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QUALITY OF ANIMAL PRODUCTS

IDENTIFICATION OF SULFONAMIDES IN COMMERCIAL HONEY

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Abstract

The contamination of honey, bees and hives are being an issue for discussion and critics from all communities, especially in tropical countries where due to hot climate conditions and higher tendency to food contamination the use of agrochemicals in agriculture systems are intense, as well as the use of antibiotics and food preservatives. In this work, a commercial kit for identification and quantification of sulfonamides (antibiotic class) in honey collected from local markets was used. Two samples of labeled organic honey were spiked with sulfonamide to validate the assay. Twelve commercial samples were analyzed and eleven presented residues of sulfonamides, ranging from 3.46 ug per kg of honey (result of organic sample) to 10.9 ug per kg of honey, from commercial honey. The method presented correlation of 0.92 (r); and specificity of 0.1 ug per kg of honey. The recovery tests using spiked samples with 10 and 5 ug per kg of honey , gave the recovery results of 95.5% and 82.0%, respectively. Although the contaminated samples have sulfonamides concentration lower than security limit, the honey analyzed (commercial and organic labeled samples) were no free from sulfanomides, which shows that honey has contamination problems, being anissue for all consumers, which includes children.

Key-words

sulfonamide, antibiotic, food safety, ELISA

IDENTIFICAÇÃO DE SULFONAMIDAS EM MEL COMERCIAL

Resumo

A contaminação do mel, abelhas e colmeias tem sido motivo de discussão critica principalmente em países tropicais, onde, devido às condições climáticas quentes e maior tendência à contaminação de alimentos, o uso de agroquímicos, antibióticos e conservantes alimentares são um problema. Neste trabalho foi utilizado um kit comercial para identificação e quantificação das sulfonamidas (antibióticos) em amostras de mel coletadas no comercio local. Duas amostras rotuladas como mel orgânico foram fortificadas com sulfonamidas para validar os imunoensaios. Das doze amostras comerciais de mel, todas apresentaram resíduos de sulfonamidas, variando de 3,46 ug por kg de mel (resultado da amostra de mel rotulado orgânico) até 10,9 ug por kg de mel, em amostra de mel comercial. O método apresentou correlação de 0,92 e especificidade de 0,1 ug por kg de mel. Os testes de recuperação de amostras fortificadas com 10 e 5 ug por kg de mel foi de 95,5 a 82,0%, respectivamente. Embora as amostras contaminadas tenham quantidades de sulfonamidas inferiores aos limites de segurança, o mel analisado (amostras comerciais e mel rotulado orgânico) contem sulfonamidas, o que mostra que o mel tem problemas de contaminação, sendo um problema de segurança alimentar para todos os consumidores, incluindo crianças.

Palavras chave

sulfonamidas, antibióticos, segurança alimentar, ELISA

INTRODUCTION

The contamination of agriculture with agrochemicals products and antibiotics is a very serious problem that needs to be solved. Bee products, such as honey, pollen, propolis are widely consumed as food and used as medicine and their contamination may carry serious people health problems. Ingestion of honey with no source of certification can be a safety issue, especially for children and infants. Honey and its co-products not subjected for analysis and sterilization should not be used for children or for medicinal purposes (AL-WAILI et al, 2012). The use of antibiotics in Bee and Honey production system is due to brood infestations and diseases. The Africanized honey bee of Brazil (COBEY et al, 2011; STRAUSS et al., 2016) is one of the few bees that disease and infestation, especially with varroosis does not demand treatment by Brazilian beekeepers, neither have relevant colony losses or economic impact (COBEY et al., 2011; STRAUSS et al., 2016,).

The reason for this phenomenon still with no explanation but is probably resulting from natural selection from which beekeepers regularly trap swarms.

