

# Characterization Of Honey: Determination Of Metal, Sugar, Acid And Moisture Content Characterization Of Honey Content

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**Abstract** — In this study, five samples of honey (4 from various beekeepers and 1 from a local supermarket) were analyzed to determine the presence of Pb, Fe, Mn, Al, Cu, Cr, Zn, Cd, As, Ni and Se content using atomic absorption spectrometry. Samples were collected from the following locations: 2 from Skopje (North Macedonia), 2 from Kumanovo (North Macedonia) and 1 from Preshevo (Serbia). HPLC (High-performance liquid chromatography) was applied to determine sugars in honey samples. Acid and moisture were also determined. The AAS (Atomic absorption spectroscopy) was applied to determine metal content in honey. The average values of concentration were from 0.033 to 0.055 mg/kg, 0.610 to 2.498 mg/kg, 0.052 to 0.377 mg/kg, 2.133 to 3.474 mg/kg, 0.060 to 1.034 mg/kg, 0.005 to 0.019 mg/kg, and 0.067 to 0.085 mg/kg for Pb, Fe, Mn, Al, Cu, Cr, and Zn, respectively. Cadmium was determined in one sample (0.040 mg/kg), while the presence of arsenic, nickel and selenium were not observed. The total amount of reductive sugars was within the standard value of min 60 g/100 g honey. The moisture was from 16.6 to 17.6% (max 20%). The acid content was recorded from 9 to 28mEq/1 kg honey (max 50mEq/1 kg honey). The results were compared with the safety standards established from the Ministry of Agriculture, Forestry and Water Economy. In general, the analyzed parameters are within the standards and honey can serve as good dietary source.

**Keywords**— atomic absorption spectroscopy (AAS); honey; high-performance liquid chromatography (HPLC); sugars

## I. INTRODUCTION

Honey is a natural substance generally obtained by honeybees from flower nectar, but also from secretions of other living parts of plants [15]. In the literature, honey is considered as a tasty and sweet food and the beneficial consumption of honey is because of its nutritional value [10, 18, 27, 1]. Honeybees suck the nectar, transform, combine it with specific substances and store it in hive cells to mature where the honey is produced [4, 23, 21].

Climatic and environmental conditions where bees live and forage affect the properties of honey during the production. The composition and type of honey depends on the types of flowers that bees feed with [22]. The content of minerals in flower honey can be variable from very low 0.1–0.2% to slightly higher about 1% [15, 22].

Honey consists of various elements and chemical components such as carbohydrates with the highest percentage of honey around 82% [17]. The fructose is between 25 and 45%, glucose from 25 to 37%, maltose in the range of 2 and 12%, and sucrose from 0.5 to 3% [16, 5]. The water content is between 15 and 18% [24]. In the honey, there are also proteins and eighteen free amino acids, where the most abundant is proline [7]. From the elements, the main one is potassium followed by sodium chloride, phosphorus, magnesium, silicon, iron and copper. The composition of these elements depends on agricultural conditions and the type of the vegetation [15, 18].

On one side, the climatic and environmental factors influence to the honey properties, and on the other the conditions such as dust, soil, water, anthropogenic factors and etc have also impact to honey. Previous studies have indicated that heavy metals are present in honey and this is related with

the geographical origin and environmental conditions [15, 22, 19, 9]. The spread of metals in vegetables, fruits or flower plants directly ends up in the food chain and the polluted pollen from metal will result in polluted honey [12]. As a result of industrialized area and steelworks the production and emission of heavy metals have increased and have reflected at high concentrations of the following elements Al, Ba, Ca, Cd, Cr, Cu, Mg, Mn, Ni, Pb, Pd, or Zn [22, 2].

According to Pohl *et al.* [22] if the honeybees are fed with syrup and sugar in that case the honey will be contaminated with high concentration of Cd, Co, Fe, K, Mg, Mn, Na, and Pb.

Honey's chemical composition exhibits various healing properties [2] in the treatment of digestive, respiratory, cardiac and rheumatic disorders, for the best effect of these healing properties, honey should not be contaminated, since any contamination is a threat to human health [22].

