Determination of streptomycin residues in honey

Determinarea reziduurilor de streptomicină din mierea de albine

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Abstract

Honey was the first sweet substance used in human food as a precursor sugar cane or beet. Honey production by bees is a complex process of transformation, from harvesting and ending with a striking honeycomb cells. Immediately after extraction, honey is always clear and liquid. This is due to the high content of fructose, which makes some items, such as locust always remain liquid. If the glucose is higher than that of fructose, honey will crystallize at temperatures below 18 ° C. Honey is easily susceptible to heat, so they should be stored at room temperature. Bees, like all living organisms, can become ill with various diseases. These, by mortality that occur among individuals of the colony, thereby reducing the number of bees and bee families are emptied and become unproductive. In many cases, it appears significant damage resulting in large losses for beekeepers. Prevention, detection and treatment of diseases with antibiotics is an extremely important factor in ensuring the health of bee.

Keywords: streptomycin, honey

Rezumat

Mierea de albine a fost prima substanţă dulce folosită în hrana omului fiind precursoarea zaharului din trestie sau din sfeclă. Producerea mierii de către albine este un proces complex de transformare, începând cu recoltarea şi terminandu-se cu căpăcirea celulelor din faguri. Imediat după extragere, mierea este întotdeauna clară şi lichidă. Acest lucru se datorează, conţinutului ridicat de fructoză, care şi face ca unele sortimente, cum ar fi salcâmul să rămână permanent în stare lichidă. Dacă conţinutul de glucoză este mai mare decât cel de fructoză, mierea va cristaliza, la temperaturi sub 18°C. Mierea este uşor sensibilă la căldură, de aceea ar trebui să fie depozitată la temperatura camerei. Albinele, ca şi orice organisme vii, se pot îmbolnăvi de diferite boli. Acestea, prin mortalitea pe care o produc în rândul indivizilor coloniei, reduc numărul albinelor şi prin aceasta familiile de albine se depopulează, devenind neproductive. În multe cazuri, se ajunge la pierderi mari ducând la pagube însemnate pentru apicultori. Prevenirea, depistarea şi tratarea bolilor cu ajutorul antibioticelor reprezintă un factor extrem de important în asigurarea sănătăţii familiilor de albine.

Cuvinte cheie: streptomicină, miere

Introduction

Resulting from the processing of honey bees to honey sweet juices or nectar harvested from other parts of plants (hand). After transport and processing, the nectar is stored in cells in the form of honey combs, bee serving as a store of food.

The processing of nectar by bees, fructose, sucrose and glucose is inverted and the water content drops to 18-20%.

Natural Honey was within three quality grades: high quality, quality I and II quality - A high quality honey may be just the honey locust tree and forest (honeydew).

Classification of honeys is based on the origin and mode of production based and / or presentation.

Depending on the origin there blossom honey - honey produced from plant nectar and honeydew honey, honey obtained mainly from excretions of insects that feed by suction on the living plant of plants or secretions of living parts of plants

Depending on the mode of production and / or presentation are: honey-comb honey stored by bees in brood cells without larvae (juveniles) or thin wall honeycomb structure made solely of beeswax and sold in whole combs or capacity sections of such combs, pieces of comb honey - honey which contains one or more pieces of comb honey, since honey obtained by draining decapped broodless, extracted honey is honey obtained by centrifuge. When the formation of rings is found in the upper third of the honey comb capacity is estimated that honey is sufficiently mature and frames with honey may be removed from the hive.

At medium and low intensity honey extraction is carried out before the end of harvest, while the main harvest long-term extractions are repeated throughout the harvest period.

After lifting the hives, bee combs comb and go in a room temperature up to 35°C to facilitate extraction.

After this, heated combs were resorting to their opening and to honey extraction, using the extractor to remove the honey from the comb by centrifugation.

Study objectives

The purpose of this study is:

- to identify streptomycin residues in honey and
- to calculate the reproducibility of measurement results.

$$\frac{\text{absorbance std. (sample)}}{\text{absorbance std. zero}} \times 100 = \% \text{ absorbtion}$$

Materials and methods

For the investigations we used six samples of honey from the same geographical area. From each sample three samples were taken which were added 20, 30 and 40 ppb streptomycin (STP) as invigorating. Further sample preparation was performed.

Extraction: Weigh 1 g of honey plus 10 ml buffer pH = 2. Shake 10 min. honey until completely dissolved. Then the solution is centrifuged 10 min. at 3000 rpm.