Bee products, including honey, are polluted via different sources of contamination such as pesticides, heavy metals, bacteria, antibiotics and radioactive materials. According to European Union regulations, honey as a natural product must be free of any chemicals (EEC, 1974). Antibiotics used in honey and other bee products are usually those used in a veterinary setting, such as streptomycin, sulfonamide, and chloramphenicol. Obviously, beekeepers use antibiotics at relatively high doses to treat infections, or at low doses as "growth promoters. Maximum residue limits (MRLs) have been established for the majority of food produced by animals treated with sulfonamides and tetracyclines. However, there are no MRLs for bee products such as honey. Honey is traded internationally, and countries generally accept standards set by the Codex Alimentarius (TILLOTSON et a.l, 2013). Antibiotics are found in honey because they are used in apiculture for treatment of bacterial diseases or during hive formations to prevent it. Because of its wide spread use, there are reports of tetracycline resistance and so, antibiotics such as erythromycin, lincomycin, monensin, streptomycin, and enrofloxacin are also being use in bee and hives treatments. In 2000-2001, streptomycin was detected. Nectar and honey samples collected from bee hives during the peak flowering seasons of rubber and banana plantation crops in southern part of Tamil Nadu were analyzed for antibiotic residues. These samples showed 4–17and 11–29 ng per kg−1 of streptomycin, 2–29 and 3–44 ng *per* kg−1of ampicillin, and 17–34 and 26–48 ng/ kg of kanamycin, respectively, according to Al-Waili et al. (2012). The use of antibiotics in beekeeping is illegal in some EU countries. However, some countries like Switzerland, UK,

and Belgium, have established level of antibiotics in honey beyond which the sample is deemed noncompliant, which lies between 0.01 to 0.05 mg/kg for each antibiotic group. For Sulfonamides, the limit ranges from 20 to 50 ug per kg of honey (DUBREIL-CHÉNEAU et al., 2014; OLIVEIRA et al., 2019)

Antibiotic residues in honey have become a major consumer concern. Some drugs have the potential to produce toxic reactions in consumers directly while some other is able to produce allergenic or hypersensitivity reactions (PETRELLI et al., 2019). Long-time exposure to antibiotic residues can include microbiological hazards, carcinogenicity, reproductive problems, and teratogenicity. Antibiotic resistance is a global public health problem and continues to be a challenging issue. The US Centers for Disease Control and Prevention (CDC, 2000) has described antibiotic resistance as "one of the world's most pressing health problems, due to "the number of bacteria resistant to antibiotics and many bacterial infections are becoming resistant to the most commonly prescribed antibiotic treatments".

Sulfonamides are a group of antibiotics discovered in 1932, known as Sulfa Drug, widely used and since World War II and known also for causing a lot of allergenic symptoms in sensible individuals.

MATERIAL AND METHODS

The tests were conducted at the Sugar Cane Laboratory, at Polo Regional Centro Sul-APTA/SAA, Piracicaba, SP.

Sampling: Ten honey samples were collected from local markets, representing different brands and types: honey labeled organic (sample 1), honey with honeycomb (sample 3), both from Piracicaba local market. Other honey samples were collectd from local markets at Rio das Pedras (samples 2 and 4) and from Piracicaba (samples 5 to 8); samples 9 and 10 from Bee Itatiba Farm belonging to US; samples 11 and 12 were honey organic labeled spiked with sulfonamide-like products. To 2g of sample it was added 1mL of PBS (Phosphate Buffer Solution) and added 7mL of acetonitrile ethyl acetate. After mixing samples were centrifuged at 4000 rpm for 5 minutes. Four mL from the upper clear organic phase were dried at 50^0C. The dry residue was dissolved in n-hexane, mixed with 1 mL of PBS Buffer. Centrifuged for 5 minutes at 4000rpm. The upper layer hexane phase was discharged and 50 uL was used for ELISA test.

A commercial kit for detection of sulfonamides was used (Elabscience, cat E- FS-E040), presenting standard curve and 2 sulfonamide-like products were used to spike samples: Veterinary commercial antibiotics Trissulphin (sulfamethoxine) and Triazoclin (sulfodiazine), at a concentration of 10 and 5 ppb, respectively.

The assay procedure was done adding 50 µL of Standards or Sample per well, then

adding 50µL of HRP Conjugate and 50 µL of antibody working solution to each well. The plate was sealed, mixed gently and incubate at 25^{0}C for 45min.The sealer was carefully removed, the liquid in each well was dispensed. Immediately 300µL of wash buffer was added to each well and this step was repeated for 5 times, 30 seconds. The plate was dried by inverting it against a thick clean absorbent paper. For Color development, 50 µL of Substrate Reagent A and 50 µL of Substrate Reagent B was added to each well. Gently oscillated for 5 sec to mixed thoroughly. Then it was incubated at 25^{0}C for 15 min with shading light. Reaction was stopped by addition of 50 µL of stop solution to each well, oscillated gently to mixed thoroughly. To determine the optical density (OD value) of each well at 450 nm a micro-plate Smart Reader Accuris was used. Samples were run in triplicate.

In the analytical method validation tests, the parameter evaluated were: accuracy (evaluated with the spiked recovery %); precision (evaluated by the variation coefficient, correlation coefficienty (R) value and sensibility (determined by the lower point of the calibration curve that could detect the antibiotic) (SANTOS et al, 2011).

