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Effect of pasteurization and storage on tetracycline levels in honey

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1. INTRODUCTION

The occurrence of residues of antibiotics in foodstuffs, arising from its veterinary use, represents a potential danger for consumers worldwide, and is related to the appearance of allergic reactions, development of bacterial resistances, modifications in intestinal flora, and possible mutagenic and/or carcinogenic power (Demoly and Romano 2005; Andersen et al. 2005).

In apiculture sanitation, the tetracyclines, antibiotics of wide spectrum that prevent the growth of bacteria by inhibition of protein synthesis, are shown to be effective in controlling American foulbrood disease (AFD) and European foulbrood disease. AFD, caused by the spore-forming *Paenibacillus larvae*, is the most widespread and destructive of the bee brood diseases. The spore stage is infectious to honeybees. Larvae up to 3 days old become infected by ingesting spores that are present in their food. Spores germinate in the gut of the larvae and the vegetative form of the bacteria begins to grow. Comb cells containing infected larvae are discolored, sunken, and have punc-

tured capping; the dead larvae have a soft, sticky, andropy consistency.

Moreover, tetracyclines can be sprayed directly to fruit trees or other plants to treat infection by *Erwinia amylovora* and *Mycoplasma* and used to control infection of seeds by *Xanthomonas campestris*. So, the contamination of the plant with high concentrations of antibiotic implies the risk of residues into hive products (Levy 1992).

Tetracyclines are not authorized for the treatment of honeybees in the EU, although some countries, based on the cascade system, include tetracyclines on the list of registered products. As an example, the use of oxytetracycline for the treatment of European foulbrood with the withdrawal period of at least 6 months is allowed in the UK (EMA/28057/2010). The cascade system allows the use of veterinary medicines for bees authorized in other countries. However, there are some obstacles for its application: cascade provisions are for exceptional use, a maximum residue level (MRL) is required for a substance to be used under the cascade, and it is also necessary to have an MRL in honey to allow for residue control (Committee on the Food Chain and Animal Health 2007; EMA/28057/2010).

However, it is certainly the case that tetracyclines are authorized for the treatment of honeybees in

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many third countries. This situation may potentially raise some problems with imports of honey into the EU.

No MRLs for tetracyclines have been established in honey (Regulation (EC) No 470/2009 and Regulation (EU) No 37/2010). In consequence, the presence of any detectable residues of tetracyclines in honey would mean that the honey cannot legally be commercialized in the EU. In other words, there is zero tolerance for the presence of tetracycline residues in honey. In 2008, the European Committee for Medicinal Products for Veterinary Use, in response to the request from the European Commission, reconsidered its previous opinion regarding the establishment of maximum residue limits for oxytetracycline in honey and agreed to recommend the establishment of a provisional maximum residue limit for oxytetracycline in honey. However, this MRL has not been set in the Regulation (EU) (No 37/2010).

In the absence of an EU MRL for tetracycline in honey, the limit of quantification may be used as the action level (the concentration of residue in food that results in action being taken). In the case of prohibited substances, any confirmed residue will be reported as in excess of the action level.

Related to this aspect, the Food Standards Agency of the UK (FSA 2010) has sought opinion from the European Commission and other competent authorities in the UK regarding setting a suitable Action level for oxytetracycline (OTC) residues in honey. Three options have been discussed: 25 µg/kg according to the FSA risk assessment; 20 µg/kg as recommended by the Community Reference Laboratory for screening for tetracycline residues in honey; and not detectable (i.e., below limit of detection). It remains to be decided as to what would constitute an acceptable limit of detection.

In order to evaluate the human risk derived from the occurrence of residues of antibiotics in foodstuffs, the joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee of Food Additives and Contaminants (JECFA), established in 1998 a group acceptable daily intake (ADI) of 0–

0.03 mg/kg body weight for oxytetracycline, tetracycline, and chlortetracycline. Therefore, it is important that analytical methods used in antibiotic residue control plans are as sensitive and reliable as possible in order to provide assurances that honey commercialized in the EU will comply with community rules.

In this context, there is a continuous need of reliable analytical methods to use in determining compliance with national regulations as well as international requirements. Many methods have been developed for the determination of tetracyclines (TCs) in honey: microbiological inhibition tests (Jinbo et al. 1992), rapid tests for antibiotic screening, Tetrasensor (Unisensor, Lieje, Belge; Reybroeck et al. 2007), and Biacore QFlex Kits (Biacore International SA, Neuchatel, Switzerland).

