# Statistical Approach to the Quality of Some Bulgarian Honeys

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***Abstract:*** *Honey is a natural product rich in biologically active substances. The current study includes HPAEC-PAD carbohydrate data (trehalose, rhamnose, arabinose, glucose, fructose, saccharose, raffinose, melezitoze, maltose), pH, moisture, ash, protein, carbon, nitrogen, P, K, Na, Mg and Zn, and presents descriptive statistic data on average, minimal, and maximal values, standard deviations and the median. The highest median levels are shown by K (647* mg $kg^{-1}$*), followed by Na (138* mg $kg^{-1}$*), P (59* mg $kg^{-1}$*), Mg (28.2* mg $kg^{-1}$*), and Zn (2.45* mg $kg^{-1}$*). Both negative and positive statistically significant correlations at $p < 0.01$ are identified. There are 20 statistically significant correlations with Pearson correlation coefficients higher than 0.95.*

***Key words:*** *honey, quality, correlation, carbohydrates, minerals, protein.*

## INTRODUCTION

Honey is a healthy natural product. Among the traditional natural foodstuff in Bulgaria, honey has been known, produced and consumed by people for centuries. Due to its composition, honey is recognized for its nourishing, antioxidant, antibacterial and other properties [4, 5, 6, 7, 10, 11, 16, 27, 29, 34, 35, 37, 39, 41, 42]. The honey sugar analysis is important for identification of the fraudulent activities i.e. the addition of industrial syrups to honey [18] and for honey's classification as a blossom or honeydew honey [9]. Carbohydrates are analyzed by means of various techniques [2, 3, 12, 13, 14, 15, 17, 19, 20, 21, 23, 24, 25, 26, 28, 30, 36, 40]. Although, the high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) is influenced by various factors [1, 15, 19, 22, 26, 33], it is the most appropriate contemporary technique.

The aim of the present study is to identify correlations between *HPAEC-PAD* carbohydrates *(*trehalose, rhamnose, arabinose, glucose, fructose, saccharose, raffinose, melezitoze, maltose), pH, moisture, ash, protein, carbon and nitrogen, phosphorus, potassium, sodium, magnesium, and zinc.

## MATERIAL AND METHODS

Honey samples. Honeys were bought directly from various local Bulgarian beekeepers. The honeys were stored at room temperature (20±2 $^{o}$C) in glass jars until analyses.

Carbohydrate analyses. An aliquot of 2 g of honey was diluted with double-distilled water (TOC = 4 ppb; 18.2 MΩ $10^{-2}$ m) and the dilution was analyzed by means of HPAEC-PAD after filtration [31, 32].

Determination of pH. An aliquot of 10 g of honey was diluted in 75 mL double-distilled water (TOC = 4 ppb; 18.2 MΩ 10-2 m) and the pH values were read by inoLAB pH720 (WTW, Weilheim, Germany) [8].

The moisture was determined using Mettler balance & moisture analyser, model LJ16, Type PJ300MB supplied with LC-P45 printer (Mettler-Toledo).

Protein, carbon and nitrogen were determined at 949 $^{o}$C, using Hellium flow-through carrier gas by TruSpec CN (Leco Corporation, USA). The apparatus was calibrated with EDTA calibation sample (Leco Corporation, USA).

Ash content determination included incineration in a muffle furnace Nabertherm (L15/11, Lilienthal, Germany) [8].

Phosphorus was colorimetrically determined based on the vanadomolybdate procedure by Stuffins [38]. The method included incineration at 550 $^{o}$C, solution in 1N nitric acid. Then, the absorbance was read by Specord 200 spectrophotometer (Analytic Jena, Germany). The data were processed by WinASPECT® 1.2 software (Analytic Jena, Germany).

The minerals K, Na, Mg and Zn were determined by flame atomic absorption spectrometer (Model AAS 5-FL, Analytik Jena AG, Germany) equipped with an AS 52 autosampler (Analytik Jena AG, Germany). A calibration standard by Merck (Germany) ICP multi-element standard solution IV (23 elements in diluted nitric acid) was used. The data were processed by WinAAS ver.3.80.

