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QUALITY EVALUATION OF HONEY FROM THE DIFFERENT REGION OF AZERBAIJAN

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A B S T R A C T

The main objective of this study was to evaluate the quality of Azerbaijan honey, verifying their compliance with international standards. Honey samples used in the research represent main honey producing regions of Azerbaijan. The physico-chemical characteristics of 29 from the 53 honey samples (54 % of all samples) analyzed in this study completely agree with the European Commission and the Codex Alimentarius indicating adequate processing, good maturity and freshness. 15 samples (28 % of all samples) did not meet characteristics established in European and Codex standards relative to the diastase activity (min. 8 Schade units), although the other physico-chemical parameters were within the range of the allowable limits. In the 53 samples analyzed, the HMF content is quite elevated 4 samples (less than 8 % of all samples) exceed the limit of 40 mg/kg, and 34 samples (64 % of all samples) show values lower than 10 mg/kg, which are typical of fresh unheated honeys, according to the current quality criteria. 2 samples (4 % of all samples) show a fructose + glucose content lower than 60 g/100g, only both of the same samples exceed the value of 5 g/100g for sucrose. 7 samples (13% of all samples) show the protein values below 0.1%.

Keywords: Azerbaijani honey, chemical composition, quality of honey, water content, sugar content.

INTRODUCTION

Honey is a complex mixture produced by honeybees from the nectar and also exudates from plants and it is consumed as a sweetener. Honey is a supersaturated solution of sugars with fructose and glucose as main saccharides. Antimicrobial effects of honey against microorganism associated with disease or infection have been reported and honey biological activity has been attributed not only to the high sugar concentration but also to different compounds such as acids, phenolics, proteins, vitamins, minerals and carbohydrates (Beatriz A. Rodriguez, Sandra Mendoza et al., 2012).

The number of honey types that are being produced depends on the geographical region and climatic conditions (Kaspar Ruoff, 2006a). This variety of physical and chemical parameters of honey does not allow establishing standard criteria. Currently used standards (The "REVISED CODEX STANDARD FOR HONEY") is intended for voluntary application by commercial partners and not for application by Governments (Codex Alimentarius. 2011). According to these standards, quality of honey is characterized by parameters; sugar profile, moisture content, acidity, diastase number, the amount of HMF and protein content.

The amount of amino acids and proteins are relatively small, at the most 0.7 % thus having relatively small nutritive effects. However these components can be important for judging the honey quality. The honey proteins are mainly enzymes and other amino acids (Stefan Bogdanov, August 2009). In general, of the total honey protein, about 1/3 relates to pollen and has plant origin and the remaining 2/3 includes enzymes and proteins with insect origin (honey bee) (Hassan Nazarian, Razieh Taghavizad and Ahmad Majd, 2010). Of the 8 to 11 proteins found in various honeys, 4 are common to all, and appear to originate in the bee, rather than the nectar (J.W. White and Landis W. Doner, 1980).

For testing of thermal treatment of honey, hydroxymethylfurfural (HMF) content in honey is usually determined. HMF in honey is formed from carbohydrates, mainly from fructose, which is thermally more labile than sacchorose and glucose.

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Fructose disintegrates at approximately 60 ^{0}C (Belitz H.D., Grosch W., 1992).

The main objective of this study was to evaluate the quality of Azerbaijan honey, verifying their compliance with international standards. Honey samples for this research were bought from sales fair and exhibition of beekeeping products, organized by the Ministry of Agriculture of the Republic of Azerbaijan. During 2012 in Azerbaijan produced 110 tons of honey. The annual production of honey samples used in the study is the 17% of the total production (http://azinterkom.com).