Besides single-class methods, multi-class methods using LC–MS/MS (Alfredsson et al. 2005; Huq et al. 2006; Hammel et al. 2008; López et al. 2008; Pancorbo et al. 2008), enzyme immunoassay (Heering et al. 1998; Pastor-Navarro et al. 2007; Jeon and Paeng 2008), spectrophotometry (Salinas et al. 1991), flow-injection chemiluminescence (Pena et al. 2000; Kaczmarek and Lis 2009), high-performance liquid chromatography (HPLC; Viñas et al. 2004; Guo-Hui et al. 2005; Li et al. 2008; Sun et al. 2009; Tayar et al. 2010), and capillary electrophoresis (Nakazawa et al. 1999) are also used for the analysis of TC residues in honey.

Several studies have shown the presence of tetracycline residues in honey. In Belgium, Reybroeck (2003) found 18% of samples contaminated with tetracycline. In a study carried out in Spain by the National Association of Consumers and Users, 5% of samples analyzed contained residues of tetracycline (OCU 2003). However, Pena et al. (2005) did not detect residues of oxytetracycline and tetracycline above detection limits (50 µg/kg) in Spanish and Portuguese honeys, nor Viñas et al. (2004) in Spain, detected levels of OTC, TC, chlortetracycline (CTC), and doxycycline (DC) above detection limits (30 µg/kg; Viñas et al. 2004). In other countries, like Japan, Jinbo et al. (1992) detected chlortetracycline

and oxytetracycline (7% of positive samples). Sabatini et al. (2003), in Italy, detected oxytetracycline and tetracycline in 4% of samples at levels up to 1.0 $\mu\text{g}/\text{kg}$ and Jürgens (1981) in a study of Romanian, Spanish, and Mexican honeys detected tetracycline residues at levels of 1.5–5.1 $\mu\text{g}/\text{kg}$ (6% positive samples).

Pasteurization and storage of honey represent two important steps of the commercial chain that could influence its quality. Pasteurization is usually applied to prevent fermentation or delay granulation. Honey can be stored at different times during processing and exposure in the market. Little information is available concerning the effect of pasteurization and storage conditions on tetracycline residues in honey. Recently, Tayar et al. (2010) evaluated the stability of the tetracyclines during 60 days of storage in commercial honey samples. The residue levels of OTC, TC, and CTC were reduced 97%, 76%, and 71%, respectively.

In relation with the effect of storage conditions, Argauer and Moats (1991) reported a high percentage of oxytetracycline decrease (50%) in honey stored at 34°C. Similar results are reported by Matsuka and Nakamura (1990) in honey stored at 35°C and 25°C.

Analytical methods to determine residues of tetracyclines in honey have been published and validated. None of them are official, which may be due to the complexity of the analysis of these contaminants in a complicated matrix such as honey.

The aim of this study was to determine the contamination of honey samples by the tetracyclines OTC, TC, CTC, and DC after pasteurization and under different storage conditions by using a reliable analytical method based on solid phase extraction (SPE) and HPLC.

2. MATERIALS AND METHODS

2.1. Materials and reagents

Analytical standard-grade of tetracycline ($\text{C}_{22}\text{H}_{25}\text{ClN}_2\text{O}_8$), chlortetracycline ($\text{C}_{22}\text{H}_{24}\text{Cl}_2\text{N}_2\text{O}_8$), oxytetracycline ($\text{C}_{22}\text{H}_{25}\text{ClN}_2\text{O}_9$), and doxycycline ($\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8\text{HCl}\cdot 0.5\text{H}_2\text{O}\cdot 0.5\text{CH}_3\text{CH}_2\text{OH}$) standards