The data were processed with the stated software packages. Statistical analyses were performed by STATISTICA statistical software package (version 7.0) and Origin 8.0. All samples were analyzed in triplicates.

## RESULTS AND DISCUSSION

According to the Bulgarian Legislation [43], various honey characteristics are subject to quality control. In Table 1 are presented descriptive data about the main properties and the contents of carbon, nitrogen, phosphorus, potassium, sodium, magnesium and zinc (Var.1-Var.11).

Table 1. Descriptive Statistics of Honey Composition and Properties

| | pH | moisture | Ash | Protein | C | N | P | K | Na | Mg | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | - | g $100g^{-1}$ | | | | mg $kg^{-1}$ | | | | | |
| | Var.1 | Var.2 | Var.3 | Var.4 | Var.5 | Var.6 | Var.7 | Var.8 | Var.9 | Var.10 | Var.11 |
| Average | 4.36 | 15.06 | 0.71 | 0.27 | 21.1 | 433 | 72 | 565 | 136 | 23.8 | 2.57 |
| Min | 3.89 | 12.58 | 0.23 | 0.17 | 18.8 | 277 | 35 | 117 | 81 | 0.0 | 0.57 |
| Max | 4.70 | 18.99 | 0.89 | 0.40 | 24.3 | 638 | 134 | 1044 | 185 | 49.6 | 4.87 |
| SD | 0.31 | 2.24 | 0.21 | 0.07 | 1.7 | 108 | 36 | 351 | 46 | 21.0 | 1.59 |
| Median | 4.40 | 14.12 | 0.78 | 0.27 | 20.9 | 429 | 59 | 647 | 138 | 28.2 | 2.45 |

Demonstrating a pH value below 5, honey was defined as an acidic product. Similar results have been reported by other authors [41, 42].

The Bulgarian legislation states different requirements for industrially- and not-industrially produced honey. The latter must not contain more than 1.0 % ash and 20 % moisture [43]. All samples meet these requirements. P, K, Na and Zn were found in all samples.

The descriptive statistics of absolute quantities of honey carbohydrates determined by HPAEC-PAD is shown in Table 2 (Var.12 - Var.22). For five carbohydrates (fructose, glucose, saccharose, maltose, and melezitose), the median values are more than 1.0 g $100g^{-1}$.

Table 2. Descriptive Statistics of Honey Carbohydrates (absolute quantities)

| | Fru | Glu | Sac | Mel | Mal | Tre | Raf | Ara | Rha | F+G | F/G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | g $100g^{-1}$ | | | | | | | | | | - |
| | Var.12 | Var.13 | Var.14 | Var.15 | Var.16 | Var.17 | Var.18 | Var.19 | Var.20 | Var.21 | Var.22 |
| Average | 33.04 | 25.39 | 273 | 1.22 | 1.37 | 0.61 | 0.04 | 0.04 | 0.04 | 58.43 | 1.30 |
| Min | 30.84 | 23.24 | 1.98 | 0.92 | 1.05 | 0.31 | 0.00 | 0.00 | 0.00 | 54.31 | 1.22 |
| Max | 38.66 | 27.06 | 3.43 | 1.69 | 1.75 | 1.06 | 0.31 | 0.27 | 0.23 | 64.78 | 1.48 |
| SD | 2.68 | 1.34 | 0.54 | 0.24 | 0.26 | 0.25 | 0.11 | 0.10 | 0.08 | 3.65 | 0.08 |
| Median | 32.15 | 25.28 | 2.84 | 1.20 | 1.35 | 0.55 | 0.00 | 0.00 | 0.00 | 57.69 | 1.29 |

Although, not found in all honeys, raffinose, arabibose, rhamnose were also detected. The average ratio between fructose and glucose (F/G) was above 1.2.

Fructose (≥50 %) was the most abundant carbohydrate (Table 3). Almost 90 % of the carbohydrates were fructose and glucose, followed by saccharose (4.28 %). The presence of maltose (more than 1.5 %) is a proof for the lack of fraudulent activities.