MATERIALS AND METHODS

Honey samples: Honey samples used in the research represent main honey producing regions of Azerbaijan. Honey samples were bought from sales fair and exhibition of beekeeping products, organized by the Ministry of Agriculture of the Republic of Azerbaijan. Honey samples coming from the regions which are covered by Greater Caucasus (North), Lesser Caucasus (West) and Talysh Mountains (South) (figure 1.). In 2012 in Azerbaijan was produced 110 tons of honey. The annual production of honey samples used in the study is the 17% of the total production. These samples were stored at room temperature in a dark place before analysis.

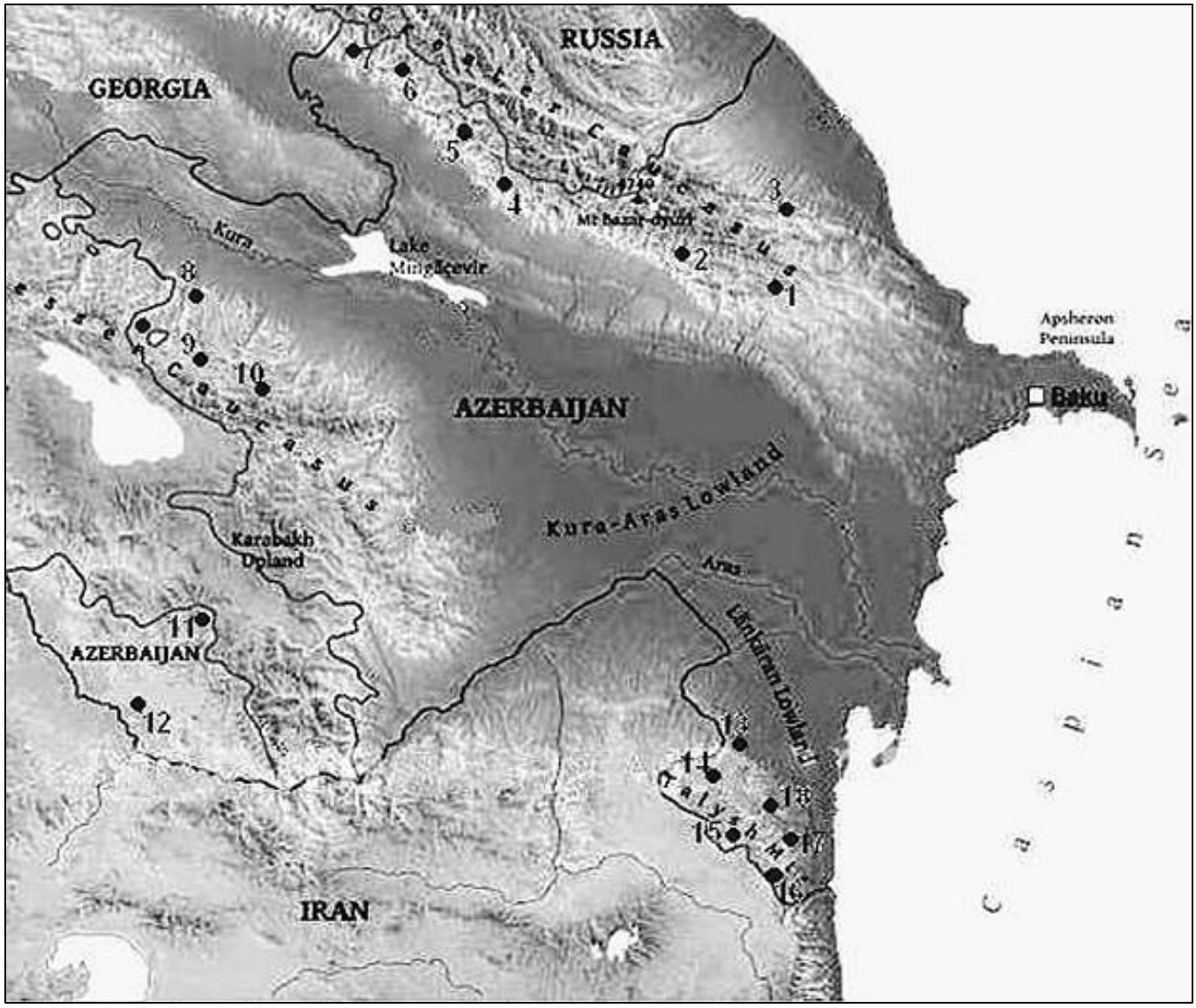

Figure 1. Location of honey bee sampling points in Azerbaijan. 1. Shamakhi, 2. Ismayilli, 3. Guba, 4. Sheki, 5. Gakh, 6. Zagatala, 7. Balaken, 8. Tovuz, 9, Gadabay, 10. Dashkasan, 11. Sharur, 12. Nakhchivan, 13. Jalilabad, 14. Yardymly, 15. Lerik, 16. Astara, 17. Lenkoran, 18. Masalli.

All chemicals (sodium hydroxide, sodium chloride, potassium iodide, iodine, bromocresol green, methyl red, sulfuric acid, hydrogen peroxide) analytical-reagent grade from Merck Chemicals. HPLC solvents were LC grade – LiChorsolv Acetonitrile and ultrapure water (Sartorius Stedim Biotech, arium 611DI, Gottingen, Germany. 18 MΩ/cm resistivity). Quality of honey was characterized by various chemical and physicochemical parameters.

Moisture content: For moisture content of natural honey max. value 20% was taken into account. Refractometric analysis method has been applied (Harmonised Methods of the İnternational Honey Commission, 2002a). (Refractometer – Bellingham Stanley, thermo controlled.)

Free acidity: The free acidity of honey is the content of all free acids, expressed in milliequivalents/kg honey, determined by following prosedure. The 10 g sample was dissolved in 75 ml of carbon dioxide - free water in 250 ml beaker. The pH measured and the solution titrated with 0.1N sodium hydroxide solution to pH 8.3 (Harmonised Methods of the İnternational Honey Commission, 2002b).

