

# Determination of crystallization tendency and level of HMF in Albanian honey products

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**Abstract**— Some attention will be given to the technologically important subject of honey crystallization, which is the precipitation of glucose from honey. The major problem resulting from glucose crystallization that the resulting increased moisture content of the liquid phase allows yeast cells, which occurs naturally in honey to multiply and fermentation to proceed. The major components of floral honey are fructose, glucose and water, averaging 38.2, 31.3, and 17.2%. Glucose and fructose are the only monosaccharides in honey and it is these sugars, combined in various forms that comprise the di- and trisaccharides fractions of floral honey. Several of these occur only rarely in nature and the trisaccharide erlose, produced by the action of honey-bee invertase on sucrose, was first discovered as a component of honey. Honeydew honey is produced by the honeybee from honeydew deposits left by various hemipterous insects on their host plant. Honeydew contains a more complex mixture of sugars than does nectar and honeydew honey is appreciably higher in reducing disaccharides and higher sugars than is floral honey. The trisaccharide melitose, not found in floral honey, is often present in levels exceeding 10% in honeydew honey. The precipitation of glucose from honey, termed granulation, is often technologically undesirable as it is sometimes followed by fermentation. Indices such as the glucose/water ratio have been used to predict granulation tendency. Small amounts of hydroxymethylfurfural (HMF) occur naturally in honey, resulting from the acid catalysed dehydration of hexoses, particularly fructose. High levels of HMF suggest adulteration of honey with acid inverted syrup and several methods are available for its determination. The conversion of nectar and honeydew to the complex array of honey sugars by the honeybee involves a variety of chemical and biochemical processes.

**Index Terms**— crystallisation, glucose, monosaccharides, disaccharides, trisaccharide melitose, glucose-water ratio, hydroxymethylfurfural, insoluble material

## 1 INTRODUCTION

Ripening is accomplished by the house bees alternately expelling and ingesting the honey sac fluid repeatedly for 15-20 min, mixing it with additional enzyme secretions and reducing its water content. When the honey is properly ripe, with nectar sucrose inverted and concentrated to about 82% solids, the comb cells are capped over by the bees and the honey stored as food. Floral honey is a mixture of glucose and fructose, with lesser amount of sucrose, maltose and an ill defined material termed "dextrin", that include a mixture of at least 22 di-, tri- and higher oligosaccharides.

Method for honey carbohydrate determination has been High Liquid Chromatography (HPLC), because both separation and detection of carbohydrates was possible with one fairly rapid analysis. Three main separation systems have been used for HPLC of honey carbohydrates. The stationary phase of these three systems are aminopropyl-bonded silica, as stationary phase, cation exchange resins and anion exchange resins. The separation system based on weak acidity of sugar hydroxyl groups and used an anion exchange system. The oligosaccharides in honey could be informative of honey composition and amount of oligosaccharides in honey is of importance because of the possibility of adulteration with a variety of less expensive sweeteners, such as corn syrups which have sugar compositions very similar to honey.

Hydroxymethylfurfural (HMF) formation results from the acid catalysed dehydration of hexose sugars with fructose being particularly susceptible to this reaction. Small amounts (0.06-0.20 mg/g) are present in fresh honey and storage result in the formation of increased amounts of HMF. They showed that in natural honey stored for one year at 25°C, the HMF level often reaches 3.0 mg/100 g honey and if heated to 60°C this level can be reached in less than 3 days. Although heat treatment of honey is essential at various stages of honey processing to prevent granulation and fermentation, it is apparent that the temperature and period of heating must be controlled, as an excessive amount of HMF is considered in many countries as evidence of overheating and a loss of freshness of the honey.

High levels of HMF also suggest the possibility that natural honey has been adulterated with invert syrup, prepared from sucrose by acid hydrolysis. Acid-invert syrup invariably contains high amounts of HMF. Prolonged storage or overheating of honey can result in an HMF level exceeding 3.0 mg/100 g and may rise to 10.0 mg/100 g or more. A content of 15.0 mg/100 g or more is taken to indicate adulteration with acid-inverted invert sugar.

## 2 MATERIAL AND METHODS

Were examined the quality of 21 honey samples representing different areas of South Albania. Method used for honey carbohydrate determination has been High Liquid Chromatography (HPLC), because both separation and detection of carbohydrates was possible with one fairly rapid analysis. Three main separation systems have been used for HPLC

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of honey carbohydrates, with 10µm Carbo Pac PA1 pellicular anion exchange columns, 250 x 4 mm columns; pulsed amperometric detection, solvent 100 mM sodium hydroxide for 4 min then linear gradient to 100 mM sodium hydroxide and 3 mM sodium acetate at 50 min and hold. Also 300 mM sodium hydroxide post-column injection at 0.8 mL/min, 85°C. 100 µL injection of 10% honey solution which first deionized and treated on a charcoal-celite column to remove most of the monosaccharides and flow rate 0.7 mL/min. The oligosaccharides in honey could be informative of honey composition and amount of oligosaccharides in honey is of importance because of the possibility of adulteration with a variety of less expensive sweeteners, such as corn syrups which have sugar compositions very similar to honey.

