Effect of Cooking Procedures on Oxytetracycline Residues in Lamb Muscle

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Data on the effect of cooking procedures on antibiotic residues in meat are rather limited and have not been reported for microwave cooking. For the present study, ground meat was used from lambs dosed intravenously with oxytetracycline 4 h prior to slaughter. The meat was mixed to ensure uniformity and formed into 100 g patties. Residues were determined by HPLC analysis. When the meat was packed in a sausage casing and cooked in boiling water, oxytetracycline was reduced 95% in 30 min. Microwave cooking to "well-done" (no red color) required 8 min, gave a final temperature of 98–102 °C, and reduced levels 60% from controls. Frying to "well-done" also required 8 min, the final internal temperature was 81 °C, and residues were reduced 17.3%. Degradation was related to the final temperature reached, which was higher for microwave cooking or for boiling than for frying. These results are consistent with other reported studies.

Keywords: *Lamb; oxytetracycline; cooking*

INTRODUCTION

Antibiotics used for treatment of farm animals may leave residues in muscle and other tissues if adequate time is not allowed prior to slaughter for the residues to dissipate. Residues are ordinarily measured on uncooked tissue. It is of some interest to determine if residues of antibiotics can be destroyed by cooking procedures. This topic was recently reviewed by one of the authors (Moats, 1988). Most of the earlier studies with antibiotics were based on loss of microbiological activity during cooking or other heat processing. It is only recently that chromatographic methods of adequate sensitivity have been developed for determination of residues of various antibiotics in tissues. There are a few reported studies on the effect of cooking on oxytetracycline (OTC) residues in muscle foods. These include reports by Yonova (1971) using chicken meat, Buncic and Dakic (1981) with rabbit muscle, Bernarde (1957) with shellfish, Scheibner (1972a,b) on sausage processing, O'Brien et al. (1981) with beef, Honikel et al. (1978) with beef, and Kitts et al. (1992) with salmon. Escanilla et al. (1959) studied the effect of cooking on chlortetracycline in beef patties and hot dogs. Only the recent study of Kitts et al. (1992) used HPLC analysis to determine residual OTC.

The present study was undertaken to determine the stability of incurred residues of OTC in lamb muscle using three different cooking procedures. Residual OTC was determined using a modification of the procedure of Moats (1986).

MATERIALS AND METHODS

Chemicals. Acetonitrile, hexane, and methylene chloride were of residue analysis grade (EM Omnisolv, Gibbstown, NJ, or equivalent). Other chemicals were of reagent grade. Sodium decanesulfonate was purchased from Aldrich Chemical Co., Milwaukee, WI. OTC used for the standards was purchased from Sigma Chemical Co., St. Louis, MO. An OTC standard solution of 1 mg/mL was prepared in 0.01 N HCl and diluted in 0.01N HCl to working standards of 100, 10, and 1 μg/mL. The OTC standard was stable for several weeks when refrigerated. A pH below 2 is optimal for stability of OTC (McCormick et al., 1957).

Equipment. A Buchler (Ft. Lee, NJ) vortex evaporator was used to draw off organic solvent under reduced pressure. Glassware including 50 mL graduated cylinders, 125 mL separatory funnels, and 15 mL graduated conical centrifuge tubes was cleaned in special detergent (Micro, International Products, Trenton, NJ) and rinsed in ca. 0.01 N HCl or H_2SO_4 and then deionized water.

Treatment of Sheep. Two sheep weighing 13.36 kg were dosed intravenously with 50 μL/kg of body weight of Liquamycin LA-200 (Pfizer) "Oxytetracycline injectable antibiotic", equivalent to 10 mg/kg. The sheep were slaughtered 4 h post-treatment. Selected muscle tissue was ground, mixed, formed into 100 g patties, and frozen at −20 °C until used.

Heat Treatment. *Cooking in Boiling Water (100 °C).* A 100 g frozen lamb patty was pressed into a plastic bag, immersed in boiling water for the specified time, removed, and cooled.

Microwave Cooking. A frozen 100 g patty was placed on a plastic dish on a turntable. The patty was cooked under full power for the specified time, removed, and allowed to cool. Temperatures were measured by inserting a thermocouple immediately after removal from the microwave oven.

Grilling. Frozen 100 g patties were placed on a grill preheated to 350 °F (177 °C) and cooked for the specified time, turning at 1 min intervals. A thermocouple was used to monitor the internal temperature.