Sugar profile: HPLC method was used to determine the sugar profile of honey. After filtration of the solution, the sugar content were determined by HPLC (Agilent 1200) with RI – detection. Peaks were identified on the basis of their retention times. Quantitation was performed according to the external standard method on peak areas (Harmonised Methods of the İnternational Honey Commission, 2002c).

For the Agilent Zorbax carbohydrate column (4.6 mm diameter, 150mm length, 5µm particle size).

The following conditions have been used to give satisfactory separation.

Flow rate: 1.3 ml/min

Mobile phase: Acetonitrile / water (75/25 , v/v)

Column and detector temperature: 30 ^{0}C

Sample volume: 20 µl

Enzyme activites: With the appointment of Diastase points an idea about the freshness of honey was obtained. Enzyme activites in honey were principally measured to evaluate possible heat defects. Even if alpha – amylase and alpha – glucosidase are derived mostly from the bees , the different honey types however show considerable differences in enzyme activities (Persano Oddo L., Baldi E., Accorti M., 1990). However, as the enzyme activites in honey decrease during storage and heat treatment, indications to botanical origin can only be obtained from fresh honeys (Kaspar Ruoff, 2006b).

The traditional method for the measurement of diastase activity in honey is the Schade procedure. One unit of diastase activity (or more specifically, α – amylase) the Schade or Gothe unit, is defined as that amount of enzyme which will convert 0.01 gram of starch to the prescribed end - point in one hour at 40 ºC. For determination of diastase activity, five grams of honey were dissolved in 15 ml water; and transferred to a 50 ml volumetric flask. According to the method, 9 different pre-defined volumes of this solution were mixed with 5 ml 0.25 % starch solution in a tube and incubated during 15 minutes and then, tubes were cooled. In each of tube were added 0.5ml KI + I solution. A standard solution of starch, capable of developing with iodine, a color in a defined rage of intensity. The diminution in the blue color is measured at intervals (V.I.Krishtafovich, I.F.Zhebeleva, 2001).

