is 3.14 mg and 62.8%, H2 is 3.43 mg and 68.6%, H3 is 3.01 mg and 60.2 %, H4 is 2.76 mg and 55.2%, H5 is 2.17 mg and 43.4% and H6 is 2.12 mg and 42.4%. Fig. 7 is representing the graph of six samples which includes weight and percent of sugar in honey.

Table 6: Showing HPLC test for H6 sample.

Peak	Retention time	Area	Relative retention time	Area %
1	2.292	220364	0.958	3.759
2	2.393	5641491	1.000	96.241
Total		5861855		100.00

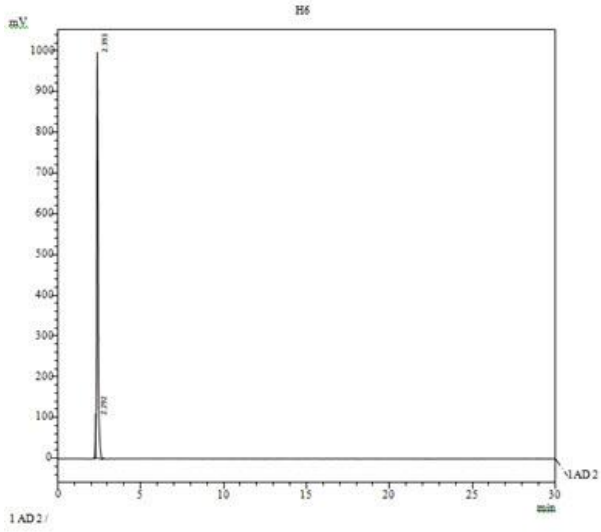


Fig. 6. Representing Sugar in H6.

Table 7: Showing the weight of sugar and percentage.

Sample	Weight (mg)	Percentage (%)
H1	3.14	62.8
H2	3.43	68.6
H3	3.01	60.2
H4	2.76	55.2
H5	2.17	43.4
H6	2.12	42.4

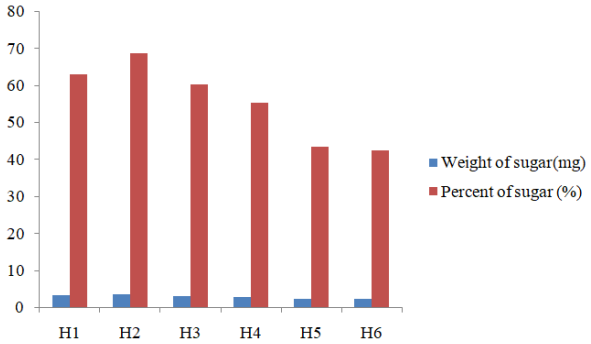


Fig. 7. Representing weight of sugar and percent of sugar/

CONCLUSIONS

Results obtained were recorded from Table 7 50% samples in this study contained more than 60% of the sugar i.e. H1, H2 and H3 and other three samples contained less then 50% of the total sugar. NHB and CAC and the European Honey Standard suggested that the minimum sugars in pure honey should be 60%. The sugar content may reduce because of the preservation techniques and some enzymes activity and the while collecting the honey. The honey collected during the season i.e. flowering, the sugar is expected to be more. Gas chromatography method was used as the study method as it determines the sugar and is highly sensitive and reliable. As this method is simple and easy. As compared with others lower in cost for analysis of sugar content in honey.

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REFERENCES

Bogdanov, S., Martin, P., & Lüllmann, C. (1997). Harmonised methods of the European Honey Commission. Apidologie, 1–59 (extra issue).

Codex Alimentarius (2001). Commission standards. Standards for honey.

Folkes, D. J. and Jordan, M. A. (1996). Mono – and Disaccharides: Analytical Aspects. Marcel A.C. Eliasson (Ed.). In: *Carbohydrates in Foods*, p. 1- 40.

Glyad, V. M., New York: Taylor and Francis. (2002). Determination of monosaccharides, disaccharides, and oligosaccharides in the same plant sample by High-Performance Liquid Chromatography. *Russian Journal of Plant Physiology, 49*, 277–282.

Pushparajah, T. and Nicholas, H. L. (2006). Adulteration of apple with pear juice: emphasis on major carbohydrates, proline, and arbutin. *Journal of Agricultural and Food Chemistry, 54*, 4861-4867.

Rogers, H. A. (2013). How Composition Methods Are Developed and Validated. *Journal of Agricultural and Food Chemistry, 61*, 8312−8316.

Yilmaz, M. T., Tatlisu, N. B., Toker, O. S., Karaman, S., Dertli, E., Sagdic, O., & Arici, M. (2014). Steady, dynamic and creep rheological analysis as a novel approach to detect honey adulteration by fructose and saccharose syrups: Correlations with HPLC-RID results. *Food Research International, 64*, 634-646.

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