Chemiluminescent analysis as perspective instrument for the honey quality assessment

M I Lesovskaya and A S Igoshin

FGBOU VO "Krasnoyarsk State Agrarian University of the Ministry of Agriculture of Russia", Mira pr., 90, Krasnoyarsk, Russia

E-mail: lesmari@rambler.ru

Abstract. Natural honey is one of highly needed end expensive nutrients, so it is rather often faked. The main problem is the deficit of quantitative methods for detecting falsifications. The results of the traditional polyparametric and innovative monoparametric ways for honey quality analysis were compared. Antioxidant activity as a measurable indicator of biological value has been considered. The results obtained in the course of a traditional chemical analysis of honey are in good agreement with the results of chemiluminescent analysis of the same samples. It was showed, that the farm honey samples contain a lot of reducing compounds and acid equivalents and had balanced antioxidant activity. Under their influence the light sum decreased no more than 50-60%. The retail honey samples had an abnormally high antioxidant or prooxidant activity. On the average retail samples contained abnormally high amount of reduced substances (ascorbic acid, protein, acid equivalents). It can be assumed that these substances were artificially add to honey, and the product was a fake. Using chemiluminescence analysis one can do express selection of samples whose quality is doubtful. This kind of control may be in-cluded in the list of modern methods for detecting fraud in the express control of honey quality.

1. Introduction

Honey is one of the functional nutrients that are traditionally included in a balanced human diet. In accordance with the generally accepted definition of State Standart 19792-2017, honey is a product intended for consumption in food, sale through the retail network and catering, as well as for use in the food industry. Honey is a source of not only natural monosaccharides, but also numerous biologically active components. The chemical composition of honey is well studied [1, 2]. Honey components have a diverse chemical nature. Moreover, they all have the same functional ability to regulate the rate of redox processes. It is well known that redox processes are the basis for maintaining homeostasis at all levels of biological organization [3]. In particular, honey contains water-soluble vitamins (first of all vitamin C), mineral components (primarily Fe²⁺ / Fe³⁺ ions), proteins as iron-containing enzymes (cytochromes) and polypeptides, as well as the polymorphic complex of organic acids, glycosides and bioflavonoids. All of them have strong redox-regulating properties and are bioregulators and adaptogens correspondingly conditions [4].

Among biological redox processes, chain reactions associated with the production of biogenic free radicals (reactive oxygen forms, ROS) are the most important. The composition of honey has a large number of components that form a variety of combinations. Therefore, the antioxidant and / or...
prooxidant activity of honey in general depends on its chemical composition [5]. An assessment of this ability is necessary to obtain information about the biological value of honey and its nutritional value in general.

In accordance with current State Standard 54644-2011, this assessment is based on a polyparametric approach, when the composition and content of chemical components are determined separately (sucrose, reducing sugars, free acidity, etc.). There is a problem here. This approach, as well as organoleptic analysis, does not allow to get inform about functional activity of honey to stimulate or inhibit free radicals. This parameter is just essential for determining the biological value of honey. It is especially important for identifying fake products. Their number is growing rapidly in the retail network when there are no reliable instrumental methods of quality control. Thus, the actual problem is the search for express and informative methods to determ the quality of honey, identify and reject falsificat sorts and products among honey ones.

A solution to this problem can be achieved using chemiluminescent (CL) analysis. The principle of the method is to evaluate the antioxidant activity of a test object by its ability to influence the production of free radicals under model conditions. The free radical decomposition of oxygen peroxide (Fenton reaction) is a simple model for comparing the antioxidant activity of various lyophilic mixtures to extrapolate data to differ bio systems level. The energy of a chain chemical reaction can be converted into the light using luminol $C_8H_7N_3O_2$. The calculation of the number of emitted light quanta can be carried out using a chemiluminometer [6].

The aim of this work was a general assessment of the quality of few sorts honey with guaranteed naturalness (farm samples) or without it (retail samples) using different parameters of the chemical composition and CL activity.