Hydroxymethylfurfural: Hydroxymethylfurfural (HMF) was determined in aclear, filtered, aqueus honey solution using reverse phase HPLC equipped with UV detection at 285 nm. The signal was compared with those from standards of known concentration. Five grams of honey samples were diluted up to 50 ml with distilled water, filtered on 0.45 µm filters and immediately injected in a HPLC equipped with a UV detector. The HPLC column was a C 18 -reversed phase material, 250_4 mm, fitted with a guard cartridge. The HPLC conditions were the following: mobile phase, 90% water and 10% acetonitrile; flow rate 1 ml/min; injection volume, 20 µl. The wavelength range was 220–660 nm and the chromatograms were monitored at 285 nm. HMF was identified by splitting the peak in honey with a standard HMF (Harmonised Methods of the İnternational Honey Commission, 2002d; M. Zappal, B. Fallico, E. Arena, A. Verzera, 2005a). HPLC separates HMF from other components and thus avoid interference in the determination (Wootton M., Ryall L. 1985). HPLC method seems to be the more appropriate for HMF determination in honey, because the presence of substances, probably derived by heat or storage damage, which interfere with the UV methods did not reveal (M. Zappal, B. Fallico, E. Arena, A. Verzera, 2005b).

Protein content: Protein content was determined with

"UDK-152 Automatic Distillation and Titration Unit" by Kjeldahl method. 1 q honey was taken for analyze, and was kept in sulfuric acid for 60 min. at 420 ^{o}C for digestion (AOAC, method 960.52, 104).

Statistical Analysis: Results represent the average of at least three replications for moisture content, free acidity, sugars, protein content, hydroxymethylfurfural (HMF) and enzyme activity. Statistical analysis was carried out by the use of Microsoft Excel Statistical Packages and GraphPad Instat program.

RESULTS AND DISCUSSION

Considering altogether the results of these physicochemical analyses, we can observe that, as far as the quality is concerned, only 54 % of the examined samples can be evaluated as wholly compliant, while about 45 % of them present some quality defect (Table 1).

Table 1. The results of physico - chemical analysis of Azerbaijani honey samples (n = 53).

Parameter	Mean value	Min – Max values	Limits of EU standards	Samples exceeding limits of EU standards
Diastase	9.69	6.5 - 23.8	min. 8 Schade units	24 samples
HMF mg/kg	14.53	0.23 - 160.68	max.40	4 samples (34 samples show values lower than 10 mg/kg)
Water %	16.52	15.2 - 18.7	max. 20	not detected
Free Acidity mmol/kg	22.26	8.4 - 43.6	max. 50	not detected
Fructose %	39.83	29.21 - 44.02	not fixed limit	
Glucose %	32.09	23.68 - 37.86	not fixed limit	
Frcts+Glcs %	71.91	52.89 - 72.91	min. 60	2 samples
Sucrose %	4.87	ND - 28.58	max.5	2 samples
Glcs/Frcts	1.24	1.02 - 1.44	not fixed limit	
Glcs/Water	1.94	1.41 - 2.44	not fixed limit	
[a]Protein %	0.31	0.01 -0.67	min.0.1	7 samples

[a]Total protein of honey is between 0.1% to 0.65% (Hassan Nazarian, Razieh Taghavizad and Ahmad Majd. (2010). Origin of honey proteins and method for its quality control. Pakistan Journal of Botany, 42(5), 3221-3228.).

Water content: For the water content, most of the samples show quite low values. Moisture shows an average value 16.5 %, this variety depends on climatic factors, season of production and maturity of honey. 20 % of moisture is the maximum allowed to avoid fermentation. (Cantarelli M.A., Pellerano R.G., Marchevsky E.J., Camina J.M., 2008.).