All cooking treatments were done in duplicate.

Analysis. *Sample Preparation.* Fifteen grams of tissue was blended with 30 mL of 1.0 N HCl and 15 mL of 0.2 M $(NH_4)_2SO_4$ slowly and then at full speed for a total of 2 min. Eight milliliters of homogenate was transferred to a 125 mL conical flask, and 32 mL of acetonitrile was added with vigorous swirling. After standing 5 min, the supernatant was decanted through a plug of glass wool in the stem of a funnel, and 20 mL of filtrate was collected. The filtrate was transferred to a 250 mL separatory funnel, and 20 mL of hexane and 20 mL of methylene chloride were added with shaking. The water layer

Table 1. Recoveries of Oxytetracycline Added to Lamb Tissues

tissue	amt added (ppm)	amt found a	amt found b	mean	mean ± SD for all tissues
muscle	0.1	0.099	0.111	0.105	
liver	0.1	0.125	0.088	0.106	
kidney	0.1	0.102	0.084	0.093	0.101 ± 0.013
heart	0.1		0.098	0.098	
muscle	1	1.02	1.04	1.03	
liver	1	1.04	0.96	1.00	1.02 ± 0.06
kidney	1	1.16	0.95	1.05	
heart	1	1.03	0.98	1.00	

[a] Determination 1. [b] Determination 2.

which separated was collected in a 15 mL graduated conical centrifuge tube. The organic layer was washed with 0.5 mL of water which was added to the water layer. After 0.5 mL of *tert*-butyl alcohol was added to suppress foaming (the *tert*-butyl alcohol may be mixed with 0.1 volume of acetonitrile to prevent freezing in cool weather), the water layer was evaporated to ca. 4 mL on the vortex evaporator and adjusted to 4 mL. It was filtered through a 0.45 μm PVDF filter cartridge into a 4 mL autosampler vial.

HPLC Analysis. The HPLC system used included a Varian (Sugarland, TX) 9010 pump, a Waters (Milford, MA) WISP 712 autosampler with a 2000 μL loop, a Polymer Laboratories (Amherst, MA) PLRP-S column (5 μm particle size, 4.6 x 150 mm, 100 Å pore size, with matching guard column), and a Waters 990 diode array detector.

The HPLC mobile phase was 0.02M H_3PO_4, 0.01 M sodium decane sulfonate (A)–acetonitrile (B). The injection volume was 1000 μL with a flow rate of 1 mL/min A gradient of 82A+18B (0–3 min)–60A+40B (25–30 min)–82A+18B (31 min) was used. Loading of the next sample was started at 45 min. Quantitation was based on the peak area compared with 1 and 0.1 μg standards run in the same manner and was based on UV absorption at 360 nm.

Recovery Experiments. Muscle, heart, kidney, and liver tissue was collected from untreated lambs and stored frozen prior to use. Fifteen grams of tissue was weighed into a blender jar. For spiking levels of 1 and 0.1 ppm, 150 μL of the 100 and 10 μg/mL standard solutions, respectively, was placed directly on the tissue and allowed to equilibrate for 30 min prior to the procedure's being done. Recoveries were based on comparisons with 15 mL of 0.01 N HCl spiked in the same manner.

RESULTS AND DISCUSSION

The extraction/deproteinization procedure used was a slight modification of that previously described by Moats (1986) for extraction of beef and pork tissues. Additional salt (ammonium sulfate) was added to reduce carry-over of acetonitrile in the water layer formed by addition of hexane and methylene chloride. Otherwise, residual acetonitrile in the sample extract caused distortion of the tetracycline peak when large volumes (1–2 mL) were injected for subsequent analysis. In the earlier procedure (Moats, 1986), multiple manual injections of small volumes (200 μL) were used rather than a single injection of 1–2 mL using an autosampler as in the present procedure. White et al. (1993), using a similar approach to analysis of milk, found that tetracyclines could be effectively separated from interferences by adding an ion pair, sodium decanesulfonate, to the mobile phase to increase retention times. This approach also proved to be effective with tissue extracts, eliminating the need for further cleanup.

Recoveries using this procedure were near 100% (Table 1) since no cleanup procedure was required. Oxytetracycline could be quantitated at levels well under 0.1 ppm. A chromatogram of control lamb muscle and lamb muscle spiked at 0.1 ppm is shown in Figure 1.