Of great importance for the resistance of honey against fermentation and granulation is the moisture content of honey. Honey can be stored for long periods and protected from microbiological activity if moisture content is low [23, 21, 9].

The aim of the study was to evaluate the quality of some samples of honey selected from relative ecological areas in Skopje, Kumanovo and Presevo and to determine metal, sugar, moisture and acid content in the samples.

## II. MATERIALS AND METHODS

### A. Sample collection of honey

A collection of 5 samples of honey were collected and analysed. 4 samples were from the Macedonian market, from different suppliers one of which was from store in Skopje and one sample from Presevo, Serbia. All natural samples examined were unprocessed honeys of casual flower type. The honey was put in tightly closed plastic containers and stored in cool place until it was analyzed. The honey samples used in this study were appropriate for commercial purposes and human consumption.

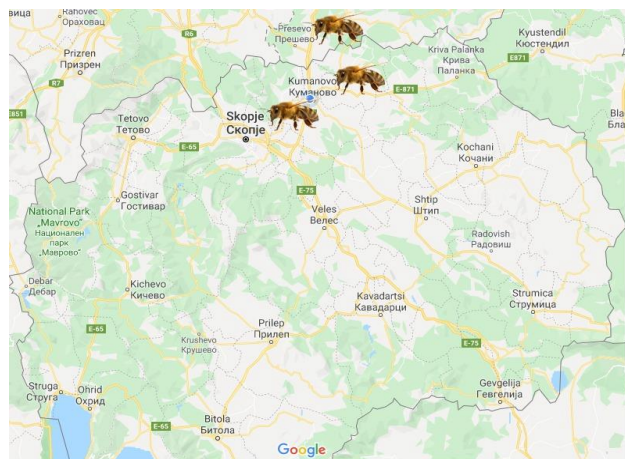


Figure 1: Map of sample locations collected in this study

### B. Reagents

All the reagents nitric acid, sodium hydroxide, and bidistilled water were with analytical grade and supplied from Merck (Germany).

### C. Apparatus

After optimization, the methods were applied for determination of moisture with digital refractometer (Zeiss, Germany), thermostated at 20 °C, regularly calibrated with distilled water. Determination of the acid content was done with a volumetric titration method; with 0.1 N NaOH and phenolphthalein as an indicator. Atomic absorption spectroscopy (AAS) measurements were performed with a Perkin-Elmer model-Analyst 600 spectrophotometer (Perkin-Elmer, CT, USA). The method was used to determine the presence of lead (Pb), iron (Fe), manganese (Mn), aluminium (Al), copper (Cu), chrome (Cr), zinc (Zn), cadmium (Cd), arsenic (As), nickel (Ni) and selenium (Se). The sample was treated with 65% HNO<sub>3</sub>. The digestion of the samples was performed in microwave Ultra Wave (Milistone, Italy). In the present work, High Performance Liquid Chromatography (HPLC) with refractive index (RI) detector with an Agilent model (USA) was used for determination of sugars. HPLC separation was performed on column (300 mm length for sugars) containing bidistilled water (0.055 μS·cm<sup>-1</sup>) as mobile phase with isocratic flow of the mobile phase. Each solution was filtered through 0.45 μm Syringe filter (Sigma-Aldrich, Germany) before chromatographic analysis.

## III. RESULTS AND DISCUSSION

In terms of quality control of honey as a dietary source, it is significant the determination of elements either described as main such as K, Na, and Ca, trace: Al, Fe, and Zn or ultra-trace elements such as Cd, Cu, Ni, and Pb. The data recorded from that type of a research can give valuable view not only for the safety consumption of honey, but also for the geographical region and botanical origin of the product [22, 13]. Atomic absorption spectrometry was applied for the determination of Pb, Cd, As, Fe, Mn, Al, Cu, Zn, Se, Cr, and Ni. The importance of analyzing the element traces especially those which are toxic such as Cd is due to the fact that the element can be achieved the nectar of the plants through the root system [3, 25]. The content of Pb, Cd, As, Fe, Mn, Al, Cu, Zn, Se, Cr, and Ni in honey samples from Skopje (2), Kumanovo (2), and from Presevo (1) is given in Table 1.

