Research on Honey Crystalization

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Crystallization of honey is a natural process and guarantee of authenticity. The aim of the study is to evaluate the influence of physico-chemical parameters on the process of crystallization and on crystal size. The interest in studying the factors influencing the crystallization process is due to the fact that most consumers, especially children, are reserved for the purchase and consumption of a crystallized honey, the commercial aspect being very important to them. Three samples of honey of different floral origins were used for the physico-chemical and microscopic analysis. The analyzes were carried out throughout 30 days every sample in triplicate. In terms of physicochemical following parameters were analyzed: acidity, diastase index, hydroximethylfurfural, humidity, invert sugar, glucose, fructose, fructose / glucose ratio and the microscopic analyze of crystals size. The parameter values resulting from the research study are in accordance with the legislation in force. Statistical analysis of experimental data highlights the influence of ratio fructose / glucose and humidity on the honey crystallization.

Keywords: crystal size, fructose, glucose, humidity

Honey is one of the most important bee products. The raw material they use for honey is spontaneous flora and that of culture. This diversity determine the diversity of honeys which are on the market. Honey is a sweet substance and high-energy food, naturally produced by the transformation and processing of the dew or the nectar by the bees, which is stored in the cells of the honeycombs [1]. This is the most important and well-known product of bees due to the large amount compared to other bee products, and nutritional value due to the high sugar content. Honey characteristics, both organoleptic and physico-chemical ones varie for each assortment and are influenced by some biotic and abiotic factors created around the bee colony, floral sources, climate conditions, soil or beekeeper practices [2-3]. Honey is a complex of compounds, both from the plants from which the bees have harvested the pollen, or directly from the bees - as a producer of honey [4]. Dry substance of honey is composed of 95% carbohydrates, the most representative of which are glucose and fructose [5]. In addition to carbohydrates, honey also contains water, enzymes, amino acids, vitamins, minerals, volatile substances, polyphenols, antioxidants and fatty acids [6]. The bioflavonoid as chrysin, pinocembrin, and galagin, pinobanksi. Pinocembrin is only found in honey and bee propolis [7]. In the digestion process, the main carbohydrates, as the glucose and fructose, are rapidly descomposed and transported into the blood, providing energy to the body [8]. The fructose and glucose concentration and their ratio are useful in the classification of unifloral honey [9]. The fructose / glucose ratio is a quality index and shows the ability of honey to crystallize [10-12]. The honey crystallization capacity is due to the lower solubility of glucose in water compared to fructose [13]. More the fructose / glucose ratio is slightly above 1, more fast crystallized the honey, and the more it touches the 1.5 value in case of the acacia honey, it crystallized harder [14]. The glucose / water ratio is considered as a parameter as important as the fructose/ glucose ratio. Honey is less crystallized when the glucose / water ratio is less than 1 and crystallizes more quickly or completely when this ratio exceeds 2 [10, 13]. Crystallization, in the case of honey, is a natural process by which honey passes from the liquid state, which flows, to a semi-solid state. Less glucose than fructose in honey makes honey to become liquid [15]. The phenomenon of crystallization of honey is superficially understood and even wrong by consumers, generally putting the result of this phenomenon on the falsification, or even altering of honey, considering it to be a non-conforming product. The interest in studying the factors influencing the crystallization process is due to the fact that most consumers, especially children, are reserved for the purchase and consumption of a crystallized honey, the commercial aspect being very important to them.

Experimental part

Materials and methods

Three samples of honey of different origins (acacia, tilia and polyfloral) from the local beekeepers in Suceava County were analysed from the physico-chemical and microbiological point of view. Several determinations were made for each parameter and for each honey assortment within the 30 days, in triplicate. Analyzes have been achieved at every 10 days, the 30 days being divided into 3 stages. The 10 days interval is considered to be the minimum period for observing crystallization. Quality standards underlie these determinations. For each of these honey assortments, were made the following determinations: acidity diastatic index, hydroxymethyl-furfural, humidity, invert sugar, glucose, fructose, fructose-glucose ratio.