2. Materials and methods
The objects of study were nine types of honey (figure 1). Five samples among them were obtained from apiary owners fermers (farm samples), four samples of honey were purchased in a retail network (retail samples). Except this, invert syrup was prepared according to the well-known method and were consisted as a «zero control».

![Figure 1. Objects of study.](image)

Figure 1. Objects of study.

The acidimetry method was used to determine the level of free organic acids in the test samples. Sucrose level was determined by the refractometric method, total protein level was determined by the biuret method, ascorbic acid level was determined by the colorimetric method by reaction with methylene blue [7]. The level of total iron ($Fe^{3+}$) was determined by photometry of the rhodane complex. The total amount of reducing compounds was determined by the permanganometric method [8].

Invert syrup as a model of fake honey («zero control») was prepared by mixing 30 g of sugar, 4 ml of water and 0.12 g of citric acid in a water bath for 20 minutes. The sucrose content, the sum of the reducing compounds and the active acidity in the invert syrup were determined. Chemiluminescent
analysis was performed using the automated software complex «Biochemiluminometer 3607». The device operates in the mode of light quanta counting. ROS were produced during the Fe$^{2+}$ induced decomposition of 0.1 mM hydroperoxide (Fenton model) [9].

The Fenton reaction mixture contained 100 μl H$_2$O$_2$ (0.01%), 100 μl solution of honey suspension (3%) in distilled water and 50 μl FeSO$_4$ (5 mM). To enhance the signal, 150 μl luminol (10$^{-4}$ M) was used. All reagents were taken from «Sigma», Novosibirsk. The method of analysis is described in detail [10].

The recording and storage of results, graphic and statistical processing of the kinetic curves (kinetograms) were performed using the BLM 07 software package (instrument operation control using a PC) and BLM 07PR software (processing of measurement results). The antioxidant or prooxidant activity of the compounds was evaluated by the direction of the change in the peak of the CL or light sum regarding control under the influence of the samples. The measurement time in the chemical model was from 5 to 10 minutes. The kinetic parameters were automatically recorded and archived as a database. Each sample was analyzed at least three times. Statistical processing was carried out using Student's criterion (the distribution of sample data was normal, the variances were comparable) at a confidence level of 0.95.

3. Different ways to determine the quality of honey

3.1. The traditional polyparametric way to determine the quality of honey

The average values of the parameters for each group of samples are shown in the petalled diagrams (figure 2). These values were multiplied for comparison with each other and connected by a dashed line.

![Figure 2](image)

**Figure 2.** Middle amount of redox active chemical components of honey samples in two groups: retail (a) and farm (b).

Resulting figures were compared with the analogue, which was compiled according to the reference data (standard, solid line). It was showed that the results for retail samples differ from the standard in all indicators, and the maximum discrepancy observed in the levels of total protein and sucrose. It is possible that falsification methods described in the literature were used in the technology for manufacturing these samples for example, the addition of starch [11] or flour suspension [12] and sugar syrup [13]. Two-fold excess of sucrose level in retail samples, even compared with invert syrup, indicates such possibility.

On the contrary, in the group of farmer samples, almost all parameters were on the same level as the standard (figure 2, b). Thus, the results indicate significant differences in the chemical composition of farmer samples and retail samples. The results were obtained using the traditional analytical methods which are described in the reglament documents (State Standard, first of all). This way is quite a long, difficult and time-consuming.
3.2. The monoparametric way to estimate redox activity as quality parameter of honey

The kinetic curves and the light sum level, which describe the change of the start level of free radicals under the influence of the studied samples, are shown in figure 3 and 4. The curves or columns below the reference level were reflected the antioxidant activity (AOA) of the test samples. Contrary, the curves or columns higher the reference level were reflected the prooxidant activity (POA) of the test samples. Reference level are marked a solid bold line (figure 3) or the first column (figure 4).