Glucose oxidase is also a very important enzyme added to honey by bees. The product of this enzyme are responsible for a great deal of the antimicrobial activity of honey. Hydrogen peroxide, one product of this enzyme has been identified as the inhibine compound in honey. The most common honey analysis involving this enzyme is a determination of the other enzymatic product δ-p-gluconolactone and its chemical hydrolysis product, gluconic acid represented in Table 1. The acidity and resulting low pH developed by the hydrolysis product also contributes to the well-established antimicrobial properties of honey.

Proline is one of the free amino acids in the greatest amount in natural honey. It is an osmoregulator when the bees add enzymes to nectar, counterbalancing the high osmotic pressure of nectar. Honey analysis for proline has been developed into a relatively simple test using ninhydrin to form color.

### 3 RESULTS AND DISCUSSIONS

Moisture analysis of honey is one of the most important measurements because honey is hygroscopic containing much more than 20-21% moisture it is likely to be fermented by ubiquitous yeast. The analysis of moisture in honey samples is carried out using a simple refractometer. These results have been showed at table 1.

Honey carbohydrate analysis involved reducing sugars showed that samples were with variation honey with floral source, age, production area and crop year and would be of great value to Albanian honey producers.

Method used for honey carbohydrate determination has been High Liquid Chromatography (HPLC), because both separation and detection of carbohydrates was possible with one fairly rapid analysis and these results are presented at Table 1. These values shows granulating tendency during the storage of same honey samples with high values of reducing sugars more 70% as Berat, and Korca. In three of four honey samples analysed, fructose content was reported to be greater than 40% and in one case the glucose content was 40.6%. These values are compiled in Table 1 and Table 2.

Ash is generally very low in honey, honey higher in ash than 0.6% can be detrimental to over wintering bees because bees eliminate the ash by excretion which must be performed outside the hive. High ash in honey can be an indication of honey adulteration with acid hydrolysis carbohydrates, which

are later neutralized. In our samples was more than 1.2% in honey samples of some farmers see Table 1 and figure 1.

Color can vary a great deal in honey from different floral sources, but commercial honey are quite light in color. It is a very important quality indicator for honey because any heating of honey leads to nonenzymatic browning. Our honey samples colors were water white, extra white, white, extra light amber, light amber, amber see Table 2 and figure 2.

The stable hydroxymethylfurfural (HMF) builds up on heating and high levels >40 mg/kg of HMF indicate abusive heating of honey or addition of an acid hydrolyzed sucrose or cornstarch product. Same of these samples as see in table 2, were more than 15.0 mg/100 g or more is taken to indicate adulteration with acid-inverted invert sugar.

Table 1.  
Determination of honey carbohydrates, ash and unsoluble material of 21 honey samples representing different areas of South Albania.

Districts Albania	Moisture [%]	Saharosa [%] Invert sugar	Glucose [%]	Fructose [%]	Ash [%]	Unsolub. Material[%]
Vlora	0.185	0.0223	0.702	35.1	0.06	0.08
Korca	14.3	0.73	62.3	31.15	1.1	0.02
Erseka	18	0.54	65.1	32.6	0.9	0.03
Pogradeci	0.172	0.0064	0.71	35.5	0.06	0.08
Gjirokastra	19.2	0.3	75	37.5	4.3	0.07
Skrapari	16	12.64	78	39	2.5	0.05
Delvina	17.3	1.87	62	31	3.1	0.04
Berat	21	3.4	65	32.5	2.7	0.08
Saranda	15.2	1.2	60	30	2.1	0.05
Tirana	17	1.7	64.1	32.05	3.2	0.07
Shkodra	0.2	0.024	61.17	30.58	0.05	0.07

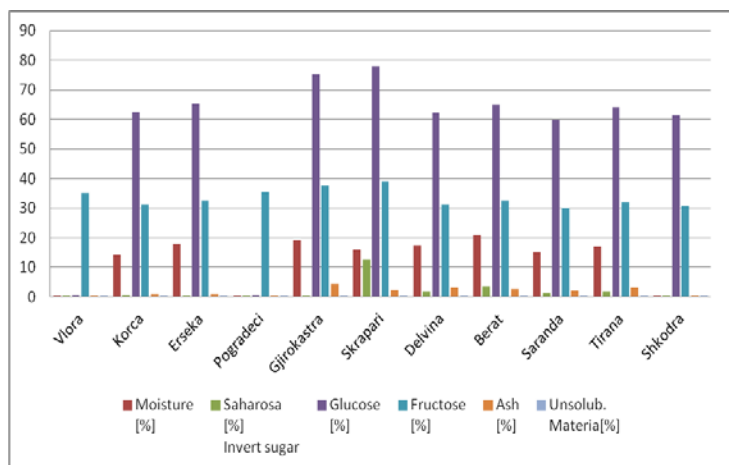


Fig. 1. Determination of honey carbohydrates, ash and unsoluble material of 21 honey samples representing different areas of South Albania.