Sugar profile: Sugar profile is quite in agreement with Bogdanov et al. (Bogdanov S. et al., 1999.) and with the international standards: only 2 samples show a fructose + glucose content lower than 60 g/100g, both of the same samples exceed the value of 5 g/100g for sucrose.

pH and free acidity values: pH values do not indicate a significant amount of honeydew. All free acidity values fall under the prescribed limit of 50 meq/ kg.

Enzyme (diastase activities): Enzyme (diastase activities) 23 samples have a diastase value lower than 8 Schade units. The EU standards establish a limit of not less than 8 Schade units for diastase. It is to be noted that the use of enzyme activities as indicators of honey freshness is often criticized, since the initial enzyme activity may be very different in the various honey types (White J.W. 1994.). The enzyme activities in honey depend on the intensity of the nectar flow and the amount of nectar processing by the honey bees. Therefore honey from very rich nectar sources e.g. often show low natural enzyme activities. When honey is adulterated by addition of inverted sucrose or hydrolyzed starch namely high fructose corn syrup (HFCS), then such dilution of honey leads to the reduction of diastase number (M.Voldrich, A.Rajchl, H.Cizková and P.Cuhra. 2009).

The measure of HMF content: It is used to evaluate honey freshness. The EU standards establish a limit of not more than 40 mg/kg for HMF and not less than 8 Schade units for diastase. Fresh honey does not contain hydroxyl-methyl-furfural. Thus HMF is not a useful criterion for the botanical classification of honey. However, before determining storage dependent measurands such as enzyme activity, one should ensure that honey are fresh and do not express any heat defects by checking the HMF content is below 15 mg/ kg.(Kaspar Ruoff. 2006c).

In the 53 samples analyzed, the HMF content is quite elevated (14.5 mg/kg on average) 4 samples exceed the limit of 40 mg/kg, and 34 samples show values lower than 10 mg/kg, which are typical of fresh unheated honeys, according to the current quality criterion. As far as enzyme activities are concerned, 23 samples have a diastase value lower than 8 Schade units.

Evaluation of the protein content: The honey proteins are mainly enzymes and other amino acids (Stefan Bogdanov, 2009). In general, of the total honey protein, has plant origin and proteins (enzyme) with insect origin (honey bee). Pollen is the major source of protein for honey bees.

During processing, sugar feed enriched with protein. Feeding honey bees sugar in syrup form is the most popular and probably most effective method in Azerbaijan too, and some beekeepers use this method for adulterating honey. In the experiments of Shenfelda (1955) protein content increased only to 0.08%, after the bee feeding syrup. But in blossom honey protein content is 0.2-0.4%. Blossom or nectar honey is derived from the nectarines of flowers and honeydew honey comes from the sugary excretion of some hemipterous insects on the host plant or from the exudates of the plants (Saxena S., Gautam S. and Sharma A., 2010). Nectar is secreted by glands at the base of the flowers, known as nectarines. Field bees collect nectar from blossom in the field. At this stage, the nectar has a high level of sucrose sugar with some laevulose and dextrose and high moisture content, with traces of other substances such as minerals, vitamins, pigments, aromatic substances, organic acids and nitrogen compounds (Goulburn, 2000). Nectar is primarily a carbohydrate source, but can contain some amino acids and lipids (Baker H.G., Baker I., 1975). The main source of the sugar in honey is nectar or honeydew. Pollen is the major source of protein for honeybees. Pollen is made up of various substances, including proteins, fats, lipids, carbohydrates, vitamins, minerals and many others. Honeybees rely on pollen as their source of protein, lipids, sterols, vitamins, minerals and certain carbohydrates (Todd F.E., Betherick O., 1942).

There are some protein compounds in honey in addition to sugars, lipids and mineral compounds. Relative quantity of proteins in honey compound is considered as a quality index. Determination of the quantity of plant origin (pollen) and animal origin (honey bee) of the proteins of honey is an important. (Hassan Nazarian, Razieh Taghavizad and Ahmad Majd, 2010).

