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High performance liquid chromatographic analysis of sugars in honeys from Western Ghats, Karnataka

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Abstract

The present investigation involved, pollen, physical and sugar analysis of five unifloral and five multifloral honeys were carried out. The main floral sources identified were coffee, coriander, neem, niger and acacia. Their sugar profile was analyzed by means of High performance liquid chromatography with refractive index detector. This method enables the determination of five main sugars of honey such as glucose, fructose, galactose, sucrose, and maltose. Sugar concentration of different types of honeys were found variable and the result obtained showed that the predominant sugar of the 5 investigated unifloral honeys and 5 multifloral honeys was fructose followed by glucose and maltose. Galactose and sucrose were present in low amount in all the samples. The sugar profile variability reflects differences n botanical origin, enzymatic activity and environmental factors. These findings not only support the use of sugar profiling and melissopalynology in honey authentication but also underscore their potential role in detecting adulteration.

Keywords: sugar composition, melissopalynology, unifloral, HPLC, refractive index, predominant

1. Introduction

Honey, the natural sweet and viscose liquid produced by honeybees from the nectar of blossoms, with nutritional and medicinal properties. It is a unique and complex product consisting of 80-85% of carbohydrates, 15-17% of water, 0.1-0.4% of proteins and minor quantity of amino acids, enzymes, vitamins and other phenolic compounds (O. O. James et.al. 2009). The composition of honey varies according to nectar source (B. O. Omafuvbe et.al. 2009), (E. Crane1980). Sugars are simple carbohydrates and have been recognized as a valuable source of energy in honey. The principal carbohydrate constituents of honey are monosaccharide, such as fructose and glucose. In addition, honey also contains disaccharides and oligosaccharides in small concentrations. It is a complex natural food and stands out as the only sweetener commonly consumed without any processing. It has been used both as a raw food and in medicine since ancient times. Primarily honey is a mixture of sugars, manly fructose and glucose. It also

contains trace amounts of various compounds, including enzymatic and non enzymatic antioxidants, glucose oxidase, catalase, ascorbic acid,, flavonoids, phenolic acids, carotenoid derivatives, organic acids, amino acids, proteins and volatile substances (Aljadi and Kamaruddin, 2004; Gheldof et al., 2002; Lachman et al., 2010). The carbohydrate in honeys is responsible for some of the key functional properties like the ability to hold moisture, to promote color and to develop the flavor. The concentration of fructose and glucose and their ratio are useful indicators for classification of unifloral honey (J. Wang and Q.X.Li 2011). High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) and proton nuclear magnetic resonance (^ 1H NMR) spectroscopy provided only qualitative data. As a result these methods did not allow for the quantification of exogenous syrup levels in the honey (Carla Egido et.al., 2024) (Zdiniakova et al., 2023). The characteristics of honey sugar may be useful for detection of honey adulteration and also to differentiate floral honey from honey dew honey (Islam et.al. 2020) (R. J. Weston, L. K. Brocklebank, 1999).

Unifloral honey is a type of honey, predominantly produced from the nectar of single plant species which have special taste and consists of substance that are beneficial to human health. This may make their commercial value greater that of multifloral honeys in the international market (S. Bogdanov et.al 1999) multifloral honey is a type of honey produced from the nectar of multiple plant species. The quality parameter of honey such as moisture content, free acidity, total solid, pH and sugar profile The Council of the European Union 2001] together with melissopalynological analysis can be used to authenticate the botanical origin of honey. Many researchers have been reported the sugar compositions of different types of honeys such as Algerian honey (L. Hayette et.al.2000), Spanish honey (F. Bosch and R Mateo, 1984), Omani honeys (A. M. Sajwan et.al.2007), Transylvanian honeydew honey (B. Victorita et.al.2008), Nigeria honey (F. Buba et.al. 2013), Tunisia honey (A. Boussaid et.al 2014), Portuguese honey (E. Mendes and E. BrojoProenc 1998), Turkish honey (M.I. Haroun et.al.2012), Lithuania honey (V. Kaskonien et.al. 2010), Estonian honey (B. Kivimaa et.al. 2014) and Romanian honey (V. Bonta et.al. 2007). On the other hand non targeted strategies such as finger printing based metabolomiic approaches are emerging as effective and powerful tools for addressing authenticity issues. These methods aim to capture as many instrumental signals as possible without requiring prior knowledge of the specific sample components responsible for those signals (CuadrosRodríguez et al., 2021; Gorska-Horczyczak et al., 2022; Jim´enez-Carvelo et al., 2021). The sugar compositions of Indian unifloral honeys were less analyzed. Hence the objective of the present study was to determine the sugar composition and melissopalynological study of honeys from different sources collected from Westren Ghat region, South India, by HPLC with refractive index detector in order to find possible relationship between carbohydrate composition and floral sources.