Table 2.

Determination of honey acidity, color, granulation and level of HMF of 21 honey samples representing different areas of South Albania.

Districts	Free acid [%] as Gluconic acid	Color	HMF [mg/100g]	Undeterm. Material[%]	Granulation [D/W]
Vlora	0.6	Light amber	15	4.1	1.89
Korca	1.3	White	8.32	3.2	2.1
Erseka	0.6	Light amber	7.1	4.4	1.81
Pogradeci	0.8	Water white	9	3.1	2.09
Gjirokastra	1.7	Light amber	16	5	1.85
Skrapari	4.77	Light amber	17	5.2	2.4
Delvina	0.6	Light amber	14.2	6	1.79
Berat	0.9	Amber	15.2	7.2	1.54
Saranda	0.6	Light amber	6	3	1.97
Tirana	2.54	Light amber	9.4	3.6	1.91
Shkodra	5.44	Light amber	17.1	4.3	1.54

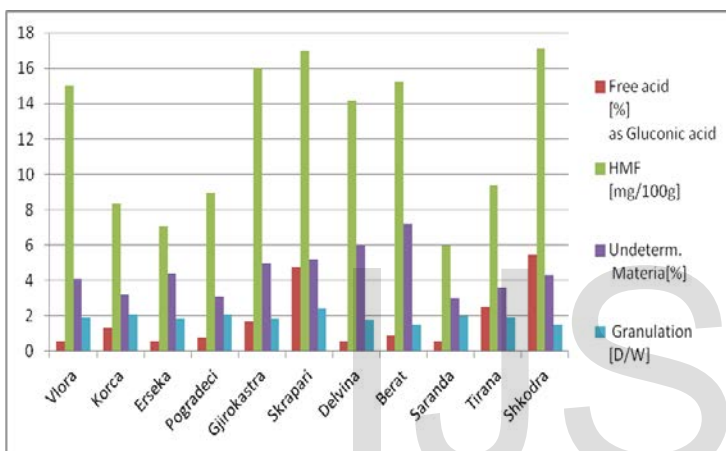


Fig. 2. Determination of honey acidity, color, granulation and level of HMF of 21 honey samples representing different areas of South Albania

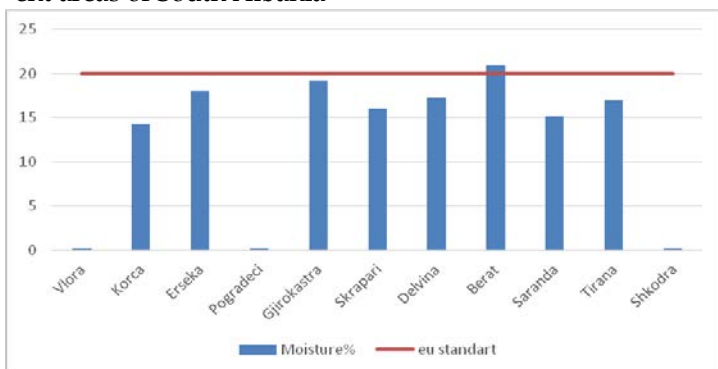


Fig. 3. Comparison of percentage moisture in honey samples with EU standart.

1. Honey carbohydrate analysis involving reducing sugars showed that samples were with variation honey with floral source, age, production area and crop year and would be of great value to Albanian honey producers.
2. These values show granulating tendency during the storage of some honey samples with high values of reducing sugars more 70% as Berat, Korca, etc.
3. Some honey samples contained monosaccharides fructose and glucose, more glucose than fructose that considers a honey adulterated if fructose is not the major carbohydrate component.
4. High ash in honey can be an indication of honey adulteration with acid hydrolysed carbohydrates, which are later neutralized. In our samples was more than 1.2% in honey samples of some farmers.
5. Moisture analysis of honey is one of the most important measurements because our honey samples were hygroscopic containing much more than 20-21% moisture it is likely to be fermented by ubiquitous yeast.
6. High levels of HMF also suggest the possibility that natural honey has been adulterated with invert syrup, prepared from sucrose by acid hydrolysis. Acid-invert syrup invariably contains high amounts of HMF. Prolonged storage or overheating of honey had been result in an HMF level exceeding 3.0 mg/100 g and may rise to 10.0 mg/100 g or more. A content of 15.0 mg/100 g or more is taken to indicate adulteration with acid-inverted invert sugar.
7. The comparison of percentage moisture in honey samples with EU standart shows that all the samples were according EU standart excepted the Berat samples.

## 5 REFERENCES

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## 4 CONCLUSIONS