Original article

Adulteration of Natural Honey and the Nutritional Effect

Steluta Radu[1], Elena Cojocaru[2], Cristi Cojocaru[2], Diana Elena Lupu[1]

[1] University of Agricultural Sciences and Veterinary Medicine of Iasi; [2] University of Medicine and Pharmacology from Iasi

Abstract. Council Directive 2001/110 / EC (1) defines honey as the sweet natural substance produced by *Apis mellifera* bees. Honey contains especially different types of sugars, especially fructose and glucose, as well as other substances, such as organic acids, enzymes and solid particles resulting from honey collection. (1). It is an aqueous solution rich in sugary substances up to 80% represented mainly by glucose and fructose that come from the floral nectar, extrafloral, manna and other sources, collected by bees and stored in honeycombs. The enzymes, which the bees introduce into the nectar, have the ability to split sucrose, maltose, melioidoses, raffinose, melibioses', this process lasting for many years. The transformation of the nectar by the bees into honey is then accompanied by the exchange and replacement of the content of useless acids, with the release of the surplus water. The pH value of the matured shoulder varies from 3,5-5.5, depending on its floristic origin. The bees depend exclusively on the plant world for their nutrition and, therefore, throughout the active season, they make sustained efforts to provide food, both for immediate needs and for reserves (2). Typically, the bees feed on nectar, honey, pollen, water and for the use of larvae and quail feeds, in addition, milkweed is used. Except for the water, the honey and pollen are stored as reserves. Artificially, a wide range of food can be used in bee feed: the sugar syrup and sherbet, the cough sugar, the powdered sugar mixed with yeast, the powdered milk, delipidated soybean meal, corn pollen, various cereal flours, medicines as well as herbal supplements. From nectar, manna or sweet juices, which are found in the different parts of plants and trees, in combination with some substances that are born in the salivary glands of bees, honey is obtained, which the bees deposit in the honeycomb cells. Subsequently, the honey is stored in the honeycomb cells and left in the air for a while, through evaporated water. Finally, these cells are tightly sealed with some wax caps (3). In this research we performed experiments on medium samples of acacia, linden, coriander, sage, manna, polyflower honey (average tests of honey from Nord East Region of Romania) and we detected minor inaccuracies related to the level of dextrin in sage honey, as well as a low level of sucrose in sage honey and acacia honey.

Key words: natural honey and adulterations

Introduction

The man is twice indebted to the bee for exploiting the fields and forests of the globe, in search of their sweetness for producing honey with its great variety of temptations for our senses and for the increased harvests that are obtained as a result of pollination, from pure chance of flowers that the collector visits. For us honey means a delicious variety, desired on merit, in our food. She is a stolen candy twice (and the stolen sweets are said to be the best) - bee-eaten, flowered (and from the owner on whose land she grows), and then stolen from bees by those who they grow them.

Honey, as found in the hive, is a special product. By the bees' processing of the natural sugar solutions known as nectar, they are transformed from a slightly alterable, thin, and sweet liquid into a stable, dense food, with high energy potential. By inverting

the sucrose in the nectar, the bee increases the density of the liquid until it reaches the required value of the final product, thus increasing the efficiency of this process in terms of its caloric potential. At the same time, the resistance of the product to microorganisms is increased, by increasing the osmotic pressure. However, honey has an increasing tendency to absorb moisture from the atmosphere, which has as a consequence the possibility of fermentation if the yeasts reach the required level. Although we produce a significant quantity of honey, we are not big consumers of this product. Honey consumption in our country is 500-600 g per inhabitant/year, three times lower than the European average. Honey is one of the healthiest foods, its benefits being doubled by the sweet taste and the many ways it can be used (4).