Table 3. Descriptive Statistics of Honey Carbohydrates (relative quantities – only carbohydrates)

| | All Sugars | Fru | Glu | Sac | Mel | Mal | Tre | Raf | Ara | Rha | F+G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | g $100g^{-1}$ | % | | | | | | | | | |
| | Var.23 | Var.24 | Var.25 | Var.26 | Var.27 | Var.28 | Var.29 | Var.30 | Var.31 | Var.32 | Var.33 |
| Average | 64.48 | 51.19 | 39.41 | 4.28 | 1.88 | 2.11 | 0.94 | 0.06 | 0.06 | 0.07 | 90.60 |
| Min | 60.42 | 50.00 | 36.60 | 2.95 | 1.52 | 1.62 | 0.47 | 0.00 | 0.00 | 0.00 | 88.58 |
| Max | 71.37 | 54.17 | 41.78 | 5.67 | 2.52 | 2.57 | 1.48 | 0.46 | 0.41 | 0.34 | 92.60 |
| SD | 3.71 | 1.40 | 1.57 | 1.02 | 0.32 | 0.31 | 0.34 | 0.16 | 0.14 | 0.12 | 1.35 |
| Median | 63.57 | 50.72 | 39.65 | 4.42 | 1.93 | 2.13 | 0.87 | 0.00 | 0.00 | 0.00 | 90.19 |

Strong positive and negative significant correlations (Pearson correlation coefficient more than 0.85) have been found at $p < 0.01$ (Table 4).

Table 4. Statistically Significant Correlations at $p < 0.01$*

| Var. | 1 | 3 | 4 | 8 | 9 | 10 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 27 | 30 | 31 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | | | | | | | | | |
| 6 | | | +++ | | | | | | | | | | | | | | | | | |
| 7 | | | | | | | | | | | | | | | | | | | | |
| 8 | ++ | | | | | | | | | | | | | | | | | | | |
| 9 | | | | | | | | | | | | | | | | | | | | |
| 10 | ++ | | | ++ | | | | | | | | | | | | | | | | |
| 11 | | | | | | | | | | | | | | | | | | | | |
| 12 | | -- | | | - | | | | | | | | | | | | | | | |
| 13 | | | | | - | | | | | | | | | | | | | | | |
| 14 | | | | | + | | | --- | | | | | | | | | | | | |
| 15 | | | | | | | | | | | | | | | | | | | | |
| 16 | | | | | | | | | | | | | | | | | | | | |
| 17 | | | | | | | | | | | | | | | | | | | | |
| 18 | | | | | | | | | | | | | | | | | | | | |
| 19 | | | | | | | | | | | | | +++ | | | | | | | |
| 20 | | | | | | | | | | | | | ++ | +++ | | | | | | |
| 21 | | | | | -- | | +++ | | | | | | | | | | | | | |
| 22 | | -- | | | | | | | | | | | | | | | | | | |
| 23 | | - | | | -- | | +++ | | | | | | | | | +++ | | | | |
| 24 | | - | | | | | + | | | | | | | | | | + | | | |
| 25 | | | | | | | | | | | | | | | | | -- | | | |
| 26 | | | | | ++ | | | --- | +++ | | | | | | | - | | | | |
| 27 | | | | | | | | | | +++ | | | | | + | | | | | |
| 28 | | | | | | | | | | | +++ | | | | | | | | | |
| 29 | | | | | | | | | | | | +++ | | | | | | | | |
| 30 | | | | | | | | | | | | | +++ | +++ | ++ | | | | | |
| 31 | | | | | | | | | | | | | +++ | +++ | +++ | | | | +++ | |
| 32 | | | | | | | | | | | | | ++ | +++ | +++ | | | + | ++ | +++ |
| 33 | | | | | | | | | | | | | | | | | | | | |

* "+" statistically significant positive correlation ("+"Pearson correlation coefficient < 0.89; "++" between 0.90-0.94; "+++" more than 0.95); "-" statistically significant negative correlation ("-" Pearson correlation coefficient < 0.89; "--" between 0.90-0.94; "---" more than 0.95).

## CONCLUSION

The data about the honey properties and the existing correlations are worthwhile for the quality evaluation of honey. In future studies the current one could be a reference point to identify regression models and coefficients.

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**The paper is reviewed.**