were purchased from Vetranal (Riedel-de Haën, Germany). Standards were dissolved in pH 3 acetonitrile/oxalic acid (15:85; v/v). HPLC analysis grade ethyl acetate, acetonitrile, and methanol were provided by Labscan (Dublin, Ireland). Oxalic acid ($(\text{COOH})_2\cdot 2\text{H}_2\text{O}$) was supplied by Panreac Química S.A. (Barcelona, Spain). EDTA ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8\cdot 2\text{H}_2\text{O}$), Na_2EDTA -McIlvaine buffer (pH 4), sodium phosphate dibasic (Na_2HPO_4), and citric acid ($\text{C}_6\text{H}_8\text{O}_7\cdot \text{H}_2\text{O}$) of analytical standard grade were from Panreac Química S.A. (Barcelona, Spain). Ultrapure water was prepared using a Milli-Q water purification system (Bedford, MA, USA). For solid-phase extraction, C_{18} (EC) 6 mL cartridges containing 0.5 and 1 g of octadecylsilica and 0.5 g of phenyl were from Isolute (International Sorbent Technology, Hengoed, UK). Ultrapure nitrogen C_{55} was supplied by Carburos Metálicos (Barcelona, Spain).

2.2. Apparatus

An analytical balance CP 224 S Sartorius (Goettingen, Germany), Temperature Bath Tectron P Selecta (Barcelona, Spain), Tembloc oven P Selecta (Barcelona, Spain), Vibratory stirrer TK3S Techno Kartell (Milan, Italy), MS2 Minishaker IKA (Staufen, Germany), and pH meter Crison GLP 21 (Barcelona, Spain) were used for preparation of samples. An SPE vacuum manifold for solid-phase extraction was supplied by Supelco (Bellefonte, USA). HPLC was performed on a Hewlett-Packard 1100 apparatus from Agilent (Karlsruhe, Germany) with a 20 μL loop and a photodiode array detector (DAD). The injection was manual and a chromatographic column ACE C_{18} (250 \times 4.1 mm I.D. \times 5 μm ; Advanced Chromatography Technologies, Aberdeen, UK) was used.

2.3. TC evolution study

Changes in tetracycline residue levels in spiked honeys were studied in two different experiments: after laboratory pasteurization (78°C/1 min) and during storage (room temperature/darkness at 30, 90, and 180 days).

2.3.1. Samples

Eleven raw Spanish multifloral honeys, with rosemary as the major component, were used for the study. Honey samples were directly provided by

beekeepers, and analyzed in our laboratory to ensure that none contained tetracycline residues.

Two hundred fifty grams were collected from each sample of raw honey and settled into a glass pot with a metal screw. Each sample was spiked with 1 mL of an acetonitrile–oxalic acid (15:85) solution of oxytetracycline, tetracycline, chlortetracycline, and doxycycline of 125 µg/mL. The final TC level in the samples was checked (487–494 ng/g honey).

2.3.2. Pasteurization study

Samples of spiked honeys ($n=11$) were subjected to laboratory pasteurization (78°C/1 min) by introducing them in a preheated Tembloc oven, so that honey samples quickly reached the pasteurization temperature. Immediately after treatment, they were cooled at room temperature in an ice bath. Samples were then analyzed for TC residue levels.

2.3.3. Storage study

Spiked honey samples ($n=11$) were divided in three 50-g subsamples, settled into a glass pot with a metal screw and stored at room temperature (20°C ± 5°C), in darkness. One 50-g subsample was analyzed at 30, 90, and 180 days.

2.4. Optimization of the analytical method for the determination of TC residues in honey

The method is based on that described by Oka et al. (1987) and consists of four different steps: a honey sample preparation, followed by an extraction of analytes, a clean-up of the extract, and the determination of the analytes. Diverse modifications were carried out in our laboratory in order to optimize the analytical method. The following parameters were tested in our laboratory: (a) solid phases (0.5 and 1.0 g of C_{18} , and 1 g of phenyl), (b) elution solvents (acetonitrile, 20:80 acetonitrile/oxalic acid, 15:85 acetonitrile/oxalic acid, ethyl acetate, 95:5 ethyl acetate/methanol, 90:10 ethyl acetate/methanol, and 80:20 acetate/methanol), and (c) elution solvent volumes (6, 10, and 13 mL of 90:10 ethyl acetate/methanol) for the SPE. For the chromatographic analysis, (a) isocratic (acetonitrile/oxalic acid in

different proportions, and 1:1.5:3 methanol/acetonitrile/oxalic acid) and gradient elution (methanol/oxalic acid and acetonitrile/oxalic acid), as well as (b) different pH (2.5, 3.0, and 3.5) of the mobile phase have been assayed.