Materials and Methods

According to the species of honey plants from which the bees gathered the nectar: mono-flower honey, wholly sourced, (or largely), from the nectar of flowers of one species: (acacia, lime, sunflower, peppermint), poly-flowers honey, derived from processing a mixture of nectar from the flowers of several species of plants. There are several types of honey according to how it is obtained: in honeycombs (it is delivered in honeycombs), free flow of honeycombs, extracted with the help of centrifuge, obtained by pressing honeycombs, melted (honeycombs are heated). After the consistency honey can be liquid (fluid), crystallized (sugary); by colour it can be: colourless, light yellow, golden, greenish, brown, reddish. After the aroma, the various kinds of honey, are appreciated by smell and tasting, indicating the name of the species of plants from which they come. The classification may also take into account: chemical composition, purity, caloric power (5). 2.1. Organoleptic analysis of honey. Sensory analysis, as a scientific method of assessing the organoleptic properties of foods, has an important role in establishing the authenticity of the products, being used especially for comparison with the reference products, in classification and standardization, as well as in detecting the freshness, defects and other shortcoming. Although the sensory analysis is still dependent on the human appreciation, having a certain degree of subjectivism, due to the professionalization of the body of tasters and statistical interpretation, it is a useful tool, and in some case it becomes irreplaceable in the assessment of quality (6).The fluid honey is examined organoleptic, initially on the sample as such. It is noted if it has foam and / or impurities. The honey is homogenized using a glass rod for uniform dispersion of impurities throughout the mass. Then, the honey is filtered through a double gauze at first use, homogenized and left to stand to remove the embedded air, until complete clarification, after which it is subjected to the complete organoleptic examination (appearance, consistency, colour, odour and taste).

The crystallized honey is examined organoleptic, initially on the sample as such. It is noted if it has foam and/or impurities, the type of crystallization (incipient, partial or total) and the characteristics of the crystals (fine, suitable, coarse). The tightly closed honey jar is subjected to fluidization by heating at 40-45°C, until the crystals are completely melted. After cooling the lid is removed, it is well homogenized using a glass rod for uniform dispersion of impurities throughout the mass (7). The appearance is appreciated by the degree of transparency presented by the honey introduced in a colourless glass tube, with a diameter of 16 mm, examined in the direct natural light. The different shades are noted in detail, such as: transparent, bright, opalescent, turbid. The consistency is assessed by the way the honey flows from a glass rod or a wooden tray, specifying the respective state: aqueous, fluid-thin, fluid-viscous, sticky.

The colour of the honey is appreciated by direct visual examination, in the daylight, on a white background, introduced in a colourless glass tube, with a diameter of 16 mm.

The quality characteristics are appreciated by the smell and taste of honey samples, too. It is noted the dominant aroma (for poly flower honey) and its intensity (pronounced, well-emphasized, moderate, discrete). Also, one appreciates the intensity of the sweet taste (pronounced, well-emphasized, moderate) and the possible secondary nuances (sour, bitter, astringent or tasteless).Honey has a number of specific sensory characteristics: no foam appearance, no visible foreign bodies, colour from slightly colourless to light yellow,

golden yellow, yellow-orange, yellow-dark, ruby, brown, brown-dark, odour and honey-specific taste, with less or more pronounced aroma, sweet taste, homogeneous, fluid, viscous, crystallized consistency (8).

Physical and chemical analysis

Chemical analyses of honey indicate that its composition includes glucose, fructose, sucrose, dextrin, water, albumin, nitrogenous substances, organic acids, mineral substances, vitamins, etc. The composition of honey varies being obtained from different types of nectar, from several honey plants, which is not the same and depends on the origin, representing the maturation and seasonal conditions (9). In France it is considered that normal honey is the one with the following chemical composition: water 17.20%; sucrose 0.40%; fructose 39.10%; glucose 34.45%; protein 1.80%; acids 1.10%; mineral substances 0.75%; wax 0.90%. The Romanian researchers P. Gabriel and P. Nicolae (1965) have researched many samples from different types of nectar honey and found that for fructose this percentage is 39.17% and for glucose 36.48%. The increased amount of glucose gives suspected falsification with industrial glucose (10).

Sample Preparation

Before carrying out the laboratory determinations, the honey is well homogenized and the crystallized one is heated to 40°C in a water bath and then homogenized. The amount of water in the honey should not exceed 20%. Otherwise, the honey begins to ferment, it forms a white foam on top, and its contents have a nice appearance due to the release of carbon dioxide.

Determination of Acidity

The acidity is determined by direct titration of the honey solution with NaOH 0,1n solution in the presence of phenolphthalein. The regulated values are max.4 for honey flowers and max. 5 for the manual one. The acidity may exceed these values in case of very advanced fermentative alteration or in the case of falsification with artificially inverted sugar syrup.

Determination of Hydroxymethyl Furfural (HMF)

HMF forms a red coloured compound with barbituric acid in the presence of p-toluidine. The intensity of the colour depends on the concentration of HMF (11). HMF forms with barbituric acid in the presence of p-toluidine a red coloured compound. The intensity of the colour depends on the concentration of HMF.

