Effect of heat treatment on oxytetracycline residues in beef

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Abstract

Literature about drug residues is mainly related to their concentrations in uncooked food. The aim of this study was to assess the effects of barbecuing and boiling treatments on the concentration of oxytetracycline (OTC) in beef samples collected from different districts in Dodoma region, Tanzania. The beef samples were boiled for 30 minutes or barbecued for 20 minutes. The OTC content was measured in raw and heated samples by using high performance liquid chromatography (HPLC). The mean concentration of OTC for boiled and barbecued beef samples was 69.45 ± 41.93 ng/g and 69.40 ± 38.91 ng/g, respectively. Both the boiling and barbecuing procedures significantly decreased the OTC levels in beef ($p<0.05$), and the boiling procedure had the highest influence on reducing OTC concentration. The OTC concentrations after the heating treatments were below the maximum acceptable residue limits (MRL). The results of this study indicate that the occurrence of violative levels of drug residues in raw meats is decreased by heating. Therefore, the occurrence of violative levels of drug residues in the food may be prevented by the heating process.

Keywords: HPLC, oxytetracycline**,** boiling, barbecuing, time, beef, Tanzania

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Introduction

Antimicrobial agents are essential drugs for both human and animal health and welfare. These agents have been used for the treatment of diseases in animals, prevention of infection in animals and to improve feed utilization and production (Heshmati *et al.,* 2013). Effective treatment of diseases in the livestock industry in sub- saharan Africa including Tanzania continues to be a challenge. The challenges have been addressed mainly by the use of antimicrobial drugs which include the tetracyclines, beta lactam antibiotics like penicillins and cephalosporins (Olufemi and Agboola 2009; Katakweba *et al.,* 2012). The lack of restriction on antimicrobial drugs availability, insufficient knowledge on drug use as well as failure to observe withdrawal period can contribute to the presence of high levels of antibiotic residues in meat (Nisha 2008). The effects caused by antimicrobial residues in foodstuff comprise carcinogenicity, bone marrow toxicity, mutagenicity, autoimmunity, (Nisha, 2008; Pavlov *et al.,* 2008). It is also important for oxytetracycline residues in meat to be controlled to the acceptable levels since it may result into allergic reactions and hazardous effect, (Shankar *et al.*, 2010; Abbasi, 2011).

To control occurrence of harmful effects of drug residues in humans and animals, various regulatory and control measures have been established. These include the setting of maximum acceptable residue limits (MRL) in animal food products (FAO/WHO, 2014) for (OTC), Chlortetracycline (CTC) and Tetracycline (TC) to be 0.2 mg/kg for muscle tissue in cattle and pigs, and 1.2 mg/kg for kidney tissue in cattle and pigs.

Behind the success story of these drugs, the issue of drug residues poses a major problem as far as consumers` health is concerned. Due to the widespread use of antibiotics for treatment of diseases in cows, much effort has been directed towards the proper management and monitoring of antibiotics usage in treatments in order to prevent contamination of raw milk and meat products (Jahed, 2007; Alica *et al.*, 2003). Several studies have been conducted on antimicrobial usage and residues in foods of animal origin such as milk, beef and eggs in Tanzania (Mmbando, 2004; Karimuribo *et al.,* 2005; Kivaria *et al.,* 2006; Simon, 2007; Mdegela *et al.,* 2009). Despite the reports by these scholars so far, there is limited information of the effect of cooking procedures on the levels of residues and this creates a scientific gap of knowledge which needs be addressed. It is of importance to address how serious the residues are after cooking and the levels be known to the public in Tanzania.

The fact on drug residues in foodstuffs of animal source is generally connected to the concentration of these medicines in raw samples. Meanwhile most of these foodstuffs are heated before ingestion. Data on the effect of heat is essential to provide a more precise evaluation on the concentration of these deposits the users may be exposed to. For example, Javadi *et al.*, (2011) showed a reduction in the concentration of doxycycline residues after boiling. Also Gratacós-Cubarsí *et al.,* (2007) stated that ordinary cooking procedure reduced the initial concentrations of TC residues by 56 to 82% using microwave and boiling, respectively. Another study conducted by Loksuwan *et al.,* (2002) revealed that antibiotic residues were reduced when heated to 63 ºC for 30 minutes. This study sought to assess the the effects of barbecuing and boiling treatments on the concentration of OTC since these methods are applied to beef during household preparation.