Briefly, the optimized method was as follows. After homogenizing the sample, 3 g of honey were weighed and dissolved in a 6 mL EDTA-McIlvaine buffer (pH 4). The resultant solution was homogenized in a vibratory stirrer at 800 rpm for 10 min. The homogenized sample was loaded onto a column of 1 g of C_{18} , previously conditioned and activated with acetonitrile (5 mL), oxalic acid (pH 3; 5 mL), and saturated EDTA solution (5 mL), surrendering to a solid phase extraction. The elution solvent consisted of 90:10 ethyl acetate/methanol (13 mL). Eluate was concentrated under vacuum and the residue was dissolved with 1 mL acetonitrile–oxalic acid to pH 3 (15:85), solvents that later will be used as mobile phase in the chromatographic determination by HPLC.

Chromatographic conditions Flow mobile phase, 1 mL/min; C_{18} column at 25°C; wavelength, 357 nm; time of analysis, 25 min; mobile phase, solvent A, oxalic acid 0.01 M pH 3; solvent B, acetonitrile; gradient elution (solvent B), time 0, 15%; 3 min, 12%; 13 min, 20%; 23 min, 25%; 25 min, 15%.

2.5. Method validation

Food and Drug Administration (FDA) criteria (FDA 2000) have been followed to validate the method, including studies of linearity, precision (repeatability and reproducibility), accuracy and sensitivity (detection limit and quantification limit). Fortified honey samples, previously analyzed for checking the absence of TC residues, were used for precision and accuracy studies. The same matrices were used as method blank, which serves to determine if analytes or any interference are present in the laboratory environment, reagents, or apparatus.

The detector's linear range has been calculated as the range of analyte weight over which the sensitivity of the detector is constant to ±5.0%, as determined from a linearity plot of response/weight vs. log weight. Studies of repeatability, reproducibility, and

recovery were also carried out. The analyses were always performed in duplicate. For the repeatability study, 18 samples were spiked at 350 ng/g honey and then analyzed by the same operator, with the same equipment, in the same laboratory, on the same day. Conditions for reproducibility study were as follows: analysis of 27 samples (spiked at 350 ng/g honey), same operator, same equipment, same laboratory, three different days within 2 weeks. Finally, the recovery study ($n=10$ samples), was carried out by spiking extracts at two concentration levels, 50 and 350 ng/g, and then samples were analyzed.

Analytical sample batches were constituted by a calibration standard solution (at three levels), a method blank, a spiked sample to check the ongoing precision and recovery, and finally, a set of four to six samples to be analyzed.

The quality of analytical data was also assured by participation in international intercomparison exercises.

2.6. Statistical analysis

A descriptive statistical analysis was applied to the validation data. A Student's t test was used to evaluate significance of differences between TC residue levels after pasteurization treatment or storage.

3. RESULTS AND DISCUSSION

3.1. Optimization and validation of the analytical method

The best results for the optimization of the method were achieved with 1.0 g of C_{18} as solid phase for SPE (recoveries of TC residues between 82% and 99%) and 13 mL of 90:10 ethyl acetate/methanol for the elution solvent (recoveries up to 98%). Best chromatographic conditions were 0.01 M oxalic acid (solvent A) and acetonitrile (solvent B) at pH 3 as mobile phase with the following gradient elution: Solvent B at time 0, 15%; 3 min, 12%; 13 min, 20%; 23 min, 25%; 25 min, 15%. Figure 1 shows tetracyclines 3D spectra according to the described method, in which optimal resolution can be observed.

Most researchers obtained good results using the non polar C_{18} solid-phase (Guo-Hui et al. 2005; Viñas et al. 2004; Pagliuca et al. 2002; Münstedt et al. 2002; Oka et al. 1994; 1993; Takeba et al. 1984). However, Nakazawa et al. (1999) used the polar solid-phase ENV⁺, while other researchers use a SPE column tandem (Pena et al. 2005; Geertsen and Pederson 2000;