Materials and Methods

A total of 10 honey samples from different botanical origin were collected directly from beekeepers in different parts of Westren Ghat region, South India. The collected honey samples were stored in sterilized polythene bottles and filtered through single thickness fine cloth to remove suspended particles like dirt, beeswax and other impurities. Later it was stored in airtight container at room temperature under hygienic conditions and used for the further study.

Pollen analysis

The identification of pollen type in collected honey samples were based on pollen analysis following the method suggested by Louveaux (J. Louveaux et.al. 1978). The percentage of specific pollen grains were counted in order to evaluate the unifloral honey samples and classified according to their frequency class as predominant pollen (more than 45% of the pollen grains), secondary dominant pollen (16-45%), important minor pollen (3-15%) and minor pollen (less than 3%). The pollen grains were compared with the reference slides for identification.

Physical analysis

All the honey samples were tested to assess their physico-chemical properties. For the estimation of moisture content, 5g of each honey samples were put in a flat dish and dried in the oven at 1050C for 3 hours, covered and cooled in desiccators and weighed. The samples were re-dried for one hour in the oven, cooled and reweighed. The process was repeated at one hour drying intervals until a constant weight was obtained (E.T.Willams et.al. 2009).Percentage total solids of each sample was determined using the following formula:

Total solids (%) = 100 – Moisture content (O. E. Agbagwa et.al.2011).

The pH value of honey samples were measured using a digital pH meter with solution prepared with 10g of honey in 75ml of distilled water (AOAC 1990). Free acidity was determined by titrimetric method (AOAC 1990). 10g of honey samples were homogenized with 75 ml of distilled water and filtered. The solution was titrated by adding 0.05M sodium hydroxide and stopped at pH 8.5. Acidity (milli equivalent of acid per kg of honey) was determined as 10 times the volume of sodium hydroxide used in titration.

Sugar composition

HPLC method was used for determining the sugar profile of 5 unifloral honeys and 5 multifloral honeys quantified for 3 mono (fructose, glucose and galactose) and 2 disaccharide (sucrose andmaltose)sugars. Methanol and water was used as mobile phase with the ratio 60:40 at the flow rate of 1ml/min with Syncronis Amino 5µm, 150 mm x 4.6 mm Column at 35o C. Preparation of honey samples for HPLC (100mg) were prepared by dissolving 1gm of honey in 4M Trifluroacetic acid (100mL), boiled at 100oC for 10 min and filtered. After acid treatment, hydrolysate was obtained by solid- through liquid separation was centrifuged at 10,000 rpm for 10 min and pH of the acid hydrolysate was adjusted to neutral. The clear supernatant was double filtered by passing through 0.45 µm syringe filter and 5µl was injected to HPLC. For quantification of saccharides, HPLC chromatograms of honey samples were compared with 5 commercial standard sugars such as fructose, glucose, galactose, sucrose, and maltose, with concentration of 1mg/ml. Sugars concentration used for the standards were 500, 750, 1000, 1250, 2500 and 5000ppm. A 25µl of sugar standards were injected in triplicate and run to check the retention time and peak area with 5µl of each prepared honey samples. The total time of analysis was up to 30 min. Honey sugars were quantified by comparing their retention time and their peak area with those of standard sugars (Harmonised Methods of the International Honey Commission 2002) (Alghamdi et.al., 2020) (Aljohar et al., 2018) (Carla Egido et.al., 2024).