First are reactions allowing protein or peptide quantification as the Kjeldahl method (IDF standard, 1964) based on the determination of the nitrogen content after a sample mineralization step. Nowadays, the evaluation of the protein content is mainly based on the Kjeldahl method.

The protein content of 53 honeys considered quite high, with an average of 0.31% and only 8 samples (15%) show values lower than 0.1% (figure. 2).

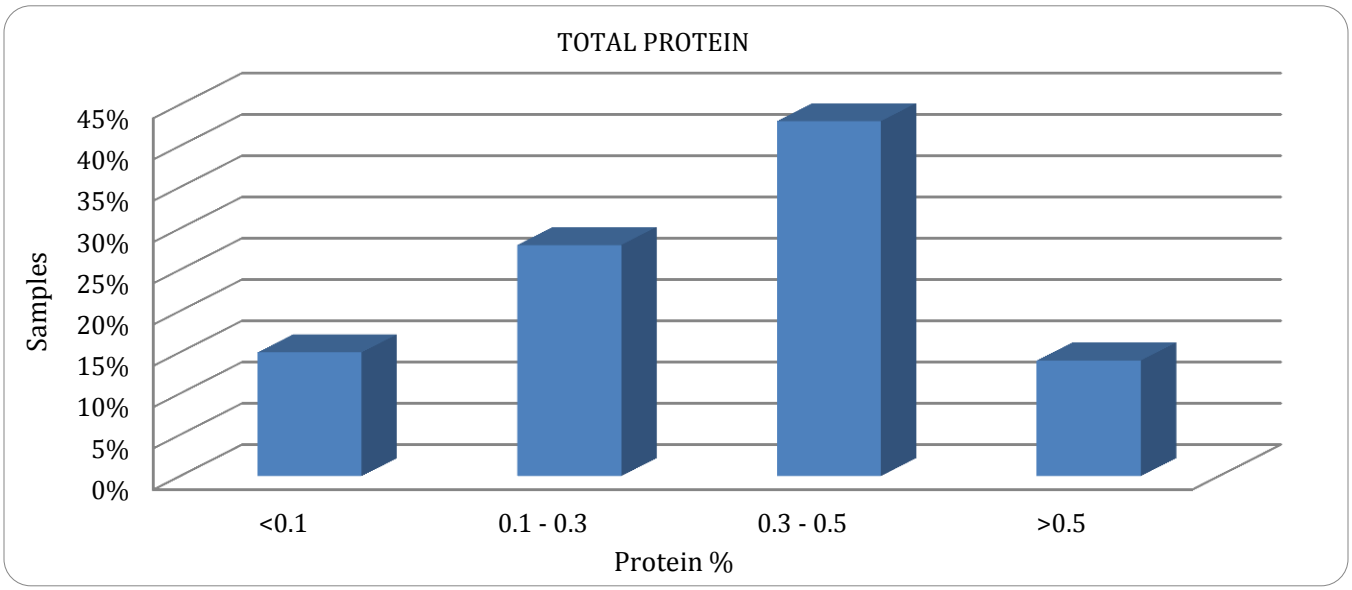

Figure 2. Distribution of the 53 honey samples according to the total protein content.

By comparing the freshness indicators, 9 unheated (HMF lower then 10mg/kg) samples are judged not to correspond to fresh honey, 6 of unheated non-fresh samples show protein values lower than 0.1 %. This can be accounted for diastase by inadequate processing or storage conditions, more feeding sugar to a colony, but partly for it could also be due to the climate of the production area. The physico-chemical characteristics of 28 from the 53 honey samples analyzed in this study completely agree with the European Commission and the Codex Alimentarius indicating adequate processing, good maturity and freshness. 15 samples did not agree with characteristics established in European and Codex standards relative to the diastase activity, although the other physico-chemical parameters were within the range of the allowable limits (figure 3).

