RESEARCHES REGARDING ANTIBIOTICS' RESIDUES PRESENCE IN HONEY

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Abstract: *The animal breeders must keep a balance between preserving animal health and assuring the quality and the safety for their products. Honey is a natural and complex product, resulted from flower nectar and enriched with proper substances, through intrinsic juice excreted by bees. This paper presents some aspects concerning antibiotics and honey properties.*

Keywords: *honey, antibiotics, animal health*

Introduction

Over 6 millenniums, honey has kept its fame as drug and food. Honey means a sweet liquid, gathered from flowers and other parts of plants, prepared and conserved in honeycomb. Honey is a natural and complex product, resulted from flower nectar and enriched with proper substances, through intrinsic juice excreted by bees.

World honey production is varying from one year to another, a medium quantity being approximately 700.000 tones. Honey is an aqueous solution, rich in sugary substances- until 80%- represented mainly by glucose and fructose, from flower nectar, extra flower nectar and other sources, harvested by bees and stored in combs. Enzymes introduced in nectar by bees can brake saccharose, maltose, raphinose, melibiose, the process taking place in a long period of time. In the same time, the transformation of nectar in honey is accompanied by transformation and replacing the worthless acids and excretion of overflow water content. The pH value for honey is in the range 3.5...5.5, depending on its vegetation provenience. Simultaneously with nectar transformation, in honey are being included proteins, albumin, organic acids (formic, malic, citric, acetic), mineral substances (calcium phosphates, iron phosphates, aluminum salts, iodine salts, silver salts, titanium salts, chrome salts, lead salts, iridium salts, inphinitesimal quantities), functional substances of organic origin (enzymes, catalase, inulinase), vitamins, natural antibiotics, hormones and pollen, thus honey definitely cannot be compared to any pharmaceutical preparat, honey being a live and directly assimilable substance.

Honey is known for its benefits in the field of human health. Researchers from Ottawa University have discovered that honey can destroy the bacteria responsable for chronic affection, sometime, more efficient than antibiotics. Scientists have studied the honey effect on 11 species of organisms, including *Staphilococcus* aureus and *Blue Bacillus*.

Antibiotics are used for treating different diseases, some of these substances could be founded in bee's products, inhibiting bacteria developing. Some examples of drugs that have antibiotics as component are: Locamycin; Codratyn; Mycocidine, Oxytetracycline, etc. Bees have on their body (head, thorax, abdomen, excretions of many glands) various antibiotics, that have breaking actions on bacteria, in fact existing differences between bee breeds (for example, bees from black kind having

less quantities of antibiotics comparing caucasian kind or italian breed), number of antibiotics being reduced in the course of ageing.

We present few examples of antibiotics and their recipe for administration.

Locamycin

It is used for larva and bees, 80-100g/3 times/per day, 3 days spacing and then 2 times, 5-7 days interval. Its active component is teramycine(oxytetracycline).

Oxytetracycline

It is administrated in form of syrup, 0.75 g/liter, 4-5 doses, and function strength ness of bees' family. In case of American or European loca disease symptoms, are used locamycin powdering, 80-100 g/bees' family.

Material and methods

We have examinated 20 samples of honey, from 4 countys: Bacau, Tulcea, Ialomita and Calarasi, five samples from each county. The honey examination and analysis are done for quality and purity appreciation, for controlling its depreciation and founding if it is debased or faked. Natural honey is considered the product resulted from combs centrifugation or draining, combs without eggs. Honey is *monoflowery*, derived from single flower specia nectar (acacia, tilia, sunflower), *poliflowery*, derived from natural combination of many flower specia nectar. From the point of view of sensitive appreciation honey may be considered inadequate when are found the following blemishes:

– honey with impurities;
– honey with bees' corpse fragments;
– honey with wax fragments;
– fermented honey;
– caramelized honey (by high temperatures heating);
– counterfeit honey.

The normal values for quality physical-chemical parameters for honey, according Romania legislation are:

- water content max.20%;
- ash max. 0.5% ;
- invert sugar min.70% ;
- saccharose max. 5% ;
- diastasic index min. 10.9 ;
- HMF max. 4% ;
- acidity, in ml NaOH/100g max. 4;
- relative density at 20°C 1.417 g/cm^3.

Honey shouldn't contain antibiotics residues, but are being accepted following limits:

- Betalactams max.12.5 µg/kg;
- Macrolides max. 10 µg/kg;
- Tetracyclines max. 5 µg/kg;
- Gentamycin undetectable;
- Streptomycin max. 5 µg/kg;
- Sulfonamides max. 10 µg/kg;
- Cloramphenicol absent.

The adulteration of honey together with microbiologic contamination and pesticides' presence are enough reasons for honey rejection.

The methods used for honey examination are:

- Water content by refractometer;
- Ash by calcinations method;
- Invert sugar and saccharose by Elser method;
- HMF content by spectrophotometry;
- Acidity by NaOH 0.1N titration;
- Antibiotics residues determination by CHARM II method that suppose a special preparation of honey extract and then the imunoenzymatic reaction between supposing antibiotics residues from honey and standard antibiotics marked with isotopes that are verified in CHARM analyser, using scintillation fluid.