Materials and methods

Study site

This study was carried out in Dodoma region in Tanzania. Dodoma Region lies at latitude 4º to 7º latitude South and longitude 35º to 37º longitude East. The region is centrally positioned in Tanzania and is bordered by four regions namely, Manyara in the North, Morogoro in the East, Iringa in the South and Singida in the West. Samples were obtained from cattle slaughterhouses and butcheries at Bahi, Kongwa, Dodoma Urban and Rural. Slaughterhouses and butcheries were selected using simple random sampling techniques.

Chemicals and Reagents

Standard of OTC and Ethylenediaminetetraacetic acid (EDTA) was supplied by Sigma-Aldrich (St Louis, MO, USA). Acetonitrile and Methanol were of HPLC grade (Merck Campany, Germany).

Sample extraction and analysis

A total of 60 beef samples of 250 g each were collected in separate polythene bags and transported on ice bags to the University of Zambia for extraction and analysis.

Antibiotic-free meat control samples (blank matrix) were collected from the Central Veterinary Research Institute of Zambia. The control and test samples were stored in a freezer at −20 °C for approximately 1 week and thawed at room temperature for eight hours before extraction and analysis of OTC residues. Meat samples which were positive for oxytetracycline were subjected to different cooking procedures; boiling and barbecue, similar to procedures applied to beef under household conditions.

Heat treatment of meat samples

Boiling procedure:

One hundred gram (100 g) weighed sample was placed into a strainer, immersed in about one liter of boiling water. Water was added during boiling time to keep the volume of water for 30 minutes. It was then allowed to cool before extraction and analysis of OTC residues.

Barbecue preparation:

One hundred gram (100 g) weighed sample was barbecued well for 20 minutes and allowed to cool before extraction and analysis of OTC residues.

Samples extraction

The extraction procedures was similar for spiked blank samples, test samples and those which were heat-treated. Samples were removed from the -20^{o}C freezer and were thawed. Approximately 10 g of muscle was weighed and mixed with 25 mg (EDTA) per gram sample. The sample and the EDTA were homogenized using a blender for one minute. The blended sample was then further ground using a mortar and pestle.

One gram (1g) of the homogenized sample was accurately weighed into a 15 mL polypropylene centrifuge tube. To the sample, 50 µL of 50 µg/mL caffeine solution, equivalent to 2500 ng caffeine, were added. Five millilitres (5 mL) acetonitrile was added using a 5 mL volumetric pipette and the mixture was vortexed for 1 minute. The sample was centrifuged for 10 minutes at 7000 rpm. The supernatant was collected into a separate 15 mL centrifuge tube by decantation. Five millilitres (5 mL) acetonitrile was added to the residue, the mixture was vortexed for 1 minute. The sample was centrifuged for 10 minutes at 7000 rpm. Both supernatants were combined into a 15 mL centrifuge tube, briefly mixed using a vortex and gently dried under a stream of nitrogen to 2 mL. After drying, 0.5 mL of HPLC grade water and 30 µL of formic acid were added, making the mixture 1.2 % acidic. Fifteen milligrams (15 mg) of Supelclean ENVI-carb active coal were added; the sample was mixed for 30 seconds using a vortex and centrifuged for 10 minutes at 7000 rpm. The supernatant was collected into a separate 15 mL centrifuge tube and dried to 0.5 mL.

Sample analysis

The determination of OTC residues was carried out according to the method of Mgonja *et al* (2016) using HPC- DAD. The HPLC apparatus was equipped with a constant flow quad pump at a flow rate of 0.5 mL/min. Elution of OTC from the analyte was done on an Eclipse XDB C-18 column 4.6 x 150 mm, 5µm I.D with HPLC grade water-acetonitrile containing 0.1% formic acid. A 100 µl injection volume of the analyte from each sample was injected to obtain average peak areas of positive samples corresponding to retention times of 5.9 minutes of the reference standard for OTC. The concentrations of OTC residues in the samples were calculated from the linear equations obtained from the standard curves (figure 1).

The Limit of Detection (LOD) documented for both boiled and barbecued beef samples were similar i.e. 18.2 ng/g for OTC. The corresponding Limit of Quantification (LOQ) value was 54.6 ng/g. The average recoveries from boiled and barbecued beef ranged from 66.6 to 75.9% for OTC (table 1).The relative standard deviations (RSD) was 8.9% which complied with the requirement of the Codex Alimentarius Commission of lower than 10%

Table 1. Characteristics of the analytical method for cooked beef samples

Analyte	Matrix	LOD (ng/g)	LOQ (ng/g)	S.L (ng/g)	R (%)	RSD (%)
OTC	Boiled	18.2	54.6	100	66.6-75.9	8.9
OTC	Barbecued	18.2	54.6	100	66.6-75.9	8.9

LOD: limit of Detection

LOQ: limit of Quantification

S.L: spiked level

R: recovery

RSD: relative standard deviations

Data Analysis

The data was analyzed by a computer Programs (SPSS Statistic 20) using T-test. A probability of $p < 0.05$ was considered statistically significant.