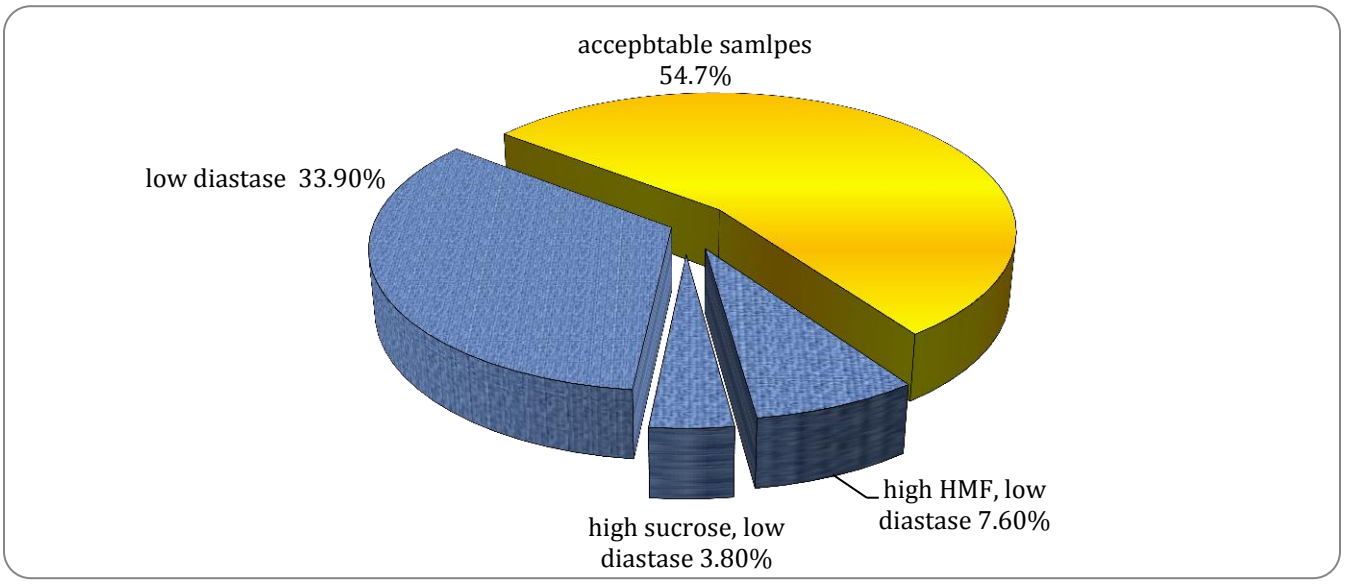

Figure 3. Defects found in the honey samples.

Table 2 is designed to study the influence of geographical conditions on the parameters of quality. Table was used to study the physical and chemical quality of honey purchased in three different regions of Azerbaijan. Means of physical and chemical results were found in samples of honey that are acceptable quality on the basis of EU standards shown Table 2.

Table 2. Physico - chemical quality of honey samples from different regions of Azerbaijan

Parameter	Regions			Total mean value	Limits of EU standards
	North	West	South		
Diastase	12.9	11	9.7	11.2	min. 8
HMF mg/kg	4.2	8.2	9.8	7.4	max.40
Water %	16.2	16.3	17.0	16.5	max. 20
Free Acidity mol/kg	24	22	24	23.3	max. 50
Fructose %	41.9	41	39.5	40.8	not fixed limit
Glucose %	32.4	31.3	32.7	32.7	not fixed limit
frcts +glcs %	74.3	73.4	71.1	72.9	min. 60
Sucrose %	0.66	0.34	0.71	0.57	max.5
glcs /frcts	1.3	1.27	1.28	1.28	not fixed limit
glcs /water	1.3	1.3	1.3	1.3	not fixed limit
[a] Protein %	0.4	0.38	0.37	0.38	min.0.1
Starch	ND	ND	ND	ND	not acceptable
Defect samples	5	12	7	Total unacceptable samples 24	

[a] Total protein of honey is between 0.1% to 0.65% (Hassan Nazarian, Razieh Taghavizad and Ahmad Majd. (2010). Origin of honey proteins and method for its quality control. Pakistan Journal of Botany, 42(5), 3221-3228.).