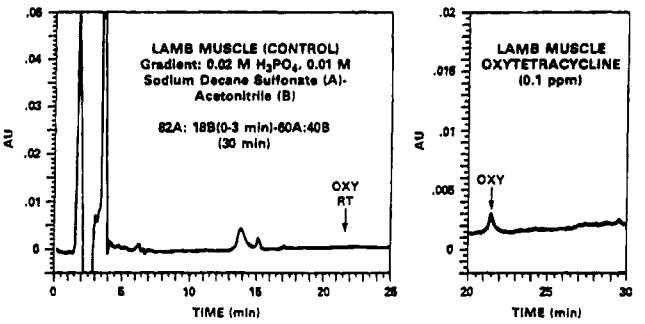

Figure 1. Chromatogram of control lamb muscle and lamb muscle with 0.1 ppm of oxytetracycline added.

Table 2. Effect of Boiling on Oxytetracycline Residues in Ground Lamb[a]

	time in boiling water bath						
	0 min	5 min	10 min	15 min	20 min	25 min	30 min
oxytetracycline found (ppm)	6.47	5.42	3.92	2.33	1.27	0.60	0.33
% reduction	0	16	39	64	80	91	95

[a] 100 g patty in plastic bag immersed in boiling water. Mean of two experiments.

Table 3. Effect of Frying on Oxytetracycline Residues in Ground Lamb Patties[a]

	time of frying				
	0 min	2 min	4 min	6 min	8 min
internal temp (°C)	frozen	16	52	64	81[b]
oxytetracycline found (ppm)	5.69	5.53	5.48	5.1	4.7
% reduction	0	2.7	3.6	10.4	17.3

[a] Frozen 100 g lamb patties were placed on a grill preheated to 350 °F (177 °C). Temperature was monitored by a thermocouple inserted into the patty. Mean of two experiments. [b] "Well-done" (no red color).

In the first experiment (Table 2), 100 g frozen lamb patties were placed into plastic bags which were then immersed in boiling water for the specified time. The results show a 95% reduction in 30 min (Table 2).

In the second experiment (Table 3), 100 g patties of minced lamb were fried on a hot plate with a surface temperature of 350 °F for varyious lengths of time. At 8 min the patties had a "well-done" appearance (no red color) and the internal temperature reached 81 °C. The reduction in residues was only 17%. In the third experiment (Table 4), 100 g patties were cooked for varyious lengths of time in a microwave oven. Although a turntable was used, it was obvious from visual inspection that heating was not uniform. The center remained red long after the edge appeared "well-done". However, after 8 min all trace of red color was gone. The final temperature of 98–102 °C was higher than that reached by frying, and the reduction in residues was correspondingly greater (60%) at the end.

The results were consistent with those of previous studies. Frying even to "well-done" did not require heating to a temperature as high as was used in other cooking or processing procedures. Reduction in residue levels was relatively small, as was also found by O'Brien et al. (1981) and Escanilla et al. (1959) using similar methods. Cooking at 100 °C for 30 min did not completely degrade residues, which is in agreement with

Table 4. Effect of Microwave Cooking on Oxytetracycline Residues in Ground Lamb Patties[a]

	cooking time				
	0 min	2 min	4 min	6 min	8 min
temp[b] (°C)					
edge	frozen	20	56	95	102
center		10	36	79	98
oxytetracycline found (ppm)	6.36	6.23	5.58	3.53	2.51
% reduction	0	2.0	12.2	44.4	60.5

[a] 100 g patties were placed on a plastic dish and rotated on a turntable during cooking. Mean of two experiments. [b] Temperature measured with a thermocouple immediately after removal from microwave oven. Patties had a uniform "well-done" appearance after 8 min.

the results of Buncic and Dakic (1981) using rabbit muscle and Honikel et al. (1978) using beef. The effect of microwave cooking on OTC residues has not been previously reported. Because of uneven heating, cooking to a higher temperature than frying was required to achieve a uniform "well-done" appearance. This resulted in greater reduction of residues.

The nature of the degradation products formed by heating OTC is unknown. No degradation products were detected by the HPLC procedure used.

The results confirm that ordinary cooking procedures cannot be relied on to completely degrade OTC in meat. Heat treatment for longer than 30 min at 100 °C or for shorter times at higher temperatures has been shown to totally degrade several tetracyclines (Yonova, 1971; Buncic and Dakic, 1981; Scheibner, 1972b; Honikel et al., 1978).

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