Result and Discussion

Melissopalynology is the most frequently used method for determination of honey botanical source. All the honey samples were tested by pollen analysis in order to assign their nectar sources (Table 1). The unifloral honeys were belongs to diverse floral origin such as Coffee (Coffea arabica), Acacia (Acacia sp.), Coriander (Coriandrum sativum), Niger (Guizotia abyssinice) and Neem (Azadirachta indica). Coffee honey was collected from Dandeli region of Westren Ghats. The melissopalynolgical analysis revealed that more than 79% of counted pollen grains were belonged to coffee and other pollens identified were from Tridax procumbens, Argemone mexicana, Canna indica, Datura sp. Emblica officinale, Ageratum and Melastoma sp.

The pollen analysis of honey samples indicated that 76% of pollen grains in acacia honey belong to acacia plant and few others from Cissus vitiginea, Evolvulus alsinoides, Gravellia robusta, Lagascea mollis and Tectona grandis. About 69% of pollen grain in the coriander honey originated from Coriandrum sativum and sample also contained pollen grains of Zea mays, Seasamum orientale, Helianthus annus, Tecoma stans, Capsicum sp. and Tropelium sp.

The analysis also indicated that 42% of pollen grains in niger honey was identified from Guizotia abyssinice, others were from Borassus sp., Areca catechu, Moringa oleifera, Brassica sp., and Amaranthus spinosus. According to the melissopalynolygical analysis, neem honey indicated 68% of pollen from Azadirachta indica plant with which Eucalyptus sp., Emblica officinale, Mangifera indica, Cestrum sp., and Mimosa pudica pollen were identified.

The result of physical parameters of studied honey samples were detailed in table 2. The moisture content of these unifloral honeys ranged from 18.18% to 21.58% however, the highest moisture content was found in coriander honey (21.58%) and niger (20.84%) honey. The lowest moisture content was in neem honey (18.18%) and there were no significant difference in coffee (19.64%) and acacia (19.23%) honey and these honey samples were within the range of required standard (21%) of International regulations of quality (Codex Alimentarius Commission Standard 2001). Highest moisture content may cause undesirable fermentation and high crystallization during the storage (O. O. James et.al. 2009). Moisture content in honey may also depend on the geographical factors and harvesting period.

All the honey samples tested were acidic in nature and the pH value ranged from3.4 to 4.7 (Table 2). Neem honey has highest pH value (4.7) followed by acacia (4.2) and niger (4.0) honey. Coffee (3.46) and coriander (3.4) honey has lowest pH value. Low pH value of honey may inhibit the growth of microorganism and acidic pH may be due to various minerals and acids (E. Mendes and E. Brojo Proenc 1998). The pH value of all the honey samples examined was in accordance with Codex Alimentarius Commission (Codex Alimentarius Commission Standard 2001).

The free acidity of honeys of the present study ranged between 8.8 and 22.0mmol/kg. All the honey samples met the standard of EU (40mmol/kg). The free acidity of honeys may explained by the presence of organic acids like gluconic, pyruvic, malic and citric acid in equilibrium with their corresponding lactones and some inorganic ions like phosphates and chlorides (A. S. Al-Khalifa and I.A. Al-Arify 1999). The percentage of total solid of the tested honey samples was found as 70.00% (Niger honey), 81.82%

(Neem honey). Coffee and acacia honey has the total solid of 80.36% and 80.77% respectively whereas coriander has 73.69 % of total solid.

Table 1: Pollen analysis of unifloral honey samples.