Acidity

Acidity was determined by the titrimetric method. It is based on the titration of the honey sample diluted with water, with 0.1 n sodium hydroxide in the presence of phenolphthalein as indicator.

Determination of diastase index

The basis of this analysis is the determination of amylase activity. The diastase index is defined as the number of milliliters of a starch solution 1% that was transformed into dextrin for one hour at 45°C at optimum pH by the amylase containing in 1 g of honey [16]

Determination of hydroxymethylfurfural (HMF)

The hydroxymethylfurfural forms, with barbituric acid, in the presence of para-toluidine a red complex [17]. The

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color intensity is proportional to the hydroxymethylfurfural content and is determined using a spectrophotometer (Spectrophotometer UV-Vis Lambda EZ 201) at 550 nm of wavelength, equipped with cuvettes with a 1 cm of layer thickness [16].

Determination of humidity

It is expressed in milligrams per 100 g of honey.The humidity was determined by method with refractometer [18]. The method with refractometer (Electronic Refractometer RE40) is based on the direct correlation between the refractive index and the content of dry substance of honey, which allows the calculation of the percentage of water [16]. This is a rapid method of determination.

Determination of reducing sugar

Reducing sugar from honey (glucose, fructose, maltose) has the ability to reduce the copper sulphate in alkaline and hot medium and convert it to cuprous oxide. The amount of cuprous oxide formed is proportional to the concentration of glucose and fructose - reducing sugars - from the solution analyzed and expressed as invert sugar [16]

Determination of glucose

When a sugar solution is titrated with iodine in an alkaline medium, only sugars containing free aldehyde groups are oxidized (glucose), and not those that contain ketone groups (fructose), [16].

$$\text{Inverted Sugar\%} - \text{Glucose\%} = \text{Fructose\%}, \quad (1)$$

Results and discussions

Physico-chemical parameters

The acidity

The increase in acidity indicates the installation of fermentative processes. The installation of this process is undesirable because honey taste changes, but may occur also the development of microorganisms that can form toxic compounds such as ethyl alcohol and carbon dioxide [19]. The acidity can influence the stability and self life of honey [13]. The acidity of the honey samples does not vary much because there not has be installed an fermentation process in the 30 days of examination. The acidity of acacia honey ranged from 0.90±0.01 mL NaOH 1 n /100g to 0.93±0.01 mL NaOH 1 n /100g, the polyfloral honey acidity increased from 1.25 ±0.01 mL NaOH 1 n / 100g to 1.35±0.01 mL NaOH 1 n /100g, and the acidity of the tilia was between 2.3±0.02 mL NaOH 1 n /100g and 2.45±0.02 mL NaOH 1 n /100g, (fig. 1).

Diastase index (ID)

The values of the diastase index decrease for all honey assortments, (fig. 2). The value of the diastase index