Table 1: Values of metals studied in honey (mg•kg<sup>-1</sup>)

Sample*	Pb	Cd	As	Fe	Mn	Al	Cu	Zn	Se	Cr	Ni
1	0.042	0.040	nd	1.152	0.071	2.133	0.113	0.080	nd	0.005	nd
2	0.039	nd	nd	2.498	0.171	3.474	0.348	nd	nd	0.019	nd
3	0.055	nd	nd	0.783	0.377	2.938	1.034	0.067	nd	0.014	nd
4	0.033	nd	nd	0.886	0.063	2.973	0.107	0.085	nd	nd	nd
5	0.051	nd	nd	0.610	0.052	3.406	0.060	0.083	nd	nd	nd

\*Sample: 1 (beekeeper 1 from Kumanovo), 2 (beekeeper from Skopje), 3 (beekeeper from Preshevo), 4 (beekeeper 2 from Kumanovo), 5 (commercial available honey in a supermarket market from Skopje), nd-not detectable

From these data, Al is the most abundant element (2.133–3.474 mg•kg<sup>-1</sup>) in the analyzed honey samples. Stankovska *et al.* [25] reported that the element content in honey is depended on various factors such as botanical origin which at the same time is at least affected by anthropogenic activity (Mn) or the amount is getting higher due to the anthropogenic factor (Cd). The higher values of Cd, Cu, and Pb content in honey samples indicated that the human activity in potential pollution in that area was present [22, 25]. The amount of Mn was between 0.052 and 0.377 mg•kg<sup>-1</sup>. Nevertheless the determination of certain elements such as Cu, Fe, Mn, and Zn is required for human health. Some of them in well-balanced amount are for good health, but some in higher concentration elements such as Cd, Cu, Pb, and Zn are toxic and can cause serious health problems [22, 14].

Maximum permitted levels of Pb, Cd, As, Fe, Cu and Zn in honey according to the Macedonian legislation are set at 0.5, 0.03, 0.5, 20, 1, and 10 mg•kg<sup>-1</sup>. All of the samples analyzed contain of Pb, Fe, and Zn were below the standard limits where the mean content of elements were found to be between 0.033 and 0.055 mg•kg<sup>-1</sup>, 0.610 and 2.498 mg•kg<sup>-1</sup>, and 0.067 and 0.085 mg•kg<sup>-1</sup>, respectively. The Cu content was found to be from 0.060 to 1.034 mg•kg<sup>-1</sup>, while Cr was found to be between 0.005 and 0.019 mg•kg<sup>-1</sup>. Cd and Cu were detected in samples 1 and 3, 0.040 mg•kg<sup>-1</sup> and 1.034 mg•kg<sup>-1</sup>, respectively above the allowed values 0.03 and 1 mg•kg<sup>-1</sup>, respectively. Elements As, Se, and Ni were not detected in any of the samples.

The beekeeping practices, as well as geographical and botanical diversities play important role in the differences in element content. In the comparison to other studies, Al was also found in a high amount in France [8] and also Pb content was found in higher amount of some honey samples from 0.28 to 1.02 mg•kg<sup>-1</sup>, while Cr and Ni were observed in some honey samples. Vorlová and Čelechovská [28] reported that in Czech honey the potential toxic

elements such as Pb, and Cd were found in relatively low concentration of 0.053 mg•kg<sup>-1</sup> and 3.3 mg•kg<sup>-1</sup>, respectively [7, 28].

The determination of sugar content in honey samples was done with HPLC method coupled with a refractive index (RI) detector. In Table 2 results concerning the sugar content of honey samples were given. The amount of present fructose was found to be the highest between 38.92 and 44.63% in different honey samples, glucose was found between 22.59 and 33.17%, while the sucrose content was from 2.41 to 7.81%.