RESULTS AND DISCUSSION

With the Competitive ELISA kit, a standard curve was built in order to calculate the sulfonamide residues in the samples tested. Figure 1 shows sulfonamides residues levels in all honey tested, including the organic labeled sample (Table 1).

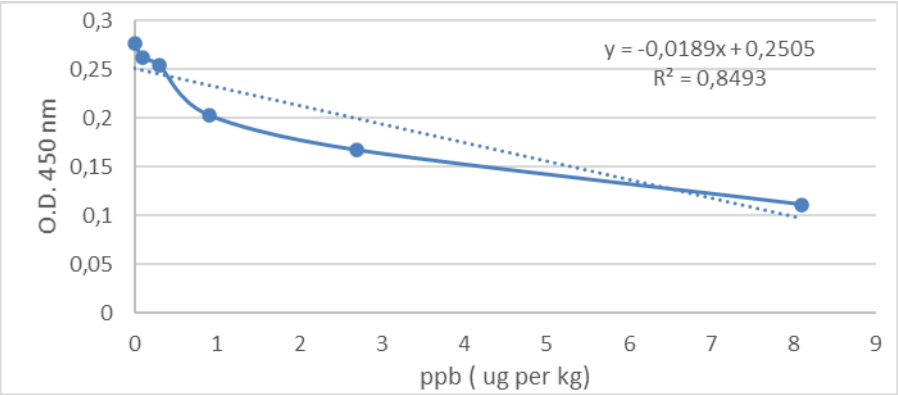

Figure 1. Standard curve, with concentrations expressed as ppb, according to fabricant (ug of sulfonamide per kg of honey) tittered by Competition ELISA kit, at 450 nm.

Recovery percentage, from spiked samples were 95.5% and 82.02 % for sulfamethoxine and sulfadiazine additions, respectively. Accuracy and precision were criticized based upon % of recovery and Variation Coefficient, respectively (Table 2).

All samples tested showed some amounts of residues, even the honey with organic label (sample 1). The method sensibility was 0.1 ppb (ug of antibiotics per kg of honey). Despite the minor sulfonamides amount measured in all samples, is indicative of the presence

Table 1. Honey samples run in EIA test for Sulfonamides residues. (*honey organic labeled; spiked samples (sample 11* with 10 ppb of sulfadimethoxine and sample 12** spiked with 5 ppb of sulfadiazine). Results expressed in ppb of sulfonamides residues in honey samples. O.D. were adjusted according to equation on Figure 1.

Honey Samples	O.D.	ppb
1	0.185	3.466
2	0.047	10.767
3	0.065	9.815
4	0.051	10.556
5	0.094	8.280
6	0.043	10.979
7	0.049	10.661
8	0.053	10.450
9	0.059	10.132
10	0.048	10.714
11**	0.07	9.550 *
12***	0.173	4.101 *

Table 2. Accuracy and precision of the assay using spiked honey samples. VC is variation coefficient.

	standard deviation	% recuperation[1]	VC[2]
Honey11 - 10ppb	0.01	95.500	0.900
Honey12- 5ppb	0.008	82.000	

1- used to criticize accuracy
2- used to criticize precision

Table 3. Sensibility test according to standard curve of antibiotics, in the fabricant manual. Concentration values expressed in ug of antibiotics per kg of honey (ppb).

standard ppb	O.D at 450 nm
0	0.277
0.1	0.262*
0.3	0.254
0.9	0.203
2.7	0.167
8.1	0.111

* sensibility

of antibiotic residues in the beehives These results should be considered as an alert to the need for quality control of all beekeeping products, such as wax, bee venom, pollen, and royal jelly, which are used either as dietary supplements or as medicinal remedies, especially for infants. Residual levels of contaminants can be changed through various production techniques; therefore training beekeepers in good managing practices and monitoring the quality of bee products is required. Only with this line of action can beekeepers enter in the market competition on these products since products comply with quality assurance and certification protocols and legislation (Al-WAILI et al, 2011).

CONCLUSIONS

All honey samples tested presented residues of Sulfonamides. From the analytical

methodology validation study results were obtained for the following parameters: sensibility of 0.1 ug per kg of honey; Precision and accuracy were high: 89% and 0.9 of variation coefficient, respectively. The overall results showed that a National Bee Program should be established to train beekeepers in the bee handling in order to guarantee the quality and food safety of the hive products.

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