Reagents. Barbituric acid, 0.5% aqueous solution: 0.5 g barbituric acid is dissolved with about 70 ml hot water and after cooling it is brought to 100 ml in a graduated flask; P-toluidine, 10% solution in isopropanol: 10 g of p-toluidine is dissolved with approximately 50ml isopropanol. Add 10 ml glacial acetic acid and make up to the mark with isopropanol. The solution is kept in the dark and can be used after 24 hours after preparation.

Equipment. Spectrophotometer adjusted to the wavelength of 550 nm and the vats with a thickness of 1 cm.

Procedure. It dissolves 10 g of honey with distilled water and add quantitatively to a 50 ml graduated flask. Take two tubes where 2 cm^3 of the prepared solution and 5 cm^3 of p-toluidine are pipetted. In the control tube, 1 ml of distilled water is pipetted and in the other tube (sample) 1 ml of barbituric acid is pipetted. The tubes are homogenized, and the absorbance is read at the wavelength of 550 nm using vats with a thickness of 1 cm.

Calculation of results. HMF = I/O × 19.2 [mg per 100 g honey; E- the value of the absorbent; The thickness of the layer; 19.2 is the conversion factor.

Determination of the Diastatic Index

In natural honey bees there are several enzymes. Amylase is the enzyme with the highest resistance to heat treatment, the last one being destroyed. Based on this characteristic, amylase can be used as a general test of appreciation (enzymatic or diastatic index) of the natural honey quality. Natural honey subjected to a brutal heat treatment will have a diastatic index with low or even zero values. The same is true of counterfeit honey. At the base of the determination of the diastatic index is the determination of the activity of the amylase. The diastatic index is defined as "the number ml of starch solution (1%) which was converted to dextrin

for one hour at 45°C and optimum pH by the amylase containing of 1 g honey (10).

Determination of the colour indicator

The colour index is determined using the special colorimeter for honey and is expressed in ml on the Pfund scale. According to the standard, each assortment of honey has a maximum limit.

Equipment. Pfund-Colour Grander.

Procedure. The tank of the appliance is filled with honey to be analysed, left to rest for the removal of the included air and for a complete clearing. It turns on the device, it inserts a glycerol tank for calibration and then read the value of the sample tank.

Identification of Industrial Glucose by Reaction with Alcohol

The identification of glucose is evidenced by precipitation with alcohol. The reaction takes place with the help of tannin, which precipitates the proteins by the appearance of a sediment after a prolonged rest.

Reagents. 10% tannin solution, obtained by dissolving 1 g tannic acid in 10 ml distilled water; concentrated hydrochloric acid (HCL) 35%; ethyl alcohol (C2H5OH) 95% vol.

Procedure. In a plastic test tube by 50 ml, it weighed 5 g of honey and it added 10 ml of distilled water. The plastic tube was stirred for dissolving honey, so that a homogeneous solution was obtained. To the solution obtaining it added 1 ml 10% tannin solution and boil for 15 min. After cooling, the solution is filtered to remove precipitated proteins. It takes 2 ml of the substance and place in a clean tube over which added 2 drops of concentrated hydrochloric acid and 20 ml of ethyl alcohol. If a turbid solution of different intensities was obtained as colour (milky) honey is suspected of falsification, respectively it contained substances from the range of sugary products. If a perfectly transparent and clear solution is obtained, then it turns out that honey did not contain substances from the range of sugary products.

Results

Humidity dynamics in the average samples studied of acacia honey, linden honey, coriander honey, sage honey, manna honey, polyflowers honey a humidity up to the admissible limit with an average mass fraction of 17.88%. preservation of honey and prevention of its crystallization.

From a statistical point of view, the results obtained are in line with the trend of superior performance, obtaining a superior positive correlation (Figure 4).