All the samples must be accompanied by positive control and negative control and also by a fortified sample, until detection limit of the kit for determination.

After repeating the positive samples by the same method CHARM, the samples must be confirmed through another method: ELISA or HPLC.

Results and discussions

The honey samples are carefully processed before proper determination; that means Charm reading, in the presence of scintillation liquid. Thus:

- the honey extracts are analyzed in the same day of preparation;
- the honey samples with high bacteria count should not be tested;
- if the honey samples are crystallized, they should be heated in water bath, until homogenization;
- there are following aspects that might be interpretive like false positive results, that means presence of antibiotics, for example: presence of HMF (resulted from honey heating), different flower sources, like: oak, eucalyptus or presence of phenols (aspect that should be controlled by $FeCl_3$ (presence is connected with black/violet precipitate).

These honey samples, in case of false positive results must be reanalyzed by HPLC method or GC/MS method.

As we could see in the table 1, all the samples from the 4 counties are negative from the point of view of betalactams, macrolides and streptomycines residues and we found one positive sample in each group: 2 positive for sulfonamides, 2 positive for tetracycline. But these 4 samples were reanalyzed by ELISA method and were found negative. Thus, they are false positive samples; the result could be influenced by a multitude of factors.

Table1. Results for antibiotics residues in honey by CHARM method

Antibiotic Sample	Betalactams [µg/kg]	Macrolides [µg/kg]	Tetracyclines [µg/kg]	Streptomycin [µg/kg]	Sulfonamides [µg/kg]
BACAU					
1	absent	absent	absent	absent	absent
2	absent	absent	absent	absent	absent
3	absent	absent	absent	absent	absent
4	absent	absent	absent	absent	**present**
5	absent	absent	absent	absent	absent
CALARASI					
1	absent	absent	absent	absent	absent
2	absent	absent	**present**	absent	absent
3	absent	absent	absent	absent	absent
4	absent	absent	absent	absent	absent
5	absent	absent	absent	absent	absent
IALOMITA					
1	absent	absent	absent	absent	**present**
2	absent	absent	absent	absent	absent
3	absent	absent	absent	absent	absent
4	absent	absent	absent	absent	absent
5	absent	absent	absent	absent	absent
TULCEA					
1	absent	absent	absent	absent	absent
2	absent	absent	absent	absent	absent
3	absent	absent	absent	absent	absent
4	absent	absent	absent	absent	absent
5	absent	absent	**present**	absent	absent

For all the antibiotics studied, we used the validation procedure that means negative honey (pre-controlled for each drug substance) was fortified at the level of

detection limit and also under this level and upper the detection limit for verifying and re-stabling the real limit of detection for each CHARM kit, as we could see in the table 2.

The conclusion is that **15 µg/kg** represent the detection limit for tetracycline kit, for honey analyses, taht means from this value the result is constantly positive.

Conclusions

Besides honey with antibiotics residues is prohibited by UE legislation, using of antibiotics substances for bees' treatment must be done with tremendous carefullness taking account following reasons:

- antibiotics' use without discernment leads bacteria to prepare substances that annihilate their effect, antibiotics' abuse presenting a great risk;

Table 2. Results for validation procedure- tetracycline determination-honey

Nr. Crt.	Sample	Level for fortification /chlortetracycline	Result
1	Honey	5 µg/kg	Negative
			Negative
			Negative
			Negative
			Negative
			Negative
2	Honey	10 µg/kg	Negative
			Negative
			Negative
			Negative
			Negative
			Negative
3	Honey	15 µg/kg	Positive
			Positive
			Positive
			Positive
			Positive
			Positive
4	Honey	20 µg/kg	Positive
			Positive
			Positive
			Positive
			Positive
			Positive
5	Honey	25 µg/kg	Positive
			Positive
			Positive
			Positive
			Positive
			Positive
6	Control	-	Negative
			Negative
			Negative
			Negative
			Negative
			Negative

– besides that, all antibiotics present somewhat toxicity, reducing treated bees life and having bad influence on hemolympha;
– when antibiotics are abusive administrate, it is produced a brutal sterilization of intestine and distruction of some microorganisms;

Bees' healing is more confident with complete hygiene and keeping strong and well maintained families; bees' families with incurable illness must be found, as soon as possible, and destroyed;

Antibiotics abuse becomes dangerous not only for bees but for people, that will consume their products, contaminated with such substances. Using of antibiotics is forbidden and honey with antibiotics residues over European limits should be refused for exportation. Thus, beekeepers must be very careful with antibiotics administration for production families, because antibiotics residues remain also in the combs from treated beehive. This is why it is recommended changing of all combs, after 2 years.

The drugs' utilization is, unfortunately, unavoided and sometimes imperiously necessary, but this generates many problems concerning the processing of materials originated from treated animals, in our example the bees.

The animal breeders must keep a balance between preservation animal health and assurance the quality and the safety for their products.

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