The moisture content of honey is highly important factor contributing to its stability against fermentation and granulation during storage (Singh and Bath, 1997). The present study also demonstrated that the average moisture content in the Northern and Western region is lower than in the South region. Southern region is hot and very humid subtropical climate, but the northern region has low humidity and very cold climate. The different moisture content of honey depends on harvest season, the degree of maturity reached in the hive and moisture content of original plant (Finola, Lasagno, Marioli, 2007).Moisture shows a mean value of 16.5 %, showing a mean value lower than that reported in Turkey - 18.9% (Guler, Z. 2005), in France - 18.1% (Devillers et al. 2004) and in Poland (Przybylowski and Wilczynska. 2001).

The diastase activity and the HMF content are widely recognized as parameters indicating the freshness of honey (Mendes et al., 1998). The total mean of diastase activity is 11.2 units. Samples from Talysh Mountains have lower values than those from Caucasus regions. Diastase activity level is very different around the world. A higher level of diastase activity were registered in France - 22.4 (Devillers et al, 2004.), Argentina – 19.7 (Cantarelli et al, 2008) also in Italy -39.1 (Esti et al.1997). No significant differences were found in the protein and acidity levels between the three regions of origin.

Samples of honey from the Greater Caucasus region showed significantly lower HMF content than the samples from the Lesser Caucasus and Talysh Mountains. The variation in the activity of diastases and HMF may be related to source of honey as well as climate of region (Singh and Bath, 1997). The mean value of HMF in this study was 7.4 mg /kg, higher than that obtained in Turkey 4.52 mg /kg (Turhan. 2007), but lower than those obtained in Argentina -14.8 mg /kg (Finola et al. 2007), as well as a report from the Italy- 7.6 mg /kg (Esti et al. 1997) showed almost the same result as our result.

In this study, the combined levels of glucose and fructose varied from 68.4% to 78.9%. Glucose + fructose means belonging Greater Caucasus (74.3%) were found less high than in other regions. The mean value of glucose and fructose was found 72.9%. Our results for glucose and fructose value showed approximately similarity with the results from Algeria - 72,6% (Ouchemoukh et al, 2007). Higher than in Turkey - 68.4% (Guler, Z. 2005) and in Argentina -68,1% (Cantarelli et al, 2008).

In this study, the mean value of sucrose was found 0.57%. This result is lower than the results from Argentina - 4,05% (Cantarelli et al, 2008), Turkey - 3,03% (Turhan. 2007), France - 0.74% (Devillers et al, 2004.) Poland - 1,23% (Przybylowski Wilczynska and 2001), Italy -1.09 (Esti et al al.1997).

Starch is important aspect in assessing the genuineness of honey, according to EU standards, the presence of starch and hydrolyzed starch is not acceptable. In this study, all samples were in the acceptable range.

CONCLUSIONS

In a study of 53 samples of Azerbaijani honey, which was produced in three different regions of Azerbaijan, some consideration may be given to the professional level of beekeepers, who sometimes do not allow high quality production and marketing of honey in the country, in fact, only 54% of the samples to achieve good quality, while about 45% of them show one or more defects (According to EU standards). An increased and more effective extension service will be necessary to improve the beekeepers' knowledge about honey quality features and adequate production and storage technologies. On the other hand, better control of the marketed honey is needed for consumer protection. The researches on Azerbaijani honey should be further developed, in order to better understand the actual extent and interpretation of some of the analytical results obtained, which may be related to bee race, environment, climate, bee forage, etc., and to learn more about the local bee flora. Moreover, the achievement of a good knowledge of the product would provide the scientific support for the introduction of a national norm for honey.

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