![Figure 3. Redox activity of the different honey samples in Fenton model.](image)

The antioxidant activity of the farmer samples was low («Achinsky» and «Lugovo») or middle («Taiga», «Diaghilev», «Raznotravny»), the light sum was 50-60% lower than the control (figure 3). On the contrary, in the group of retail samples of AOA, the results were abnormally contrasted from excessive inhibition of CL to increased production of ROS. The honey samples «Potapov Fagopyrum» and «Minusinsky» had excessive antioxidant activity, the light sum was 95–80% lower than the control. These results are in good agreement with the chemical analysis data described above.

![Figure 4. The light sum level of the CL reaction under the influence of various honey samples.](image)
This fact indicates that retail samples contain more of reducing compounds (protein, sucrose, ascorbic acid), apparently of artificial nature. So, ХЛ-analisis allows to get the same information about biological value, at that time the complexity of the CL analysis is lower, the reproducibility and accuracy are much higher. The results obtained in the course of a traditional polyparametric chemical analysis of honey are in good agreement with the results of chemiluminescent analysis of the same samples. The farmer’s honey samples «Diaghilevy», «Taiga» and «Raznotravny» contained a lot of reducing compounds and acid equivalents and had middle antioxidant activity. Under their influence the light sum decreased no more than 50–60%. The retail honey samples «Potap Fagopyrum» and «Minusinsky» had an abnormally high antioxidant activity, while the samples «Sloe Fagopyrum» and «Potap Acacia» had prooxidant activity. Under their influence, the light sum decreased by 85–90%. The content of reducing compounds in these samples (ascorbic acid, sucrose, total protein) significantly exceeded the reference values by 25%, 100%, and 200%, respectively. It can be assumed that these substances are artificially added to honey, and the product is a fake. In contrast to traditional chemical analysis, CL-analisis help quickly to select samples whose quality is doubtful. In prospect, samples with abnormal antioxidant or prooxidant activity should be investigate to a more detailed analysis to identify fake additions.

4. Conclusion

Different chemical fraud that reduces the quality of honey can be detected using CL analysis with an advantage in speed, visibility and informativity.

Results of CL analysis were obtained under the conditions of a single experimental model. Therefore, they are well comparable and interpreted.

Chemiluminescent analysis is recommended to be included in the list of modern methods for detecting fraud in the express control of honey quality.

References

[1] Dubtsova E A 2008 Clin. gerontol. 1 38-41
[2] Romanov A V and Larionov O G 2007 Sorption and chromatographic processes 7(5) 719-25
[3] Proskurnina E V and Vladimirov Y A 2015 Free radicals as participants in regulatory and pathological processes Basic science for medicine (Biophys. medical technology vol 1) ed A I Grigoriev and Y A Vladimirov (Moscow: Max Press) 38-71
[4] Lesovskaya M I 2015 Fundamental research 2-6 1211-15
[5] Voloboy N L, Zverev Ya F et al 2011 Bulletin of Siberian Medicine 5 41-4
[6] Lesovskaya M I and Shaporova Z E 2016 Bull. of Omsk State Agrarian University 1(21) 226-35
[7] Vasiliev V P Analytical chemistry in 2 volumes 2004 Physical and chemical methods of analysis (Moscow: Drofa vol 2) 384
[8] Vinogradova A A Melkina G M and Fomicheva L A 1991 Laboratory workshop on the general technology of food production (Moscow: Agropromizdat) 335
[9] Lesovskaya M I 2015 Influence of nutrients on the free radical balance of blood in vitro (Moscow: Publishing House Academy of Natural History) 94
[10] Vladimirov G K, Sergunova E V, Izmaylov D Y and Vladimirov Yu A 2016 Bull. of Russian State Medical University 2 65-72
[11] Zvyagin A A, Lesnikova E P and Zvyagina A P 2013 Food industry 6 30-1
[12] Kalinina I V Kobyakova A Y 2015 Bull. of the South State University. Ser. Food and Biotechnology 3(3) 69-74
[13] Lysenko S E 2015 News of agricultural science of Tauris 2(165) 157-61