Purification: C18 column using Baker. Wash column with 2 ml methanol (100%) and 2 ml distilled water. Then apply 5 ml sample flow of 15 drops per minute. Wash column with 3 ml distilled water. Column's drying for 2 min. air flow or nitrogen.

Elution: Elute the sample with 1 ml methanol in stream 15 drops per minute. It is completely evaporated (to dryness) elute to a temperature of 45^{0}C. Dissolve residue in 10 ml PBS-buffer. The analysis uses 50 µl samples. Standards and reagents are prepared according to package the kit ELISA - Enzyme Linked Immunosorbent Assay.

Test procedure:

a) insert a sufficient number of wells of microplates frame for testing standards and duplicate samples, recording their positions;

b) Add 50 µl enzyme conjugate and 50 µl sample or standard separate wells in duplicate,

c) Add 50 µl antibody to each well and incubated 120 min at room temperature,

d) Pour the liquid from the wells and beat vigorously upside down on absorbent paper to remove fluid from the wells completely

e) Fill the wells with 250 µl of distilled water and liquid flows Repeat 2 more times,

f) Add 50µl and 50µl chromogen substrate to each well shake vigorously and incubate 30 min at room temperature in the dark;

g) Add 100 µl of stopping solution to each well, shake well and measure the absorbance at 450 nm against a blank (air blank) is read within 60 min.

Results and discussion

Calculated values are stored in a standard coordinate system logarithmical compared with equivalent concentration of streptomycin in µg/kg.

Calibration curve should be virtually linear in the 2-32 µg/kg (ppb).

By extrapolating the standard curve, determine the equivalents of Streptomycin in µg/kg.

In order to achieve effective Streptomycin concentration in µg/kg of a sample, the concentration read from the standard curve must be multiplied by the appropriate dilution factor, honey 20.

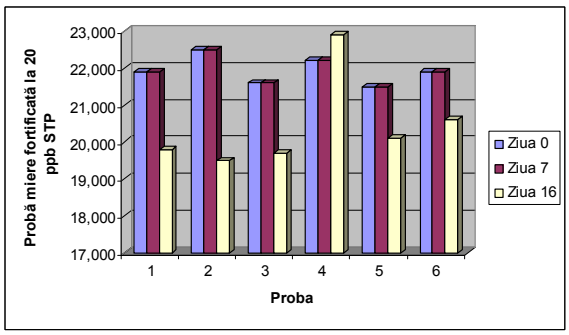

Figure 1. Evolution in the 20 ppb STP fortified sample concentration compared for three days

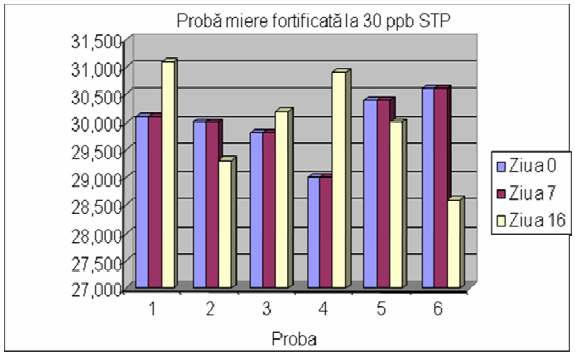

Figure 2. Evolution in the 30 ppb STP fortified sample concentration compared for three days

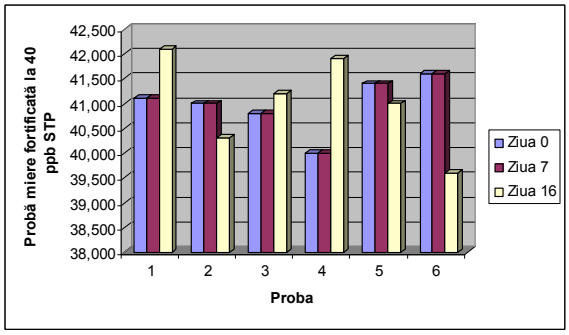

Figure 3. Evolution in the 40 ppb STP fortified sample concentration compared for three days

In the case of samples fortified with STP (Figures 1, 2 and 3) shows that at day 0 and day 7 were approximately equal amounts of antibiotic and notes the presence of additional quantities of 1933 ppb average antibiotic.

Note some changes in antibiotic content only after 16 days of storage.

Figures 4, 5 and 6 are represented on the day of the actual quantities present in STP samples. It shows the presence of antibiotic in samples fortified with 20 to 30 ppb.