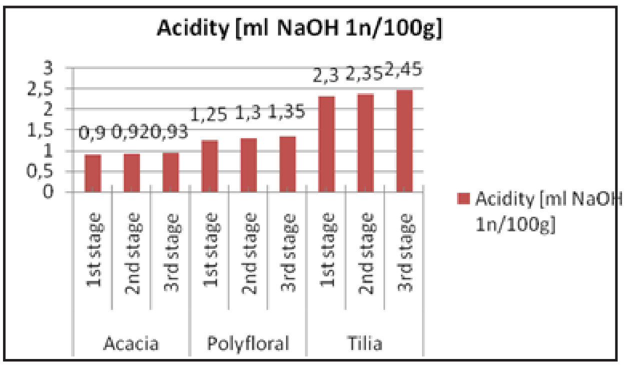


Fig. 1. Variation of acidity

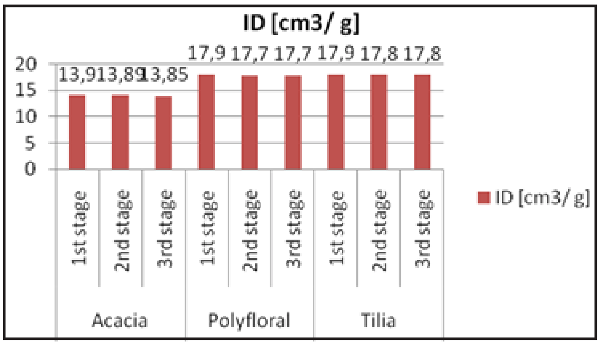


Fig. 2. Variation of diastase index

decreases for acacia honey from 13.9±0.63 cm^3/g to 13.85±0.59 cm^3/g. The polyfloral honey ID varies between 17.9±0.93 cm^3/g and 17.7±0.78 cm^3/g, and at the tilia honey ranges from 17.9±0.46 cm^3/g to 17.8±0.53 cm^3/g.

Hydroxymethylfurfural determination

The determination of hydroxymethylfurfural gives us information on the degree of freshness of honey and the thermal treatments applied to honey [20-21]. The hydroxymethylfurfural content has grown to all honey varieties (fig. 3). HMF content of Acacia honey increases from 0.6±0.03 mg/100g to 0.64±0.02 mg/100g, of polyfloral honey between 0.46±0.01 mg/100g and 0.47±0.01 mg/100g, and of tilia honey from 0.43±0.02 mg/100g to 0.47±0.03 mg/100g.

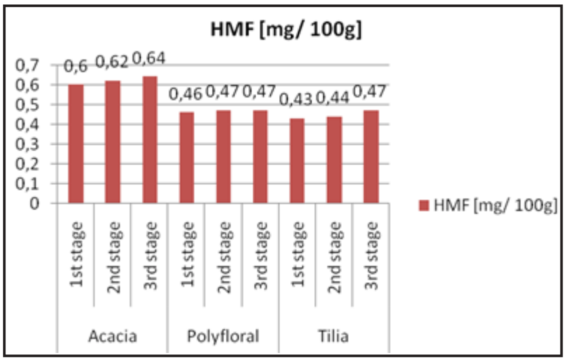


Fig. 3. Variation of HMF

Humidity

The moisture content is an important parameters, because that influence the shelf stability of honey and a higher content of moisture in honey determine a fermentation process and degradation product [22-23]. The water content of the 3 types of honey analyzed falls within the values stipulated in the norms and thus not have been installed degradation or fermentation processes during the determinations. The values of humidity content of the three varieties of honey decreased during the stage.The water content of acacia honey decreased from 16.3±0.04% to 16.05±0.06%, polyfloral honey from 16.8±0.03%to 15.9±0.04%, and at the tilia honey between 16.9±0.05% and 16.15±0.03%, (fig. 4).

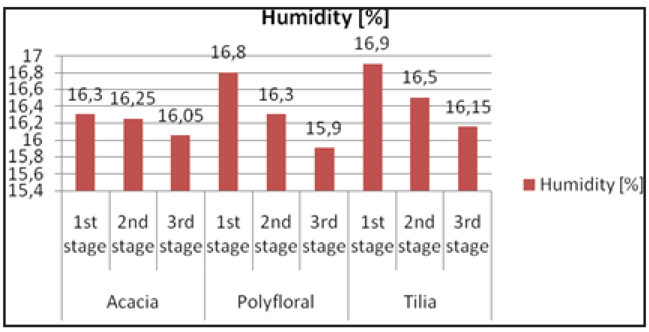


Fig. 4. Variation of humidity

Invert sugar

The amount of invert sugar varies from one assortment to another, (fig. 5). The value of invert sugar in the case of acacia honey increases from 74±0.10% to 75.25±0.11% at the third stage. In the case of polyfloral the amount of invert sugar increases from 75.5±0.12% to 76.2±0.12%, and of tilia honey, varies from 73.5±0.10 to 75.25±0.11%.