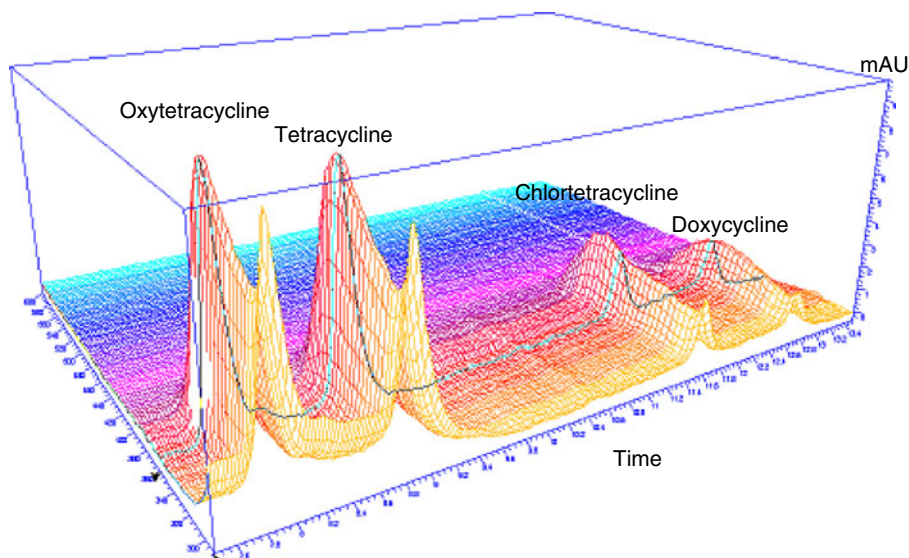


Figure 1. 3D absorption spectra of oxytetracycline, tetracycline, chlortetracycline, and doxycycline.

Oka et al. 1987, 1994). Regarding the amount of packing material, 500 mg cartridges are mostly used, although other authors reported the use of 60 mg (Pena et al. 2005), 100 mg (Oka et al. 1987), or 200 mg of packing material (Pagliuca et al. 2002; Oka et al. 1987).

As for the elution solvent, acetonitrile, methanol, oxalic acid, water and ethyl acetate, individually or mixed, are the mostly employed, depending on the class of tetracycline analyzed and the packing material used for the SPE (Guo-Hui et al. 2005; Pena et al. 2005; Viñas et al. 2004; Pagliuca et al. 2002; Münstedt et al. 2002; Geertsen and Pederson 2000; Nakazawa et al. 1999; Oka et al. 1987, 1994; Takeba et al. 1984).

For the chromatographic analysis of tetracyclines, an acid/organic solvent solution (80:20) pH 2–4 is commonly used as mobile phase, being methanol, acetonitrile or both on them the organic solvents chosen (Guo-Hui et al. 2005; Viñas et al. 2004; Pagliuca et al. 2002; Geertsen and Pederson 2000; Nakazawa et al. 1999; Oka et al. 1994; Jürgens 1981).

Validation results are indicated in Table I. Results of linearity obtained in our laboratory ranged between 5 and 500 ng/g honey for DC and CTC and between 5 and 700 ng/g honey for OTC and TC. Accuracy and precision results satisfy the international criteria (FDA 2000; Horwitz and Albert 1996). Recoveries obtained in spiked samples are very high for all tetracyclines (82–102%) for both tested levels, particularly for assays carried out at 350 ng/g honey

(around 100%). This is in agreement with the FDA that accepts recovery-ranges between 80% and 110%, and the current European regulations that accept 70–110% (Commission Decision 2002). Recovery results reported in literature range between <80% (Nakazawa et al. 1999) and 81–97% (Oka et al. 1987). Precision results, expressed as relative standard deviation (RSD), are also very good and were always less than 9%, according to the recommendations of Horwitz and Albert (1996; RSD<15% for the repeatability and RSD<31% for the reproducibility, respectively).

The detection and quantification limits for the four analytes were 1 and 5 ng/g honey, respectively, which indicates a good sensitivity of our method, similar to those obtained by Guo-Hui et al. (2005). Other methods reported higher detection and quantification limits (15–50 and 24–72 ng/g honey, respectively; Geertsen and Pederson 2000; Münstedt et al. 2002; Pagliuca et al. 2002; Viñas et al. 2004; Pena et al. 2005).

3.2. Study on the effect of pasteurization treatment on tetracycline residues

Levels of antibiotic residues in honey before and after the pasteurization treatment were compared. The analysis of honey samples after pasteurization revealed a decrease of all tetracycline levels from 10% to 18%. Specifically, tetracycline levels decreased from 490.73 to 441.66 ng/g honey (10.2% of reduction) for oxytetracycline, from 493.45 to 401.18 ng/g

Table I. Validation study results.

