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Residues of Oxytetracycline in Cultured Rainbow Trout

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Abstract: Nowadays, antibiotics are widely used in aquatic animals to control and treatment of infections or as food supplement for growth increase and animal output. With increasing use of veterinary drugs in food production, there is global consideration about the consumption of antimicrobial residues in aquatic foods and their effects on human health. This study was aimed to evaluate the Oxytetracycline (OTC) residues in Rainbow trout meat in Shahre-kord (Iran) markets before and after frying. After randomized collection of 50 samples of fish in Shahre-kord markets in a six months period were examined. The prepared samples were examined for OTC residues using HPLC analytical method before and after frying. Results showed that 3 (6%) of the samples before frying and 12 (24%) after frying were having lower than Maximum residual limits (MRLs) in Codex alimentarius. However, mean OTC residues before and after frying samples were above MRLs. The mean amounts of OTC were 2260±1090 and 1110±930 ng g^{-1} before and after frying, respectively. These findings show that the frying of fish reduces OTC residual. Nevertheless, the usage of OTC should be reduced to an acceptable level in fishery industry.

Key words: Oxytetracycline, rainbow trout, residue, HPLC

INTRODUCTION

Antibiotics are essential drugs considered as the final strategy to treat human infections. Their effectiveness is, however, threatened by wide and unfit use, not only in medicine but also in agriculture (Smith *et al*., 2009). Currently, more than forty thousand types of antibiotics have been identified and 80 of them are consumed in the agricultural and aquaculture industries (Martos *et al*., 2010). In the veterinary practice, antibiotics are used for treatment and prevention of disease as well as to promote growth in the livestock and fish farms (Lopes *et al*., 2012).

In the USA, more than 70% of the total antibiotic production in a year (nearly 35 million pounds) is used in chattels for non-medical benefits (Lopes *et al*., 2012). The incorrect use of these antibiotics in livestock and fishery farms as well as lack of attention for withdrawal time after treatment have resulted in their release into the environment as the main compounds and their metabolites. In addition, the presence of antibiotic residues in foods might cause hypersensitivity reactions in some persons and creation of antibiotic-resistant bacterial strains (Bogialli and Di Corcia, 2009; Mastovska, 2011).

Tetracyclines are a group of wide spectrum antibacterial compounds that are widely consumed in the aquaculture industry (Wen *et al*., 2006). Oxytetracycline (OTC), a tetracycline antibiotic, is used to treat different bacterial infections in food-fish and ornamental aquarium fish (Wang, 2009). The Codex alimentarius (Anonymous, 2006), European Union (Anonymous, 2010a), Canada (Anonymous, 2010b) and Japan (Anonymous, 2011) in order to increase the safety of food supply for consumers and simplification of international commerce, have established MRLs for OTC residues in muscle of fish which are 200, 100, 200, 200 and 200 ng g^{-1}, respectively.

In Iran, there has been an increase in the production and consumption of freshwater fish reared in aquaculture systems in recent years, mainly the Rainbow trout (*Oncorhynchus mykiss*). Therefore, the drug residues in the fish tissues have reduced the quality and safety of fish as food. However, there is less attention to the residual drugs in cultured fish than in domestic animals (Ueno *et al*., 1999). Therefore, monitoring of OTC residues has a major role to ensure the safety of food. In foods, tetracyclines the most detected by microbiological methods but these methods are twisted, time-consuming and lack specificity. On the other hand,

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the High-performance Liquid Chromatography (HPLC) technique has gained much attention in this context, due to its high sensitivity and accuracy (Wen *et al*., 2006). This study was aimed to evaluate the Oxytetracycline residues in Rainbow trout meat in Shahre-kord (Iran) markets before and after frying.

MATERIALS AND METHODS

Chemicals and reagents: Oxytetracycline hydrochloride was purchased from Sigma-Aldrich Corporation (St. Louis, MO., USA) and analytical or HPLC grade of citric acid, methanol, acetonitrile and nitric acid from Merck Inc. (Darmstadt, Germany).