Table 2: Characterization of honey samples

Samp le*	Moistur e (%)	Total amount of acids (50 mEq/1 kg honey)	Suc rose (%)	Gluco se (%)	Fruct ose (%)	Total amount of reductiv e sugars (%)
1	17.00	13	7.81	22.86	44.63	67.49
2	17.20	16	5.83	22.59	33.05	55.64
3	17.60	28	2.41	31.87	41.49	73.36
4	17.40	16	3.48	33.17	38.92	72.09
5	16.60	9	3.00	31.90	41.20	73.10

\*Sample: 1 (beekeeper 1 from Kumanovo, R.of North Macedonia), 2 (beekeeper from Skopje), 3 (beekeeper from Preshevo, Serbia), 4 (beekeeper 2 from Kumanovo, Republic of North Macedonia), 5 (commercial available honey in a supermarket from Skopje, R.of North Macedonia)



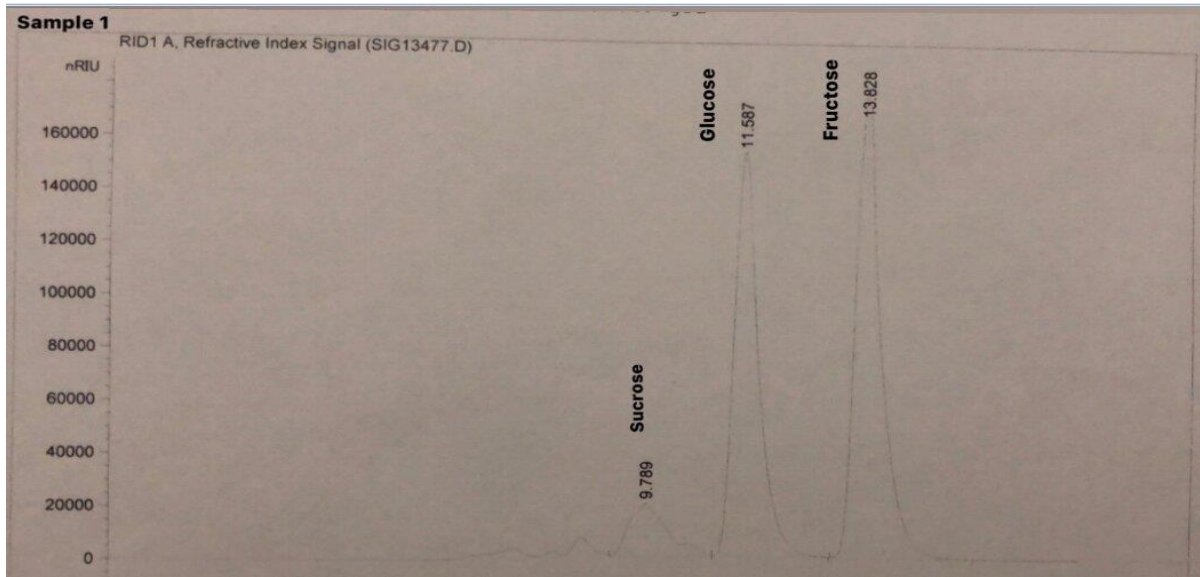


Figure 2: Chromatogram of analysed honey samples: 1 (beekeeper 1 from Kumanovo, Republic of North Macedonia)