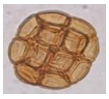
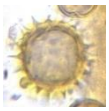
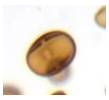
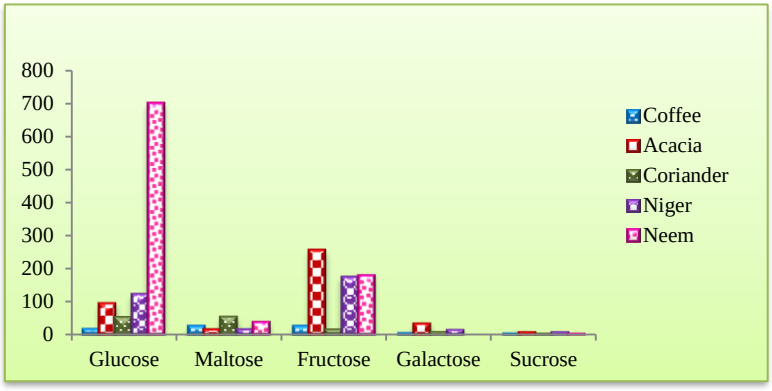
Sl. No	Sample	Location	%age of pollen	Source	Other pollen sources
1	Coffee 	Dandeli	79%	*Apis cerana*	*Tridax procumbens, Argemone mexicana, Canna indica, Datura* sp. *Emblica officinale, Ageratum and Melastoma* sp.
2	Acacia 	Belgam	76%	*Apis cerana*	*Cissus vitiginea, Evolvulus alsinoides, Gravellia robusta, Lagascea mollis andTectona grandis*.
3	Coriander 	Sagara	69%	*Apis cerana*	*Zea mays, Seasamum orientale, Helianthus annus, Tecoma stans, Capsicum* sp. *and Tropelium* sp
4	Niger 	Desipura	49%	*Apis cerana*	*Borassus* sp., *Areca catechu, Moringa oleifera, Brassica* sp., *and Amaranthus spinosus*.
5	Neem 	Lakkavalli	68%	*Apis cerana*	*Eucalyptus* sp., *Emblica officinale, Mangifera indica, Cestrum* sp., *and Mimosa pudica*

Table 2: Physical properties of Honey samples:

Sl. No.	Honey sample	P^H	Acidity	Total moisture	Total solid
1	Coffee	3.46	11.54	19.64	80.36
2	Niger	4.0	8.8	20.84	70.00
3	Neem	4.7	13.2	18.18	81.82
4	Coriander	3.4	9.0	21.58	73.69
5	Acacia	4.2	22	19.23	80.77
6	B.R.Hils	3.65	8.0	7.69	92.31
7	Agumbe	4.12	20.1	15.38	84.62
8	Theerthahalli	4.67	25.0	18.75	81.25
9	Kalasa	3.34	12.6	14.28	85.72
10	Shankkaraghatta	4.09	7.0	20.58	79.42

Fig: 1, Comparison of sugar concentrations of unifloral honeys



The collected honey samples were analyzed for sugars by HPLC with refractive index detector and results are detailed in table 3.The honey samples (5 unifloral and 5 multifloral) were analysed for sugars by HPLC with refractive index detector. Fructose and glucose were the major monosaccharides in all the honeys examined. The results showed that, the fructose content varied between 1.90µg/ml (B.R.Hils) to 254.22µg/ml (Acacia honey), whereas in Niger (172.8µg/ml) and Neem (177.86µg/ml) honeys specifically contained a moderate amount of fructose. Theerthahalli (21.43µg/ml) contained low concentration of fructose. The glucose content of the samples was within the range of 13.17µg/ml (Coffee honey) to 705.36µg/ml (Kalasa). The amount of glucose content was 701.11 µg/ml, 120.76µg/ml, 92.94µg/ml and 50.14µg/ml in Neem honey, Niger honey, Acacia honey and Coriander honey respectively. Galactose content was in the range of 1.76µg/ml to 31.77µg/ml. Moreover, the study confirms that the galactose was not detected in Agumbe (multifloral) and Neem honey (unifloral) and it was detected as 1.76µg/ml (coffee honey), 21.22µg/ml (Kalasa) and 31.77µg/ml (Acacia honey). Among the disaccharides in the tested honey samples, the content of maltose was the most abundant and varied from 13.97µg/ml (Niger honey) to 67.05µg/ml (Kalasa). The other honey samples like Coffee (21.89µg/ml), Shankaraghatta (27.89µg/ml) and Acacia (14.35µg/ml) were also reported for the presence of significant amount of maltose. The sucrose content was in the range of 0.21µg/ml to The sucrose content of honey samples studied was in the range of 0.23µg/ml to 5.39µg/ml and the good quality honey should not contain more than 5gm/100gm sucrose according to Codex Alimentarius Commission[Codex Alimentarius Commission Standard 2001]. The value obtained for sucrose content of studied honey samples were within the limits of International standards except acacia honey which showed little higher amount of sucrose content (Table 3). Such in the case with rice sugar syrup which produce chromatograms that closely resemble those of authentic honey samples albeit with different relative absorbance intensities. This similarity can make it challenging for many authentication methods to detect adulteration as their effectiveness often depends on the specific type of sugar based adulterant used (Zdiniakova et al., 2023).