Fish: Fifty samples of live cultured fish Rainbow trout (*Oncorhynchus mykiss*) were collected in a six months period (July-December 2011) in Shahre-kord markets in Iran. Then 200 g of fish muscle samples were removed and divided into two parts. One of the sample parts was examined as raw material and another part was pan-fried in soybean oil for ten minutes.

HPLC analysis: The HPLC system consist of a Hewlet Packard Series 1100 Liquid Chromatograph with a 7725 rheodyne injector (20 μL loop), HP UV-Vis detector, vacuum degasser, gradient pump module and column compartment oven. The HPLC column used was Hypersil BDS C18 (5 mm, 250×4 mm) (Germany). The mobile phase was distilled water (pH = 2.1 with H_2SO_4): acetonitrile, 85:15(v/v). The flow rate was 1.5 mL min^{-1}. The detection wavelength was set at 360 nm. The injected volume was 20 μL and chromatography was performed at 24°C (Senyuva *et al*., 2000).

Samples of 2 g fish muscle were homogenized in a blender for two minutes. Then to this mixture 0.1 g citric acid, 1 mL nitric acid (30%), 4 mL methanol and 1 mL deionized water were added, respectively. The suspension was mixed in a vortex and kept in an ultrasonic bath for 15 min. After centrifugation for 10 min at 5300 rpm, the supernatant was filtered through a 0.45 μm nylon filter. Twenty microliter of solution was injected into HPLC for analysis. The preparation of frying muscle samples were the same as the described above. The recovery of the method for OTC was determined by adding known amount of OTC to the samples (Senyuva *et al*., 2000). Standard solutions of OTC were prepared using concentrations of 0.25, 0.5, 1.5, 2.25, 3.0 and 6.0 mg OTC L^{-1}. The working solution was prepared from the daily prepared stock solutions and treated as above (Senyuva *et al*., 2000).

Statistical analysis: Statistical analyses of the data were carried out using of Paired t test using mean and standard deviation with 95% confidence interval. The results of before and after frying samples were compared with the MRLs and analyzed by SPSS 16 software using the Single- sample t test.

RESULTS

Fifty samples before frying and 50 samples after frying were analyzed during this study. All samples (100%) before frying and 44 (88%) samples after frying had detectable levels for oxytetracycline residues. The method recovery result for before or after frying was 91.3%. Three samples (6%) before frying and 12 samples (24%) after frying were lower than MRLs in Codex alimentarius.

The maximum and minimum amounts, mean and standard deviation for Oxytetracycline residues are shown in Table 1. Oxytetracycline residues in before frying samples (2260 ng g^{-1}) were significantly more than after frying ones (1110 ng g^{-1}) ($p<0.0001$). Furtheremore, the mean of Oxytetracycline residues in before and after frying samples were significantly more when compared with MRLs in Codex alimentarius ($p<0.0001$). Standard curve and one chromatogram of sample before and after frying are shown in Fig. 1 and 2, respectively.

Table 1: Amounts of Oxytetracycline residues in Rainbow trout meat samples before and after frying

Oxytetracycline	No. of samples	Min amount	Max amount	Mean	standard deviation	p-value
Before frying	50	80.00	5020	2260	1090	<0.0001
After frying	50	0.00	3440	1110	930	<0.0001