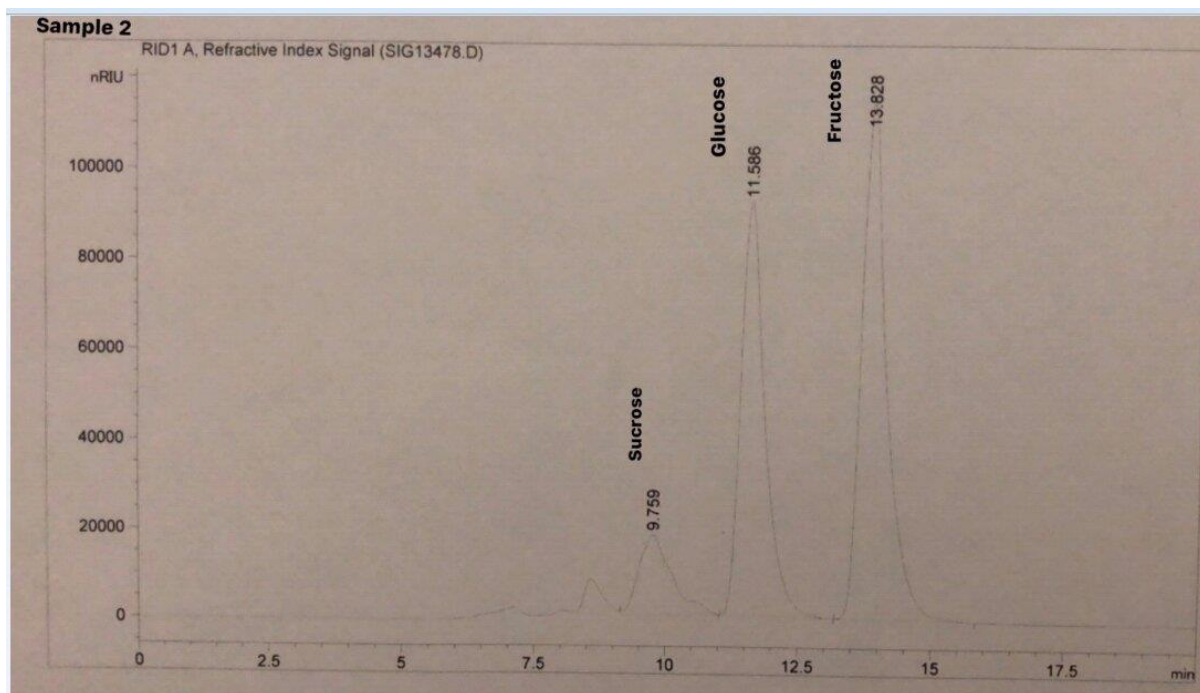


Figure 3: Chromatogram of analyzed honey samples: 2 (beekeeper from Skopje, Republic of North Macedonia)

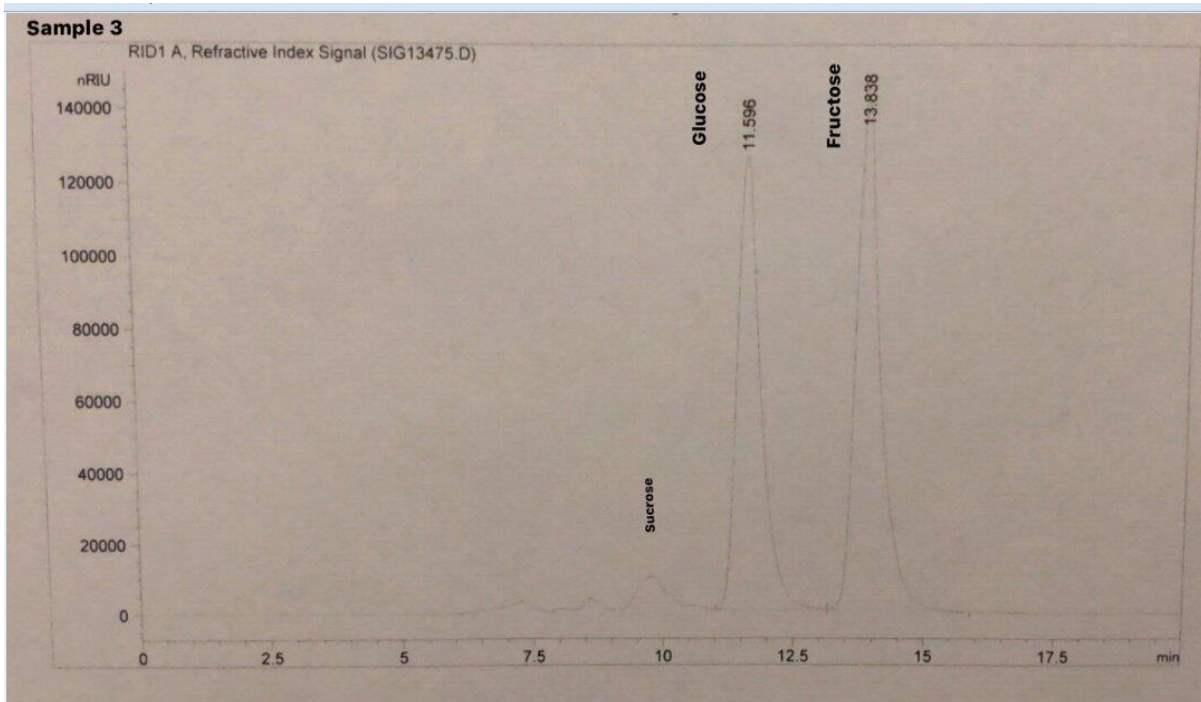


Figure 4: Chromatogram of analysed honey samples: 3 (beekeeper from Preshevo, Serbia)