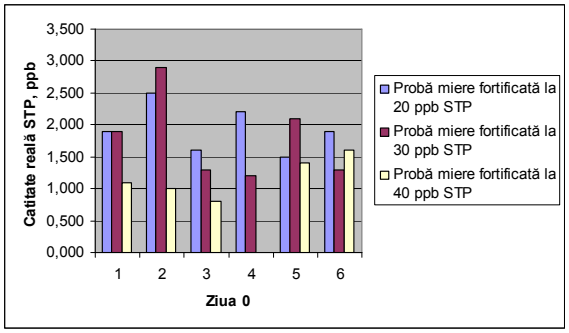

Figure 4. STP real change compared to day 0 samples fortified with 20, 30, 40 ppb

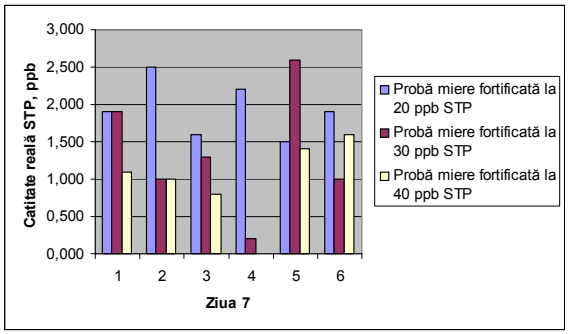

Figure 5. STP real change compared to day 7 samples fortified with 20, 30, 40 ppb

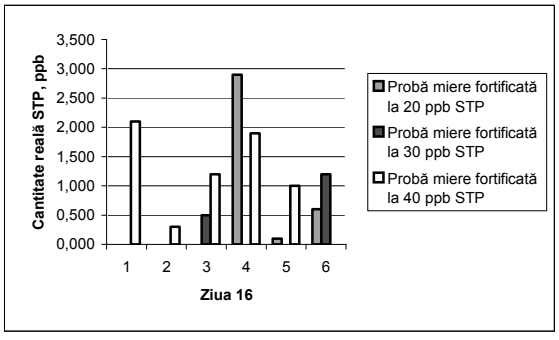

Figure 6. STP real change compared to day 16 samples fortified with 20, 30, 40 ppb

In examining the amounts of antibiotics (in our case streptomycin) in the six honey samples we used in calculating the coefficient of variation (CV %) for the case of samples fortified with 20 ppb, 30 ppb and 40 ppb. He obtained a good model, with an accuracy of 0.99 gauges.

Standard deviation is a value to a data set and is expressed as data on their media. Standard deviation, SD was calculated using the formula:

$$SD = \sqrt{\frac{n\sum x^2 - \left(\sum x\right)^2}{n(n-1)}}$$

where: n - number of tests,
x - determining the current value.

The coefficient of variation, CV:

$$CV = \frac{n \cdot \sum SD}{\sum_{i=1}^{k} x_i} \cdot 100 \quad [\%]$$

where: k = m * n,
m - number of days,
m = 3 (0, 7, 16).

The reproducibility of the measurements results, is the agreement between the closely, measurements performed by varying conditions of measurement.

To analyze and verify the quantity of antibiotic in honey samples, determined experimentally for the three concentrations of invigorating in Figure 7, the following feature:

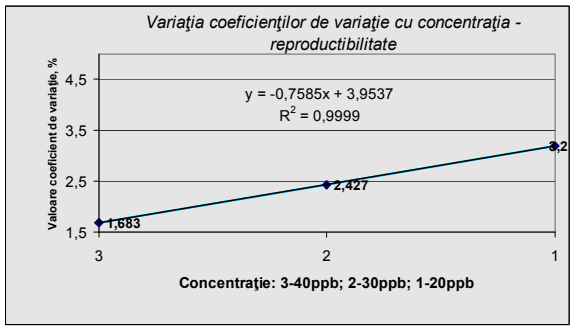

Figure 7. Evolution of the concentration coefficient of fortifier variation

Correlation coefficient of variation to the amount of fortifier added linear character shown in Figure 7 confirms that the reproducibility of the measurements are consistent with expectations.

Conclusions

From the investigations the following conclusions:

1. Accuracy of a series was determined from the results of three different tests.
2. Coefficients of variation (% CV) values obtained for the absorption are plotted

according to standards appropriate concentrations of streptomycin.

3. Small coefficients of variation provides a good test reproducibility.
4. Since the detection limit is 20 ppb for honey, I preferred that the determination be made with larger amounts of invigorating.
5. The optimal dose in terms of qualitative and quantitative strengthening STP is about 30 ppb.
6. From the analysis of honey samples collected from beekeepers in the area there is a small charge antibiotic - STP.

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