	Lineal range (ng/g honey)	Repeatability (% RSD)	Reproducibility (% RSD)	Recovery (%)		DL (ng/g honey)	QL (ng/g honey)
				50 ng/g honey	350 ng/g honey		
OTC	5–700	4.4	8.9	99.1	99.6	1	5
TC	5–700	6.9	8.4	98.9	102.4	1	5
CTC	5–500	7.7	8.4	95.8	102.2	1	5
DC	5–500	7.3	8.6	82.2	100.4	1	5

OTC oxytetracycline, TC tetracycline, CTC chlortetracycline, DC doxycycline, DL detection limit, QL quantification limit, RSD relative standard deviation

Table II. Evolution of tetracycline levels in honey during storage at 20°C±5°C and darkness.

Time of storage (days)	OTC (ng/g honey)	TC (ng/g honey)	CTC (ng/g honey)	DC(ng/g honey)
0	490.73	493.45	490.73	486.82
30	410.45	391.73	381.55	386.18
90	258.73	256.09	169.00	130.73
180	85.36	105.00	71.91	57.09

honey (18.7%) for tetracycline, from 490.73 to 426.94 ng/g honey (13.0%) for chlortetracycline and, finally, from 486.82 to 437.65 ng/g honey (10.1%) for doxycycline. The statistical analysis (ANOVA test) demonstrated that these reductions were significant ($P=0.0001$). To our knowledge, there are no similar studies in honey.

As most European countries have zero tolerances, even the low concentrations of residues detected after pasteurization (401.18–441.66 ng/g honey) cannot be accepted.

3.3. Study on the effect of storage time in darkness on tetracycline residues

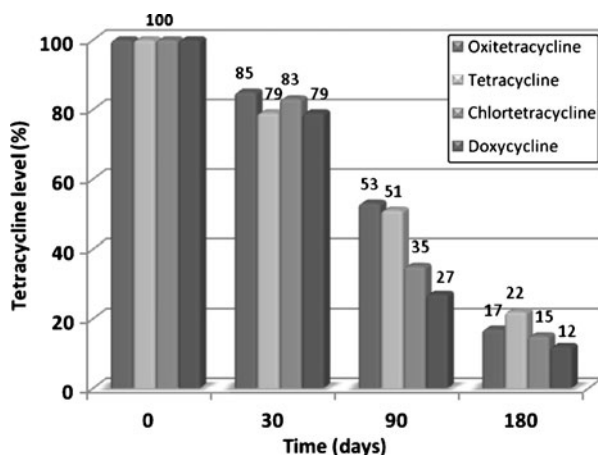
Results of the effect of storage time on tetracycline levels are shown in Table II. A continuous decrease was observed for all tetracyclines. At day 30, a slight decrease (15–21%) similar for all tetracyclines was observed. This

trend continued after 90 days, although was most pronounced for CTC and DC, which showed reductions of 65% and 73%, respectively.

After 180 days of storage, all tetracycline levels were reduced by 78–88% (Figure 2). In addition, the statistical analysis (Student's *t* test) of results revealed that the observed reduction was significant for all tetracyclines/oxytetracycline ($P=0.006$), tetracycline ($P=0.0001$), chlortetracycline ($P=0.0093$), and doxycycline ($P=0.007$).

Matsuka and Nakamura (1990) obtained a 50% decrease of oxytetracycline levels after 70 days in honey stored at 25°C, and after 42 days when stored at 35°C. Similarly, Argauer and Moats (1991) reported a 50% decrease of oxytetracycline levels in honey after 12 days of storage at 34°C. These earlier decreases of tetracycline levels could be attributed to the higher storage temperature.

Liang et al. (1998) investigated the stability of tetracycline on methanol solution. Tetracy-

**Figure 2.** Evolution of TC levels in honey during storage at 20°C±5°C and darkness.

cline decomposed rapidly under the influence of light and atmospheric oxygen.

Tayar et al. (2010) reported residue levels of OTC, TC, and CTC were reduced 97%, 76%, and 71%, respectively, after 60 days of storage for commercial honey samples.

4. CONCLUSIONS

In conclusion, we have validated a simple, fast, and sensible analytical method, according to international criteria, which could be used for routine analysis.

Pasteurization treatment and storage conditions influence the tetracycline residue levels in honey. In fact, all tetracycline levels decreased after the pasteurization, with oxytetracycline showing the largest decline. In addition, after 90 days of storage, a 50% decrease of tetracycline levels was observed, with up to 70% decrease for CTC and DC. However, it is not possible to conclude that, after pasteurization or the periods of storage used in this study, honey is free of TC health-related problems.