Table: 3, Sugar analysis of Honey samples.

Sl. No.	Name of honey samples	Honey type	Sugar (µg/ml) content of honey samples				
			Glucose	Maltose	Fructose	Galactose	Sucrose
1	U_1 (Coffee honey)	Unifloral	13.17	21.98	21.43	1.76	0.34
2	U_2 (Acacia honey)	Unifloral	92.94	14.35	254.22	31.77	5.39
3	U_3 (Coriander honey)	Unifloral	50.14	50.74	13.54	5.59	0.76
4	U_4 (Niger honey)	Unifloral	120.76	13.97	172.58	12.22	5.00
5	U_5 (Neem honey)	Unifloral	701.11	36.49	177.86	0.00	0.54
6	M_1 (B.R.Hils)	Multifloral	15.60	21.84	1.90	2.09	0.82
7	M_2 (Agumbe)	Multifloral	68.94	41.53	25.34	0.00	2.14
8	M_3 (Theerthahalli)	Multifloral	28.81	31.97	18.54	8.06	0.21
9	M_4 (Kalasa)	Multifloral	705.36	67.05	54.13	21.22	4.13
10	M_5 (Shankaraghatta)	Multifloral	47.12	27.89	124.67	4.97	0.72

Conclusion

Honey is a high energy carbohydrate food which can be easily digestible. From the results it is concluded that the honey samples did not exhibited a significant variation in moisture, pH, free acidity and total solid. Basically honey is considered to be a unifloral if maximum of 45% of the pollen in it derived from one plant and multifloral honeys derived from different plants. Melissopolynological analysis method used in detecting the origin of unifloral honey. The content of mono and disaccharide in honeys from different botanical origin assayed by HPLC-RI detector shows a significant variability. Though fructose was predominant in all the honey samples, coriander and neem honey contained low concentration of fructose and higher concentration of glucose. All the samples showed relatively high concentration of maltose, whereas sucrose and galactose were present in low amount. The variability of sugar composition probably depends on the factors of their origin, botanical sources, enzymes present in nectar and enzyme reaction. The quantitative sugar test is cited as help in detection of honey adulteration with sugar coming from different sources.

References

1. Boussaid, M. Chouaibi, L. Rezig, R. Hellal, F. Donsì, "Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia",Arabiian Journal of Chemistry, 30, pp 1-10, 2014.
2. M. Sajwani, A. Elsadig,E. A. Eltayeb, A. Sardar, S. A. Farook,A. Patzelt, "Sugar and Protein Profiles of Omani Honey from Muscat and Batinah Regions of Oman", International Journal of Food Properties,), pp 675-690, 2007.
3. A.S. Al-Khalifa, I.A. Al-Arify, "Physicochemical characteristics and pollen spectrum of some Saudi honeys", Food Chemistry, 67, pp 21–25, 1999.