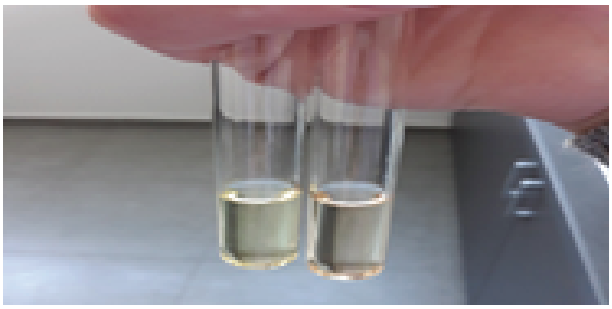

Figure 1. HMF within the legally allowed limit

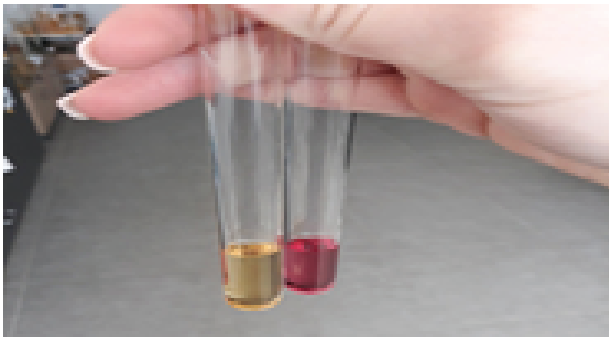

Figure 2.HMF that exceeds the legally allowed limit

Figure 3. Identification of industrial glucose

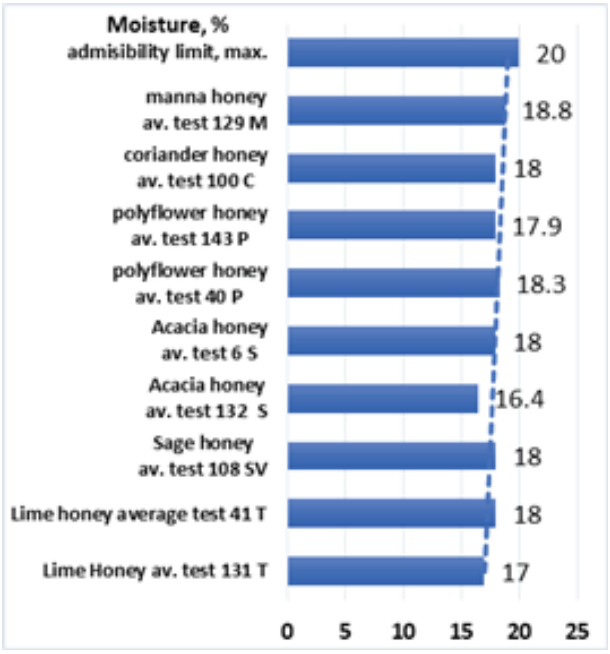

Figure4. Dynamics of moisture in honey versus the maximum allowable level accepted

However, there are also situations in which the samples that performed better in the first determination, obtain a lower performance in the second determination, without the pairs of determinations aligning perfectly. They may tend to group around a straight line when we have a higher negative correlation (Figure 5).

Aqueous honey is a defect that results either from uncoated honeycombs or through improper storage. To avoid this situation and to remedy the problem, the producers must quickly ensure the recovery or reconditioning, by mixing with a low humidity, because the water product should not exceed 20%. We will refer to crystallized honey. This seems to be a relative defect, as it does not totally affect, but only some of the product's features. We refer to the next characteristics: colour, consistency, viscosity, etc. Depending on the intensity of the phenomenon, the following can be encountered: beginning of crystallization, partial crystallization, mass, and bulk crystallization, with the separation of a liquid part. After storage time any honey crystallizes. The most resistant to crystallization were found to be acacia honey and forest (dew) honey. The fastest crystallization occurs in rapeseed honey, sunflower honey, and linden honey, which can sometimes crystallize even in honeycombs if extraction is delayed.