When conducting a risk assessment of tetracyclines in human health, the presence of these contaminants in honey and other bee products should be considered as a potential source, depending on levels of contaminants and the daily intake of honey.

In order to protect consumer health, good sanitary practices by beekeepers should be employed and monitored by technicians and public health authorities. The results of this study suggest that pasteurization and storage according to the assayed conditions do not guarantee the absence of tetracycline residues in honey.

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Effets de la pasteurisation et du stockage sur les teneurs en tétracyclines dans le miel.

miel / tetracyclines / pasteurisation / stockage / risque sanitaire

Zusammenfassung – Der Einfluss von Pasteurisierung und Lagerung auf den Gehalt von Tetracyclinen in Honig. Tetracycline sind Antibiotika mit einem breiten Wirkungsspektrum, die das Bakterienwachstum durch eine Hemmung der Proteinsynthese verhindern. In der Imkerei haben sie sich als wirksame Mittel zur Kontrolle der amerikanischen und europäischen Faulbrut erwiesen. Allerdings kann das Auftreten von Rückständen der Tetracycline in Honig ein Risiko für die Verbraucher weltweit darstellen (allergische Reaktionen, Entwicklung resistenter Bakterienstämme, Veränderungen der Darmflora sowie mutagene und/oder karzinogene Effekte; Demoly and Romano 2005; Andersen et al. 2005). Tetracycline sind daher nicht für die Behandlung von Honigbienen in der EU zugelassen und es wurde auch kein "maximal residue level"(maximaler Rückstandswert: MLR) festgesetzt, obwohl einige Staaten auf der Basis eines Kaskadensystems Tetracyclin in die Liste der zugelassenen Produkte aufgenommen haben. Dieses Kaskadensystems erlaubt die Verwendung von Tierarzneimitteln, die in anderen Ländern zugelassen sind. Die FAO/WHO setzte im Jahr 1998 Werte für die maximale tägliche Aufnahme (acceptable daily intake, ADI) von 0–0,03 mg/kg Körpergewicht für Oxytetracyclin, Tetracyclin, und Chlortetracyclin fest. Es ist daher wichtig, dass die Nachweismethoden für die Rückstandsanalytik von Antibiotika so empfindlich und zuverlässig wie möglich sind, um sicherzustellen, dass der in der EU vermarktete Honig die Anforderungen der EU erfüllt.

In dieser Studie wurde eine Methode für den Nachweis der Rückstände von Oxytetracyclin, Tetracyclin, Chlortetracyclin und Doxycyclin in Honig entwickelt und validiert. Dabei wurden Honigproben in EDTA-McIlvaine-Puffer (pH 4) gelöst und die homogenisierte Probe auf eine C₁₈ Säule geladen, die zuvor mit Acetonitril, Oxalsäure (pH 3) und gesättigter EDTA-Lösung konditioniert und aktiviert wurde. Danach wurde die Probe nach einer Festphasenextraktion (SPE) über eine 90:10 Ethylacetat/Methanol Lösung eluiert. Das Eluat wurde unter Vakuum konzentriert und danach in Acetonitril-Oxalsäure (15:85) bei pH 3 gelöst. Diese Lösung wurden später als mobile Phasen für die HPLC-DAD verwendet. Nach der Validierung der Nachweismethode untersuchten wir die Entstehung von Tetracyclin-Rückständen in Honigen unter zwei unterschiedlichen experimentellen Bedingungen: Pas-

teurisation im Labor (78°C/1 min) und Lagerung (Raumtemperatur/Dunkelheit über 30, 90, und 180 Tage). Die Tetracyclin-Rückstände nahmen nach der Pasteurisation um 10–19% ab. Die Tetracyclin-Rückstände in den gelagerten Proben nahmen um 18,5% nach 30 Tagen, 58,5% nach 90 Tagen und 83,5% nach 180 Tagen ab.

Zusammenfassend kann man feststellen, dass angesichts der "Nulltoleranz" in Europa (kein MLR für Tetracyclin) die Pasteurisation und die Lagerung von Honig keine Garantie dafür sind, dass so behandelte Honige danach unbelastet von Tetracyclin-Rückständen sind.

Honig / Tetracycline / Pasteurisierung / Lagerung / Gesundheitsrisiko

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