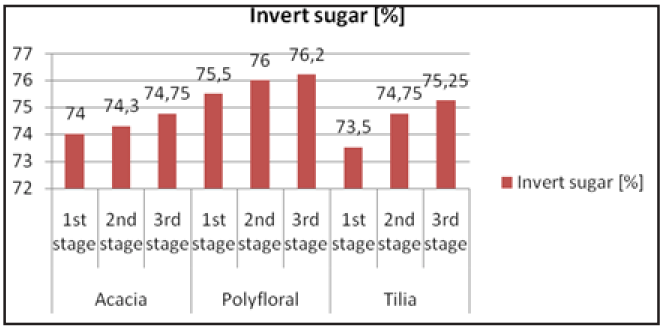


Fig. 5. Variation of invert sugar

The glucose content

The amount of glucose is increasing for all sorts of honey, (fig. 6). The glucose content of acacia honey increased from 29.85±0.09% to 30.2±0.07%, of polyfloral honey from 32.9± 0.10% to 33.3±0.09%, and of tilia from 33.3±0.09% to 34.9±0.11%.

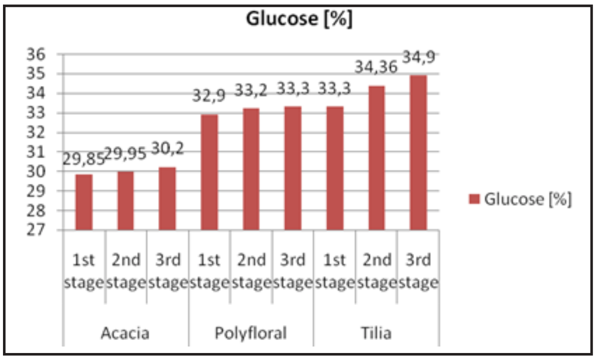


Fig. 6. Variation of glucose content

The fructose content

The fructose content increases in the case of all three honey assortments,(fig. 7). In acacia honey, fructose ranges between 44.15±0.11% and 44.55±0.11%. In the case of tilia honey the fructose increases from 40.2±0.08% to 40.35±0.08%, and for polyfloral from 42.6±0,10% to 42.9±0.10%.

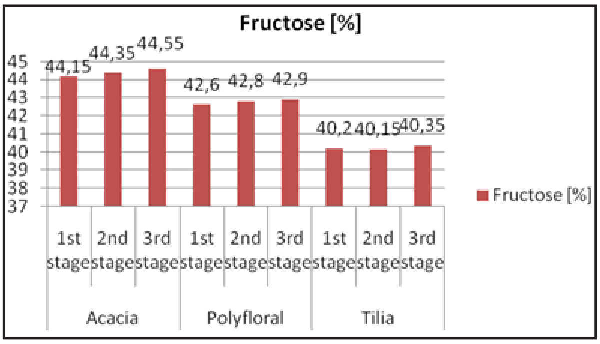


Fig. 7. Variation of fructose content

Fructose/glucose ratio (F/G)

The fructose / glucose ratio varied differently from one assortment of honey to another, (fig. 8). In the case of acacia honey, the fructose / glucose ratio is nearly unchanged. In the case of polyfloral honey, the ratio is constant, and for tilia honey decreased from 1.2 to 1.16.