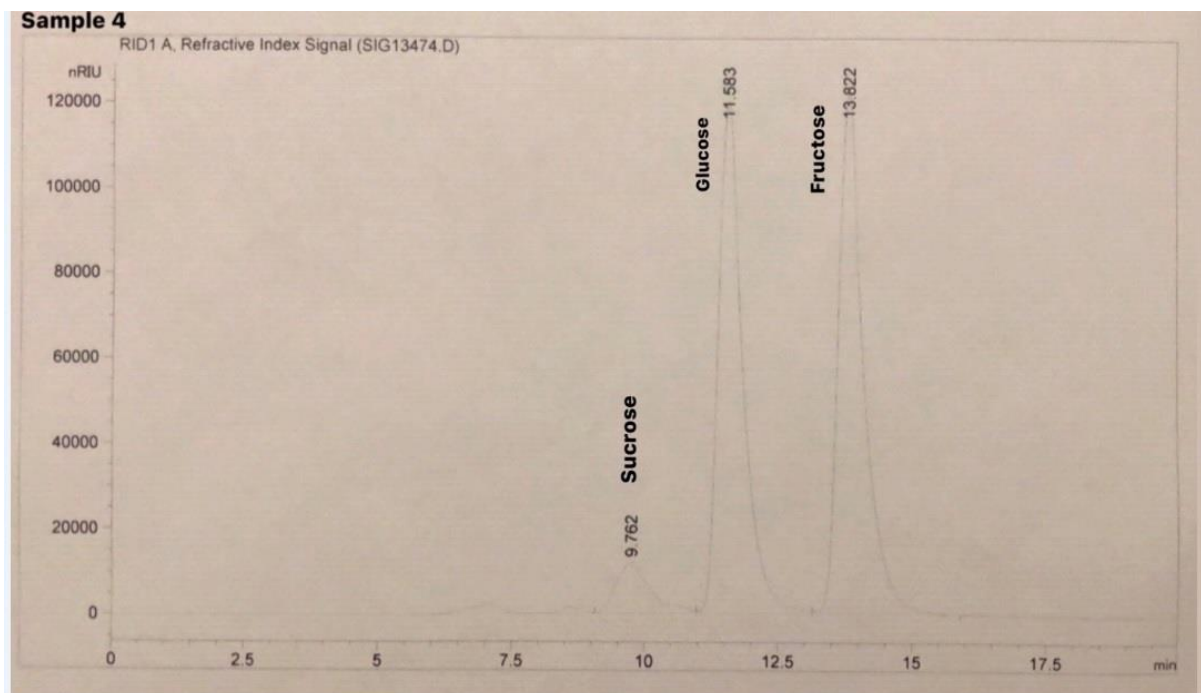


Figure 5: Chromatogram of analysed honey samples: 4 (beekeeper 2 from Kumanovo, Republic of North Macedonia)