4. Alghamdi, Elham Shasho Alshumrani, Muneerah Saad Bin Saeed, Ghufran Mustafa Rawas, Norah Talal Alharthi, Mohammed Nabih Baeshen, Nawal Mohammed Helmi, Mohammad Zubair Alam Mohd Suhail (2020)- Analysis of sugar composition and pesticides using HPLC and GC–MS techniques in honey samples collected from Saudi Arabian markets Budour. Saudi Journal of Biological Sciences 27, 3720-3726
5. Aljadi, A.M., Kamaruddin, M.Y., 2004. Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. Food Chem. 85, 513–518
6. Aljohar, H.I., Maher, H.M., Albaqami, J., Al-Mehaizie, M., Orfali, Rawan, Orfali, Razan, Alrubia, S., 2018. Physical and chemical screening of honey samples available in the Saudi market: An important aspect in the authentication process and quality assessment. Saudi Pharmaceut. J. 26, 932–942
7. AOAC, Official methods of analysis of the Association of Official Analytical Chemists. Ed. Sidney Williams, 15th Edn., Arlington, AOAC., Inc, 1990,
8. Kivimaa, Seimanb A, Palla R, Sarapuua E, Martverka K, Laosa K, "Characterization of Estonian honeys by botanical origin", Proceedings of the Estonian Academy of Sciences, 63(2),pp 183–192 ,2014.
9. O. Omafuvbe, O. O. Akanbi, " Microbiological and physico-chemical properties of some commercial Nigerian honey," African Journal of Microbiology Research, 3(12), pp. 891-896, 2009.
10. Victorita,L. Marghitas, L. Stancin, O Laslo, D. Dezmirean, O, Bobis, " High performance Liquid Chromatographic analysis of Sugars In Transylvanian Honeydew Honey", Bulletin UASVM Animal Science and Biotechnologies, 65 pp1-2, 2008.
11. Carla Egido, Javier Saurina, Sonia Sentellas, Oscar Núnez (2024)- Honey fraud detection based on sugar syrup adulterations by HPLC-UV fingerprinting and chemometrics. Food chemistry Vol 436-(13775), 1-10
12. Codex Alimentarius Commission Standard– 24th Session, July 2001, "adopting the draft revised standard for honey", Alinorm 01/25, Appendix II: 22-24, 2001.
13. Cuadros-Rodríguez, L., Ortega-Gavilan, ´ F., Martín-Torres, S., Arroyo-Cerezo, A., & Jim´enez-Carvelo, A. M. (2021). Chromatographic Fingerprinting and Food Identity/ Quality: Potentials and Challenges. Journal of Agricultural and Food Chemistry, 69 (48), 14428–14434.
14. Crane, "A book of honey". International Bee Research Association, Oxford University Press, Great Britain1980.
15. Mendes, E. BrojoProenc, "Quality evaluation of Portuguese honey", Carbohydrate Polymers, 37, pp 219-223, 1998.
16. E.T.Willams,J. Jeffrey, J.T. Barminas,I. Toma, "Studies on the effect of honey of 2 floral type (Ziziphus Spp. and Acelia Spp.) on organism associated with burn wound infections", African Journal of Pureand Applied Chemistry, 5, pp099- 101, 2009.
17. Bosch, R Mateo, "Study on electrical conductivity and pH of some types of Spanish monofloral honeys", El Campo, Bol. Inf. Agrar., 93 pp 97 -100, 1984.
18. Buba,A. Gidado, A. Shugaba,"Analysis of biochemical composition of honey samples from North East Nigeria", Biochemistry and analytical Biochemistry, 2, pp 3-10, 2013.
19. Gheldof, N., Wang, X.-H., Engeseth, N.J., 2002. Identification and quantification of antioxidant components of honeys from various floral sources. J. Agric. Food. Chem. 50, 5870–587