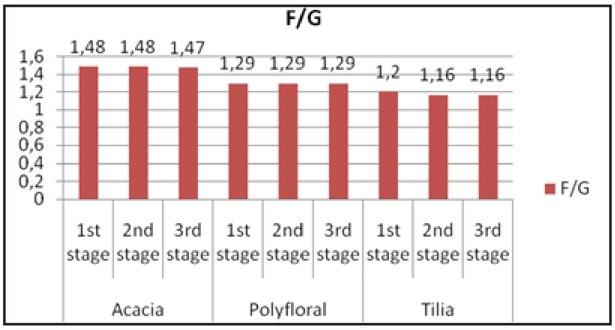


Fig. 8. Variation of fructose/glucose ratio

Size of crystals

The size of the crystals increased for the three types of honey analyzed, (fig. 9). Crystals of acacia honey increased from 0.0357±0.001 mm to 0.1269±0.007 mm, of polyfloral honey from 0.0427±0.002mm to 0.0635±0.004 mm and tilia from 0.0486±0.002mm to 0.2053±0.005 mm.

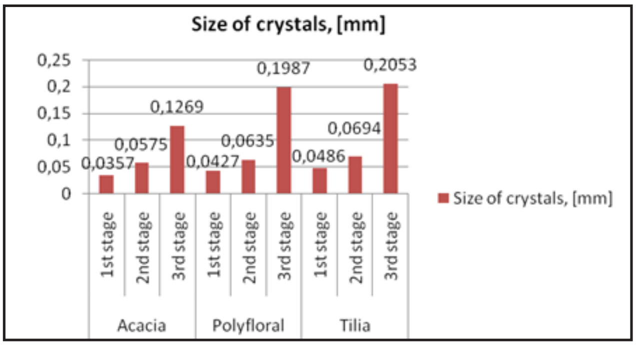


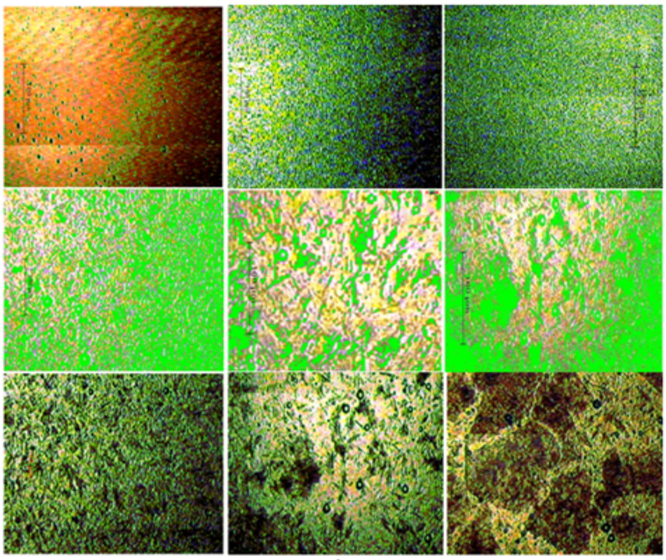
Fig. 9. Variation of size of crystals

Microscopic analysis of honey crystals

The size of the crystals was analyzed by means of a stereomicroscope. In figure 10, the differences between the crystals of the three honey assortments can be observed for each stage.

From the presented images, the finely and uniformly distributed crystals can be observed at the first stage, agglomerating at the second and at third stage forming larger crystals and unevenly distributed. Following the statistical analysis regarding the degree of influence of each physico-chemical parameter in the crystallisation process and the size of the crystals, the following correlations were obtained for each assortment of honey analyzed.

In the case of acacia honey the parameter that most influences the size of the crystals, according to the statistical analysis, is the glucose content, (table 1). In the case of polyfloral honey the most important is the fructose / glucose ratio, tabel 2 and in the tilia honey the fructose content, (table 3). For all honey assortments, the second parameter that contributes to the size of the crystals is the humidity.



a b c

1-st stage 10 days

2-nd stage 20 days

3-rd stage 30 days

Fig. 10. Microscopic images of crystal honey during the three stages, a)accacia, b) polyfloral, c) tilia