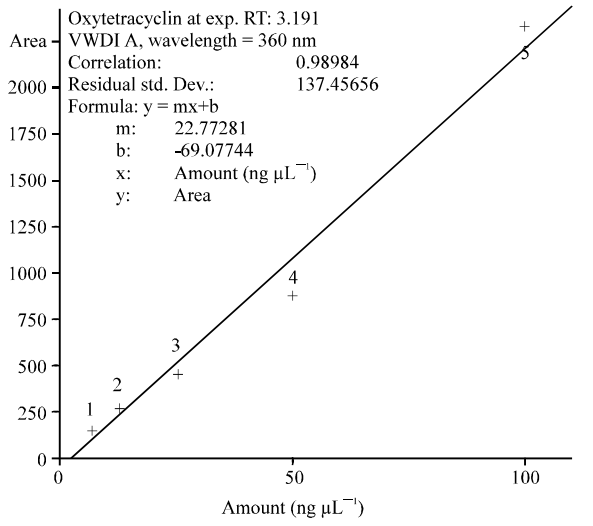

Fig. 1: Standard curve for oxytetracycline

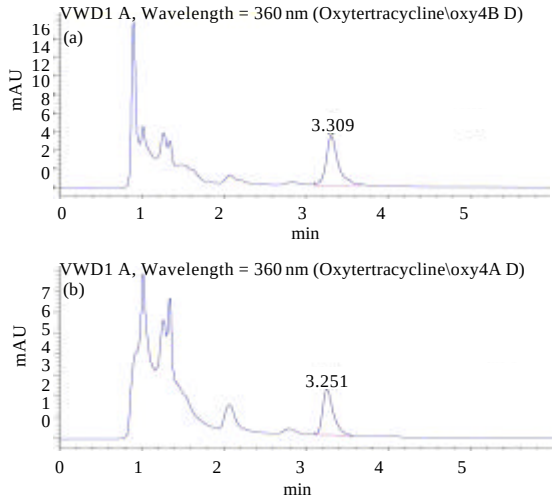

Fig. 2: Chromatograms obtained from (a) Before and (b) After frying samples

DISCUSSION

The presence of antibiotic remains in meat and edible viscera of food-producing animals has attracted extensive worldwide attention from national and international public health agencies (Salehzadeh *et al*., 2006; Asperger *et al*., 2009). Many reports have shown that bacterial resistance to antibiotics may be due to animal exposure to these drugs form and the resistance gene may be transmitted to human pathogenic bacteria. Furthermore, consumption of a large amount of various animal products containing antibiotic residues by human may cause undesirable changes in bowel microflora and create immunological reaction in susceptible persons (Mottier *et al*., 2003; Salehzadeh *et al*., 2006; Nafisi *et al*., 2008). In the present study we examined the muscle samples of Rainbow trout for presence of Oxytetracycline residues. In this study, all before frying samples contained detectable OTC residues but frying the samples caused a significant reduction in OTC residues.

The maximum Accepted Daily Intake (ADI) of OTC for individuals designated by Food and Agriculture Organization (FAO), World Health Organization (WHO), Veterinary Medicines Directorate (VMD) of the Europe, should not be more than 3 μg (Salehzadeh *et al*., 2006).

The results of (Senyuva *et al*., 2000) for detection of OTC residue in cured meat using HPLC in Turkey showed that 7 out of 10 meat samples marketed in Turkey, had higher OTC residue than the levels accepted by the EU and FDA. Also HPLC was a rapid and specific method for the determination of OTC with recoveries from 78 to 100%. Using this method in the present study, the level of OTC in 47 samples (94%) before frying and in 38 samples (76%) after frying were higher than MRLs.

Previous studies have suggested a withdrawal time of 10.5 to 24 days for depletion of OTC in various sea foods as sea bream, cat fish and salmon (Bernardy *et al*., 2003; Balta and Cagirgan, 2010). Also it was shown that during the frying process, the moisture in the fish meat leached out and replaced by the frying oil. The exchange of moisture and oil could remove OTC out from the fish meat (Ismail-Fitry *et al*., 2008). Because the results are affected by the kind and method of tests, different apparatus or equipment and health regulations in countries, comparing the results related to antibiotic residual levels in various animal meats is difficult (Salehzadeh *et al*., 2006; Adewuyi *et al*., 2011).

CONCLUSION

OTC is usually used in fish farms and based on the results obtained from this study, it seems that the monitoring and implementation of the recommended withdrawal time may be insufficient for this drug. Therefore, we recommend more control by monitoring the usage of OTC on fish farms, implementation of withdrawal period, restrictive regulation for the use of antimicrobial drugs in the fishery industry and the inspection of fish for residues prior to marketing.

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