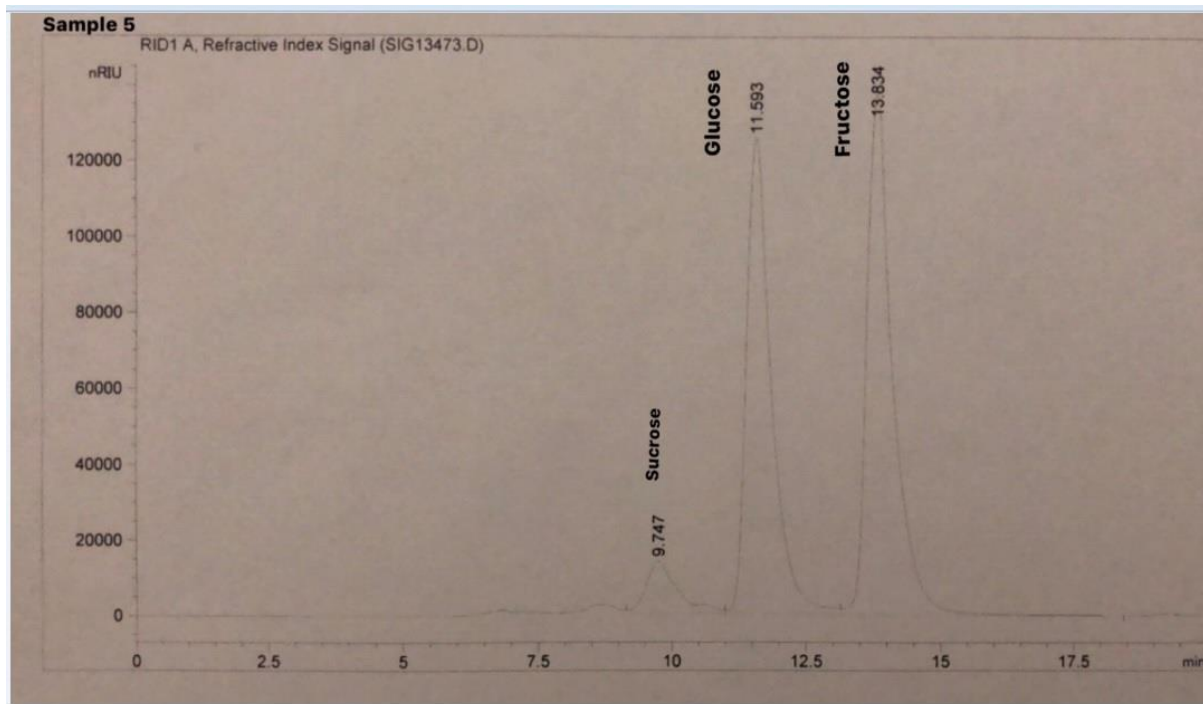


Figure 6: Chromatogram of analysed honey samples: 5 (commercial available honey in a supermarket from Skopje, Republic of North Macedonia)

The identification of honey sugars was obtained by comparison of their retention times with the standard sugars. Calibration curves for each sugar were established for quantification purposes of the analysis. The retention times for fructose, glucose, and sucrose were 13.8, 11.6, and 9.8 min, respectively.

The volumetric method was implemented in order to determine total amount of acids expressed as mEq/1kg honey. According to many authors, honey is slightly acidic as a result of the role of enzymes derived from sugars [20]. The values of total amount of acids in the analyzed honey samples were between 9 and 28 mEq/1000 g honey and the recorded values were within the max allowed values to 50 mEq/1000 g honey [26].

The acid content test can be considered as storage honey conditional test. At higher temperatures and during the time over 20 months the sugar fermentation as a result of the action of honey yeasts can be present and spoil the quality of the product [20]. The higher acid amount influences to the colour and flavour of the honey. With the storage, it was noticed that the sugar content was increased [20, 6]. This can affect on honey spoilage due to higher probability to grow yeasts in the presence of moisture [23]. This resulted in the sensory honey quality. According to the standards, the total amount of reductive sugars (total amount of glucose and fructose) has to be no less than 60 g/100 g honey. The legislative and laws are harmonized with EU directive [11].

Five different samples of honey have a satisfactory quality depended on the sugar amount. It was observed only in one honey sample (from a

beekeeper from Skopje) that the reductive sugar content was below than the prescribed value.

Based on the obtained results from the honey samples, the concentration of tested substances and parameters can varied depended on the origin of the honey, geographical location, climatic conditions, and storage conditions. It is notable to say that not all beekeeping practices were known which can affect to the final result. The chosen regions were ecological acceptable resulted in not exceeding of metals.

#### IV. CONCLUSION

In this work the content of eleven elements (Pb, Fe, Mn, Al, Cu, Cr, Zn, Cd, As, Se and Ni) was determined in 5 honey samples. On one hand moisture and the total amount of acids were determined, and on the other sucrose, glucose, and fructose content was determined. In general, the results for honey samples were acceptable compared to the standard values.

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