Table 1
THE CORRELATION BETWEEN PHYSICO-CHEMICAL PARAMETERS AND THE SIZE OF CRYSTALS IN ACACIA HONEY

	Acidity ml NaOH 1n/100g	ID cm3/ g	HMF mg/ 100g	Humidity %	Invert sugar%	Glucose%	Fructose%	F/G	Size of cristals, mm
Acidity ml NaOH	1								
ID cm3/ g	-0.866025404	1							
HMF mg/	0.981980506	-0.944911	1						
Humidity%	-0.866025404	1	-0.9449	1					
Invert sugar	0.953820966	-0.976221	0.9934	-0.976221	1				
Glucose%	0.907841299	-0.995871	0.97073	-0.995871	0.9918698	1			
Fructose%	0.981980506	-0.944911	1	-0.944911	0.9933993	0.9707253	1		
F/G	-0.755928946	0.981981	-0.866	0.9819805	-0.917663	-0.960769	-0.866025	1	
Size of cristals, mm	0.885694783	-0.999168	0.95747	0.9891682	0.9842491	0,9987443	0.9574734	-0.97346	1

Table 2
THE CORRELATION BETWEEN PHYSICO-CHEMICAL PARAMETERS AND THE SIZE OF CRYSTALS IN POLYFLORAL HONEY

	Acidity ml NaOH 1n/100g	ID cm3/ g	HMF mg/ 100g	Humidity %	Invert sugar%	Glucose%	Fructose%	F/G	Size of cristals, mm
Acidity ml NaOH	1								
ID cm3/ g	-0.866025404	1							
HMF mg/	0.981980506	-0.944911	1						
Humidity%	-0.866025404	1	-0.9449	1					
Invert sugar	0.953820966	-0.976221	0.9934	-0.976221	1				
Glucose%	0.907841299	-0.995871	0.97073	-0.995871	0.9918698	1			
Fructose%	0.981980506	-0.944911	1	-0.944911	0.9933993	0.9707253	1		
F/G	-0.755928946	0.981981	-0.866	0.9819805	-0.917663	-0.960769	-0.866025	1	
Size of cristals, mm	0.885694783	-0.999168	0.95747	0.9891682	0.9842491	0,9987443	0.9574734	-0.97346	1

1 = positive correlation; 0 = no correlation;-1 = negative correlation (inversely proportional).

Conclusions

Concentrations of glucose and fructose, but also their ratio are important indicators for the quality of honey [24-25], and its crystallization. As a result of the researches, the parameters that influence the crystallization of honey are: for acacia honey, the most important is the glucose content, for polyfloral honey is the fructose / glucose ratio, and the fructose content is for the tilia honey. For the three honey assortments analyzed, the second parameter that contributes to the size of the crystals is humidity. The values obtained for the quality indexes were within the limits allowed by the law and those specific to honey varieties presented in the literature.

Table 3
THE CORRELATION BETWEEN PHYSICO-CHEMICAL PARAMETERS AND THE SIZE OF CRYSTALS IN TILIA HONEY

	Acidity ml NaOH 1n/100g	*ID cm3/g*	*HMF mg/ 100g*	*Humidity%*	*Invert sugar%*	*Glucose %*	*Fructose*	*F/G*	*Size of crystals, mm*
Acidity ml NaOH 1n/100g	1								
ID cm3/ g	-0.75592894	1							
HMF mg/ 100g	0.99587059	-0.693375	1						
Humidity%	-0.97398537	0.884615	-0.9493907	1					
Invert sugar %	0.90784129	-0.960768	0.86602540	-0.9792452	1				
Glucose%	0.93028487	-0.943381	0.89314029	-0.9892133	0.9983712	1			
Fructose%	0.83862786	-0.277350	0.88461538	-0.6933752	0.5329387	0.5803	1		
F/G	-0.75592894	1	-0.69337524	0.8846154	-0.960768	-0.9433	-0.27735	1	
Size of crystals, mm	0.97782954	-0.6020836	0.93280210	0.9849388	0.7999086	0.8328	0,994098	-0.602	1

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