Results

The effect of different heat treatment on the concentration of OTC residues in beef samples are shown in tables 2 and 3. The results revealed a reduction of OTC with boiling by 9.1-90.9.% in 30 minutes and barbecued resulted in 26.1 – 87.8% in 20 minutes. The mean concentration of OTC was significantly lower for boiled beef samples than for barbecued beef samples (69.45 ± 41.93 ng/g versus 69.40 ± 38.91 ng/g; $p < 0.05$). The reduction percentage was lower for the boiled beef than for the barbequed beef. The different levels of OTC residues between the raw, boiled and barbecued beef samples (table 2) were statistically significant ($p<0.05$) while the different OTC levels between the boiled and barbecued beef samples (table 3) were not statistically significant ($p>0.05$).

Table 2. Percentage reduction of OTC before and after cooking process

PLACE COLLECTED	RAW (Conc.ng/g)	RAW %	BARBECUED (Conc.ng/g)	BARBECUED % Reduction	BOILING (Conc.ng/g)	BOILING % Reduction
Dodoma Urban	322.28	100	93.77	70.9	71.10	77.9
Kongwa	25.06	100	14.69	41.3	22.48	10.3
Dodoma Urban	370.42	100	45.21	87.8	33.37	90.9
Dodoma Rural	167.32	100	45.46	72.8	32.37	80.6
Chamwino	262.16	100	134.07	48.5	134.07	48.5
Dodoma Rural	121.19	100	70.68	41.7	92.01	24.1
Kongwa	110.83	100	81.93	26.1	100.77	9.1

Table 3. Effect of cooking methods on OTC residues in beef

Treatment	Time	OTC Levels		
		Raw samples (ng/g)	After heat-treatment (ng/g)	% Reduction
Boiling	30 minutes	196.75 ± 124.75	69.45 ± 41.93	(26.1-87.8)%
Barbecued	20 minutes	196.75 ± 124.75	69.40 ± 38.91	(9.1- 90.9)%

The higher concentration of oxytetracycline was associated with a higher peak. The correlation coefficient associated with the linear regression for the oxytetracycline standard, is represented by R^2 = 0.94 (Figure 1).