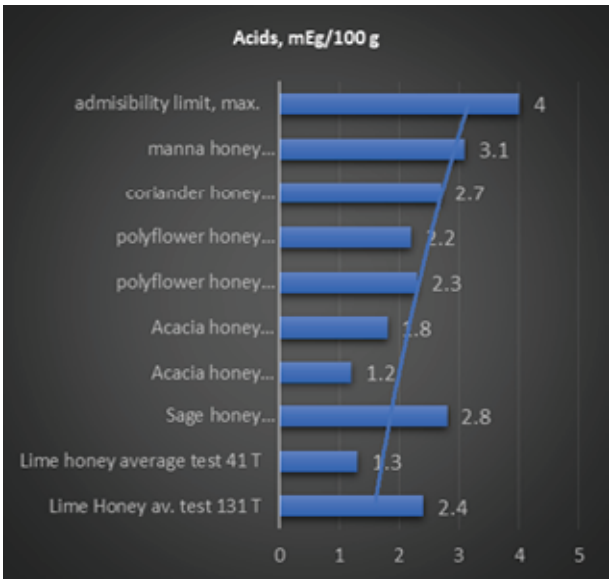

Figure 5. Dynamics of acidity in honeybees to the maximum accepted level

Processing bees change not only the nectar's sugars, but also its acids. Thus, nectar acids transformed into polyflower honey at a concentration of hydrogen ions PH of 3.48-4.8. Instead, PH ul of hand honey is 5.9-6.9, so less acidic. All varieties of honey have an acid reaction, at most the acidity can be in the neutral range. Honey acids are organic in nature from floral nectar and fermentative enzymatic processes. Due to its citric and malic acid content, polyflowers honey is superior to manna honey. Honey acids which allow its long-term preservation, preventing the action of bacteria are the following: succinic, tartaric, lactic, formic, propionic, butyric, valerian, caproic acid, as well as pantothenic acid in a proportion of 0.5-9% which has an effect on pathogens (Figure 5). This is an important advantage for the human health. Invertase and amylase are the enzymes that contribute essentially to the formation of honey from the sweet substances harvested by bees, by enzymatic hydrolysis, transforming polysaccharides into monosaccharides-glucose and fructose. There are situations in which the samples analysed for the sucrose, invert sugar levels performed better in the first determination and a lower performance in the second determination, without the pairs of determinations aligning perfectly. In this case the tendency is to group around a straight line, obtaining a higher negative correlation (Figure 6).

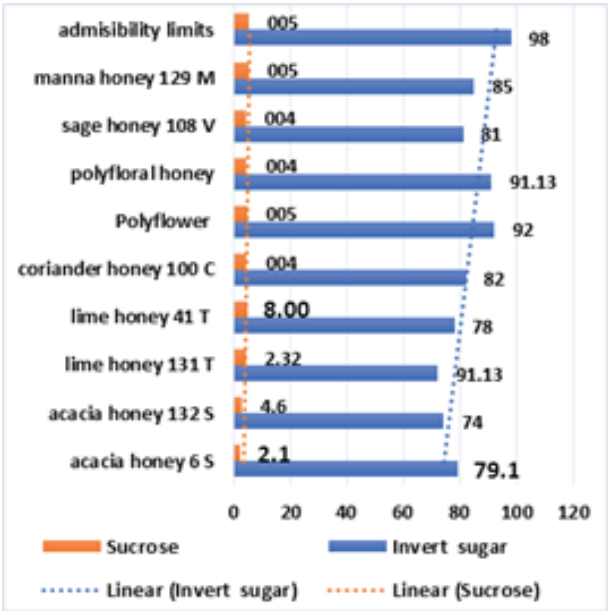

Figure 6. Dynamics of sucrose, invert sugar versus the maximum level of admissibility