20. Gorska-Horczyczak, ´ E., Zalewska, M., & Wierzbicka, A. (2022). Chromatographic fingerprint application possibilities in food authentication. European Food Research and Technology, 248(4), 1163–1177.
21. Harmonised Methods of the International Honey Commission. FAM, Liebefeld, CH 3003: 45-48, 2002.
22. J. Louveaux, A. Maurizio, G. Vorwohl, "Methods of Melissopalynology", Bee World, 59, pp139 -157, 1978.
23. J. M. Álvarez-Suárez, S.Tulipani, D. Díaz, Y. Estévez, S. Romandini, F. Giampieri, "Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with colour, polyphenol content and other chemical compounds", Food and Chemical Toxicology, 48 pp2490–2499, 2010.
24. J. Wang, Q. X. Li,"Chemical composition, characterization, and differentiation of honey botanical and geographical origins",Advance Food Nutrition Research, 62, pp 89-137. 2011.
25. J.F.Cotte, H.Casabinca, S. Chardon,J.L.Lheritier, M. F. GrenierLoustalot, "Chromatographic analysis of sugars applied to characterization of monofloral honey", Analytical and Bio-analytical Chemistry, 380, pp698-705, 2004.
26. Jim´enez-Carvelo, A. M., Martín-Torres, S., Cuadros-Rodríguez, L., & Gonzalez-Casado, ´ A. (2021). Nontargeted fingerprinting approaches. In C. M. Galanakis (Ed.), Food Authentication and Traceability (pp. 163–193). Academic Press Inc.
27. L.Hayette, O.Salim, P.Schweitzer, "Physicochemical Characteristics and Pollen Spectrum of Some Algerian Honeys", Food Control, 18(1) pp 52-58, 2000.
28. Lachman, J., Orsák, M., Hejtmánková, A., Kovárˇová, E., 2010. Evaluation of antioxidant activity and total phenolics of selected Czech honeys. LWT - Food Sci. Technol. 43, 52–58.
29. M.I.Haroun,E.S.Poyrazoglu,N.Konar, N.Artik, "Phenolic acids and flavonoids profiles of some Turkish honeydew and floral honeys". Journal of FoodTechnoogy, 10, pp 39–4, 2012.
30. M.M. Cavia,M.A. Fernandez-Muino, E,Gomez-Alonso, M.J. Montes-Perez, J.F. Huidobro, M.T. Sancho, "Evolution of fructose and glucose in honey over one year: Influence of induced granulation", Food Chemistry, 78, pp157–161. 2002.
31. O. E. Agbagwa, T.V. Otokunefor, Frank-Peterside, Nnenna, "Quality assessment of Nigeria honey and manuka honey", Journal of Microbiologyand Biotechnology Research, 1 (3),pp20-31, 2011.
32. O. O. James, M. A.Mesubi,L. A. Usman, S. O. Yeye, K. O. Ajanaku, K. O. Ogunni ran, O. O. Ajani and T. O. Siyanbola"Physical characterisation of some honey samples from North-Central Nigeria", International Journal of Physical Sciences, 4(9), pp. 464-470, 2009.
33. R.J. Weston, L.K. Brocklebank, "The oligosaccharide composition of some New Zealand honeys", Food Chemistry, 64, pp33-37. 1999.
34. S. Bogdanov, T. Jurendic, R. Sieber, P. Gallmann, "Honey for nutrition and health.A review", Journal of American. Collegeof Nutrition, 27,pp 677-689, 2008.
35. The Council of the European Union, Council Directive 2001/110/EC of December 2001 relating to Honey, Official Journal of the European Communities. L10, pp 47-52, 2002.
36. V. Kaskonien, P.R. Venskutonis, V. Ceksteryt, "Carbohydrate composition and electrical conductivity of different origin honeys from Lithuania",LWTFood Science and Technology, 43, pp 801–807,2010.

37. V.Bonta, L.A. Mărghitaş,O. Bobiş, D. Dezmirean,"HPLC sugar profile in quality and authenticity determination of Romanian honey" Apiculture – From Scienceto Agribusiness and Apitherapy,Cluj-Napoca,3-5 pp 58-63, 2007.
38. Zdiniakova T., Loerchner, C., De Rudder, O., Dimitrova, T., Kaklamanos, G., Breidbach, A. Maquet, A. (2023). EU Coordinated action to deter certain fraudulent practices in the honey sector, EUR 31461 EN. Publications Office of the European Union.