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Effect of cooking process on level of Enrofloxacin in pork

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Abstract
The present research was conducted to study the effects of cooking process like boiling deep-frying and microwaving on Enrofloxacin (ENR) level in Pork. Pork samples incurred with known concentration of ENR were subjected to these cooking processes. The cooked samples were then analyzed to record the level of ENR using Ultra High Performance Liquid Chromatography (UHPLC) system. The results showed the reduction in concentration of ENR residue after different cooking processes. The most reduced level of ENR in cooked meat samples was observed in microwaving followed by deep-frying and then boiling. The result shows significant decrease in ENR level in pork after cooking. It may be concluded that cooking of meat leads to decrease in the concentration of ENR.

Keywords: cooking, enrofloxacin, microwaving, pork, residue

Introduction
Fluroquinolones are a group of synthetic antimicrobial agents that have a wide spectrum of activity and high efficacy against various microbial infections. They act by inhibiting the DNA-gyrase which affects the stability of the DNA configuration of the bacterial DNA molecule during cell division (Xu *et al*., 2006) [14]. They are commonly used for the treatment of urinary tract and enteric infections in humans (Salehzadeh *et al*., 2007) [12]. These agents are normally used for treatment and prevention of infectious disease in farm animals (Maraschiello *et al*., 2001; Dipeolu *et al*., 2002) [9, 3]. They are also used as growth promoters (Okerman *et al*., 1998) [10]. Antibiotic residues in food can cause hazardous effects to human health. Allergic reactions, imbalance of intestinal microflora, bacterial resistance to antibiotics are some of the adverse effects (Cunha, 2001; Kirbis, 2006) [2, 7].
Enrofloxacin (ENR) is a synthetic fluoroquinolone antimicrobial agent which is administered to farm animals like swine for the treatment of infections of the respiratory and alimentary tract (Posyniak *et al*., 2001) [11]. Levels of drug residues in raw meat and animals products is regulated. Codex Alimentarius Commission (2012) [1] have established maximum residue limit (MRL) of 0.1 µg/g for ENR in meat. Since most foods of animal origin are cooked before consumption, ENR levels in the tissue are dependent on the type of cooking (Lolo *et al*., 2006) [8]. Thus the present study was undertaken to see the cooking effects on ENR level in cooked meat.

Materials and Methods
Preparation of samples
About 100 g of pork sample free from residue was taken and minced and fortified with 1.0 µg/g of ENR standard. The mixtures were then made into portions of 20 g meat balls.

Cooking procedure
Cooking procedures such as boiling, deep-frying and microwaving was performed to study the effects. The meat balls were boiled at 100º C for 5 and 10 mins respectively. In case of deep-frying, the meat balls were fried in a pan with sunflower cooking oil at 170º C for 3 and 6 mins respectively. In case of Microwaving, the meat balls were placed at the turntable of a microwave oven. The samples were cooked under full power (800 W) for 1 and 2 mins respectively. The temperature during cooking was 100ºC. Samples were then processed and analyzed using UHPLC to record the level of ENR.

Extraction and clean up
HPLC grade water was added to the cooked meat sample and then homogenized. About 5 g of the sample was transferred to a glass test tube and added 2 ml of 0.1 M phosphate buffer (pH 7.2) and then mixed. After adding 10 ml of dichloromethane, the mixture was sonicated in an ultrasonicator. The sonicated sample was then left undisturbed for 15 mins for allowing the extract to dissolve in the solvent. The sample was then centrifuged at 10,000 rpm for 10 mins at 0° centigrade in a refrigerated centrifuge machine. The supernatant was separated and filtered through a Whatman filter paper No. 42.
Cleanup of the extract was done by using Solid Phase Extraction (SPE) method. The filtrate was loaded on a C_{18} polymeric cartridge preconditioned with 2.5 ml of methanol and 2.5 ml of HPLC grade water. The cartridge containing the sample was washed with 3 ml of water and then finally eluted with 3 ml of methanol.
The extract so obtained was filtered through a syring filter (0.2µm). Finally, 20µl of the eluted sample was then injected into the UHPLC system for analysis.

Chromatographic condition
A mobile phase of Water: Acetonitrile (70:30 v/v) was used. The flow rate was kept at 1.0 ml/min keeping mode as isocratic. The wavelength for the detector was set at 277 nm.

Quantification
About 10 mg of pure Enrofloxacin standard was dissolved in 100 ml of HPLC grade water with drop of HCl until complete dissolution to obtain a concentration of 100µg/ml. Further

dilutions were made from this solution in the descending concentration of 5.0, 4.0, 3.0, 2.0 and 1.0 µg/ml respectively. An aliquot of 20µl each of these solutions were injected into the UHPLC system. Peak areas were recorded. A standard calibration curve with coefficient of determination of 99.95 % was obtained by plotting concentration of standard solutions against peak areas.

Results and Discussion

Microbiological methods are the preliminary screening methods for detection of antibiotic residues in food of animal origin (Hussein, 2004) [6]. Screening methods allow preliminary detection of a wide spectrum of antibiotics (Haasnoot *et al*., 1999) [5] but they cannot be used for quantitative analysis for antibiotic residues. A positive result should be confirmed with more precision methods like chromatographic technique (Ferrini *et al*., 2006) [4]. Thus, the present study was performed with Ultra High Performance Liquid Chromatography (UHLPC). The present method revealed that calibration curves showed good linearity (r^2) of 0.999 over the range of 1.0 to 5.0 µg/ml. Accuracy and recovery was in the range of 95-99% in meat indicating that the method was a validated method.

Lolo *et al*., 2006 [8] reported that when the meat samples were boiled at 100°C for 10 min and microwaved at 800 W for 3.5 min there was a decrease in concentration. The results of boiling and microwaving in this research confirm the findings of our study about the decrease of ENR activity after cooking. As shown in Table 1, after 1 min of microwaving, ENR level in the meat samples was found to be 0.426 ± 0.019µg/g. The samples which were micro waved for 2 mins showed further decrease in the level of ENR which was 0.257 ± 0.023µg/g. Similarly, ENR level in the samples after 5 mins and 10 mins of boiling was found to be 0.648 ± 0.020µg/g and 0.443 ± 0.021µg/g respectively. Similarly, after 3 mins of deep-frying ENR level in the meat samples were found to be 0.651 ± 0.016 µg/g. The meat balls which were deep-fried for 6 mins showed further decrease in the level of ENR to 0.416 ± 0.019 µg/g. It also corroborated well with the findings of Van Egmond *et al*., 2000 where it was reported that Enrofloxacin residue in pork reduced to 68% after cooking.

Table 1: Effect of cooking process on ENR level in Pork

Cooking Process	Boiling		Deep Frying		Microwaving	
Time (min)	5	10	3	6	1	2
ENR Concentration (µg/g)	0.648 ± 0.020	0.443 ± 0.021	0.651 ± 0.016	0.416 ± 0.019	0.426 ± 0.019	0.257 ± 0.023

n=6

Conclusion

It can be concluded from the present study that cooking processes causes significant decrease in the level of ENR in pork. It was also found that cooking time and temperature played a major role in reducing the level of ENR. Microwaving can be regarded as the best cooking process followed by deep-frying and then boiling for reducing ENR level.

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