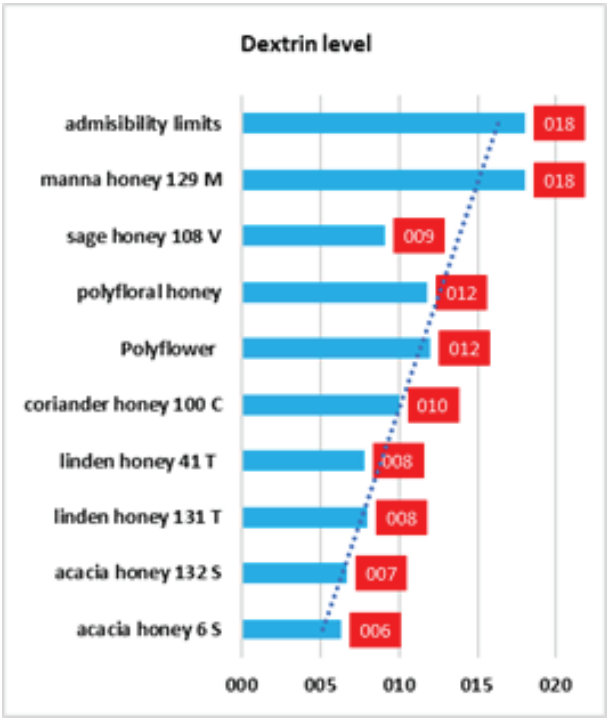

Figure 7. Level dextrin of honey versus the maximum level of admissibility

Adulteration with sugar syrup-signs: the existence of thickening substances, the supersaturated solution gives a rapid crystallization; after cooling, two phases are observed: one liquid, one above, one more consistent at the bottom of the vessel; the crystals are whitish and have a strong consistency; if the substitution is complete, the colour of the honey is very light; if the caramel is used, it is detected by specific chemical analysis; amylase is missing; increase of water content over 20%; hydroxymethyl furfural is missing. Adulterations with artificially inverted sugar syrup are detected when: honey is extremely liquid, the colour is orange-brown-reddish, the specific aroma is missing, the taste may be caramel, the sucrose content increases (12).

Figure 6 shows that the average samples of acacia honey, linden, coriander, polyflower, sage, manna, were not falsified with sugar syrup or invert sugar syrup, because from the research carried out the sucrose level does not exceed the maximum allowable limit. Adulteration with corn syrup or other enzymatic hydrolysed of starch was indicated by the presence of dextrin. The starch in contact with iodine forms a blue colour that is highlighted only in cold. In the hot solution, the blue colour never appears, even if the starch is present in a large quantity (Figure 7). Dextrin's level has not exceeded the admissibility limits, and this is very

Table 1. Content and limits of physico-chemical indices in average honey samples

Indices	*Admisibility limits*	*X±Sx*	*V, %*	*Limit(min.-max.)*
Quantity of honey	-	23045, 5 Kg	-	50-20800
Moisture, %	Max.20	17,83±0,233	8,11	16,4-18,8
Mass fraction of invert sugar, %	Min.70	83,71±1,149	7,67	74-92
Mass fraction of sucrose, %	Max.5	4,28±0,209	54,66	2,1-4,78
Diastasic index, un Gote	Min. 8	17,63±0,771	27,17	16,1-19
Acidity, cm^3 NaOH sol. Meg/100 g honey	Max.4	2,2±0,072	24,07	1,2-3,1

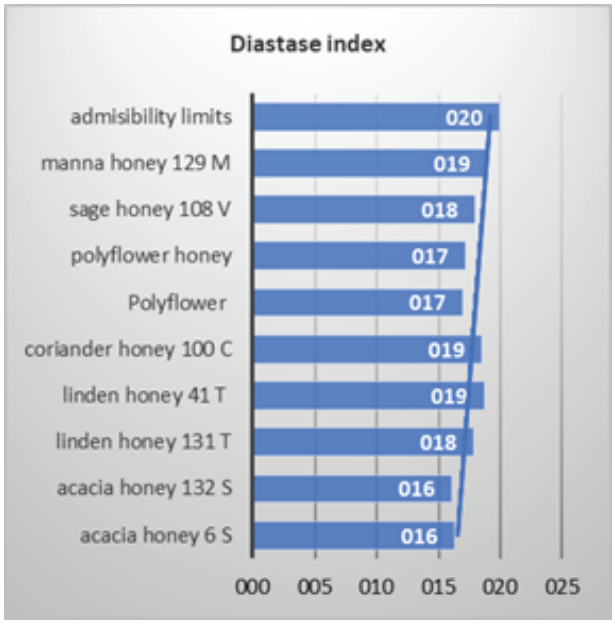

Figure 8. Dynamics of diastase index in the polyflower, acacia, linden, coriander, sage, manna honey

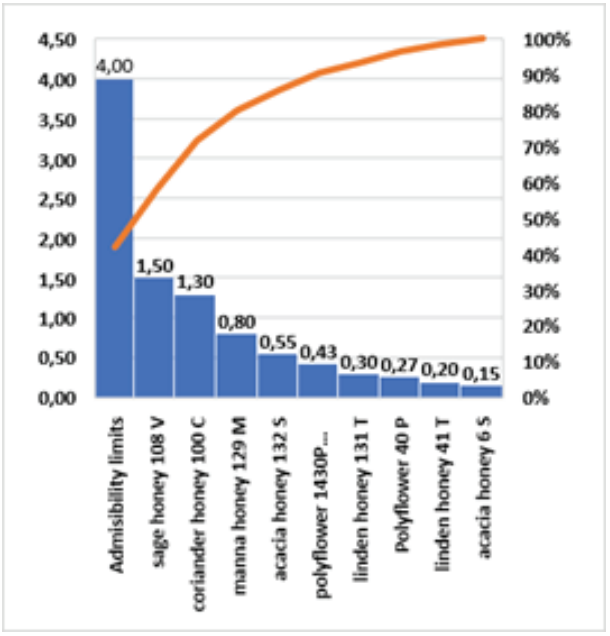

Figure 9. hidroxymethilfurfural, mg/kg in the polyflower, acacia, linden, coriander, sage, manna honey