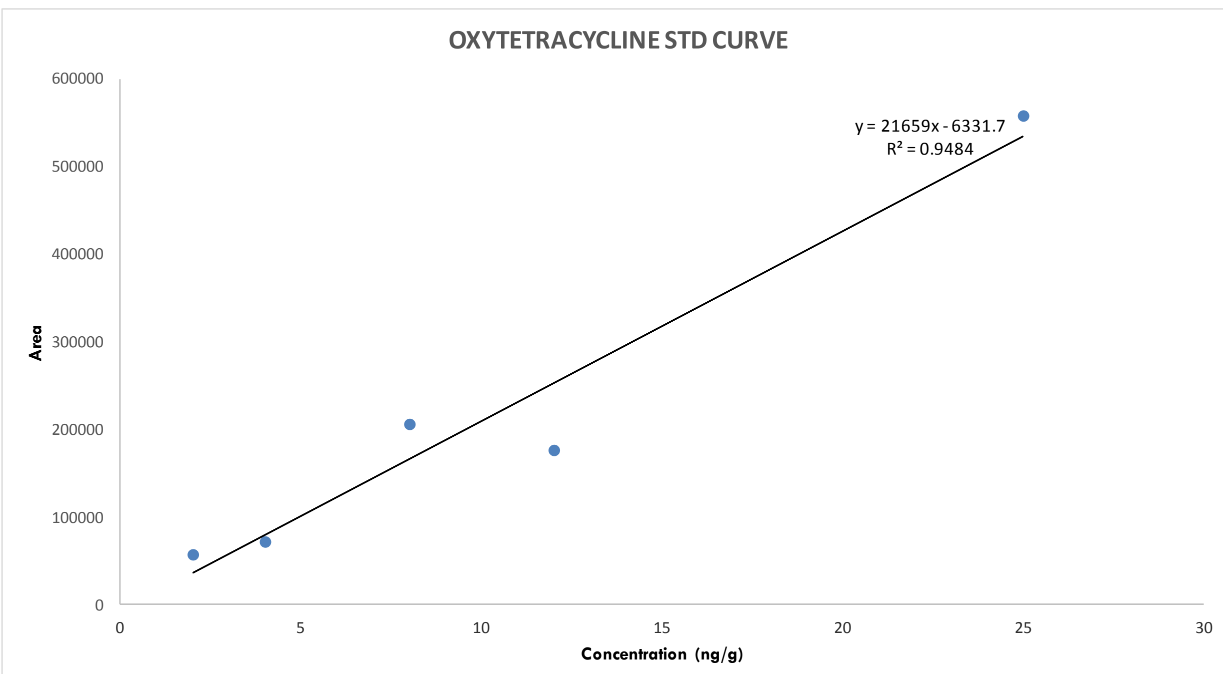

Figure 1: Calibration curve of Oxytetracycline standard curve.

Discussion

The present study determined the effect of cooking procedures on the OTC concentration in beef samples collected from slaughterhouses and butcheries in the Dodoma region in Tanzania using HPLC. Both boiling and barbecuing cooking procedures significantly reduced OTC concentration in beef in the present study. The data revealed that the reduction in the OTC content of beef boiled for 30 mins was 87.8% while for beef barbecued for 20 mins it was 90.9% (table 3). The decrease in OTC concentration observed in this study is consistent with earlier studies in which heat treatments decreased the concentration of antimicrobial drug residues in foodstuffs. For example, Rose *et al.* (1996) investigated the effect of cooking procedures including microwaving, boiling, roasting, grilling, braising and frying on OTC residues in animal tissues and observed 94% net reduction in OTC. Another study by Ibrahim and Moats (1994) reported that OTC level was reduced by 95% when meat was boiled for 30 minutes. Our results are in agreement with Salah *et al.* (2013) who found 73.6% OTC reduction in meat by boiling for 30 minutes.

It was also observed that none of beef samples had the OTC residues of above the MRLs (200 ng/g) after the barbecuing and boiling processes. These results are in line with a study conducted by (AI Ghamdii *et al.*, 2000) who found a decrease of OTC residues below MRLs after boiling for 20 minutes. There was however, no difference in OTC concentration in boiled and barbecued beef. The decrease in OTC concentrations during the boiling process was due to migration of the OTC from the meat to the cooking medium (water) while the decrease during the barbecuing process was due to juice oozing out from the meat (Rose *et al.*, 1995; Rose *et al.*,1996). The general loss of OTC residues was due to decomposition of TC compounds (Rose *et al.*, 1995; Rose *et al.*,1996; Javadi *et al.*, 2011). These findings demonstrate an extra benefit of cooking as a food processing method.

The high concentration of OTC residues in beef observed in Dodoma Municipal and Chamwino District compared to Dodoma Rural and Kongwa Districts (table 2) may be due to slaughtering animals before the end of the withdrawal period of 7 days (Aiello & Moses 2010). Availability of antibiotics such as OTC, lack of awareness and knowledge on proper use of guidelines from manufacturers may lead to mismanagement and overuse of the antibiotics. This may result to the failure to observe withdrawal periods and contribute to the high levels of antibiotic residues in meat (Nisha, 2008). The variation in OTC concentrations observed in the current study may be due to different types of beef samples and local animal farming practices. Our results are similar to the findings by Muriuki *et al.* (2001) who reported residue level variations even from the same district which indicates the variation in animal husbandry practices.

Therefore, policy- and decision-makers should emphasize on the proper use of antimicrobial agents in the treatment, prevention and control of diseases as well ensuring effective implementation of regulatory measures. The withdrawal period should be observed carefully before the animals are slaughtered or before allowing milk for human consumption. Also, the public should be educated on appropriate cooking preparation of meat while health education should be provided to communities to avoid feeding on raw beef or blood practice. In addition, livestock keepers should be educated on the basic knowledge and skills on the concept of antibiotic resistance.

Conclusion

Heat treatment generally may decrease the concentration of OTC in meat, and boiling is more effective than barbecuing in reducing the concentration of OTC. The decrease in OTC concentrations below the MRLs suggests the advantage of reducing violative levels of drug residues in raw meat by heating for a longer time.

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Ethical issues

None to be declared.

Competing interests

The authors declare no conflict of interests.

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