important for the scientific nutrition because cannot influence the hydrolysed of starch into sugars.

In the dextrin's level, a higher performance was achieved at the first determination and a lower performance at the determination of the sage honey sample, so the pairs of determinations do not align perfectly. In this case the tendency is to group around a straight line, obtaining a higher negative correlation (Figure 7).

Diastase is the invertase secreted by bees that metabolizes other sugars such as trehalose, raffinose, melioidosis (Figure 8). There are situations in which the samples analysed for the diastase index performed better in the first determination and a lower performance in the second determination, without the pairs of determinations aligning perfectly. In this case the tendency is to group around a straight line, obtaining a higher negative correlation (figure 8). Adulterations with artificially inverted sugar syrup generate the high HMF content (10-100mg) (Figure 9).

Caramelized honey is a defect that results from reconditioning by heating at high temperatures. In this case, problems can be detected both in colour and taste, by inactivation of enzymes and formation of hydroxymethyl furfural (HMF).

This type of honey is used at a lower quality, corresponding to the degree of depreciation. The Pareto diagram graphically represents the distribution of data in descending order of frequency with a cumulative line on a secondary axis, as a percentage of the total (figure 9).

Conclusions

1. It was revealed that the mass fraction of water in the average samples of honey investigated is 17.88%, invert sugar is 79.96%, sucrose is 4.055%, diastase index 17.63%, hydroxyl methyl furfural HMF content -0.611 mg / kg, total acidity of 2.2 mEg / 100 g honey, and the ash - 0.258%.

2.The research showed that the average samples of acacia honey and lime honey were not falsified with sugar syrup because the water level did not exceed the permissible limit, the falsification with invert sugar syrup was not detected and the sucrose level did not rise above the standard, did not exceed the permissible limit (Figure 7).

3. The falsification of honey with corn syrup can be detected according to the dextrin level, determined

experimentally, having an admissible limit of 18.03, the level of uncertainty being higher for manna honey.

4. The samples of natural honey not falsified with sugar, invert sugar, corn syrup, have a small fraction of sucrose, which constitutes an important nutritive effect for population health.

Acknowledgements: Experiments were carried out by SC Apis Rom Ltd and the University of Agricultural Sciences and Veterinary Medicine of Iasi.

References

1. Council directive 2001/110 / EC of 20 December 2001;
2. Daniela Guaiti, Honey, properties and uses, Health from bees on honey;, Home Publishing House); 2013;
3. Marin M.,Popa AL., Popescu N., Sutianu A., The dietary, therapeutic and dietary value of bee products, Agro-Silvic Publishing House; 1966;
4. Eva Craine, Honey a comprehensive study, Apimondia Publishing House, London, 1979;
5. STAS 784/1- Honey bees Technical quality conditions when taken from beekeepers, 2009;
6. STAS 784 / 3-2009 Honey bees Methods of analysis, 2009;
7. STAS 784 / 3-1989 Honey bees Methods of analysis. 1989;
8. Bondoc I., Șindilar E., Veterinary health control of food quality and sanitation, Publishing House "Ion Ionescu de la Brad", Iași, 2002;
9. Order 522, annex II: Characteristics and composition of honey;
10. Popescu N., Meica S., The bee products and their chemical analysis, Diacon Coresi Publishing House, Bucharest, 1997;
11. Gonnet M. Composition, properties and conservation of honey.B.T.A., 1974, 3/74, 43-45;
12. .BonvehI I.,Serra J.,Pajuelo G., Determination de la falsification possible du miel avec des produits sucrants.B.T.A., 1984, 11, (4), 49, 195-02;

Correspondence
Steluta Radu
University of Agricultural Sciences and
Veterinary Medicine of Iasi
Email: stelaradu2010@yahoo.com