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Effect of Ordinary Cooking Procedures on Tetracycline Residues in Chicken Meat

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ABSTRACT

The abundant misuse of tetracyclines (TCs) in poultry production results in the presence of their residues in edible tissues, intended for human consumption, causing a health threat. Hence, the stability of TC residues in chicken tissues under cooking conditions is an important research area, which provides valuable information related to health safety aspects. This study aimed to present the changes by different cooking processes on TCs in chicken meat, and determine the cooking time required to make the cooked sample safer for consumption. Chicken breast and thigh incurred with TC were cooked by boiling, roasting and microwaving for different durations of time and analyzed by high-performance liquid chromatography with diode array detection. The losses of TC residues in chicken meat were depended upon the cooking procedure, cooking time and TC agents. Microwaving was more effective than boiling and roasting. The losses of TC residues increased with prolonged cooking time. Doxycycline was the most heat stable of TCs, while oxytetracycline was the most heat labile. The time required to destroy 90% of the initial TC level was 23.9, 53.2 and 101.6 min for microwaving, boiling and roasting, respectively. Generally, sufficient cooking temperature and time can have a significant effect on the losses of TC residues and provides an additional margin of safety for consumers.

Key words: tetracycline residue, chicken meat, cooking, *D*-value, health safety

INTRODUCTION

Antibiotics are among the most commonly used compounds in livestock production. Tetracyclines (TCs), including oxytetracycline (OTC), tetracycline (TTC), chlorotetracycline (CTC) and doxycycline (DOC), are considered the main antibiotics used. TCs are licensed for use in a wide range of livestock species including chicken, and can be administrated either orally or by injection. Reliable data on the consumption of veterinary drugs in general, and of antibiotics in particular, are not easy, especially in developing countries. However some figures can be used to indicate the orders of magnitude of the consumption of antibiotic agents[(1)]. A lot of antibiotics were used in veterinary medicine in the EU countries and TCs were among the most frequently used [(2,3)]. Moreover, the largest amounts of antibiotics were sold in the USA in 2007 and 2009 for use in food animals and the largest classes were TCs[(4,5)]. Accordingly, TC residues in edible products can and do appear from both disease treatment and nutritional uses, as well as foods derived from all livestock, regardless of species, posing residue problems. Consequently, the US Food and Drug Administration (FDA) announced that it will request livestock producers, drug companies and veterinarians to curb the use of antibiotics to promote growth in food-producing animals[(6)].

The existence of TC residues in chicken products and their transfer to consumers are the cause of some effects such as bacterial resistance, allergic reactions, toxicity, carcinogenic effects and disturbance of natural intestinal micro-flora. Therefore, maximum residue limits (MRLs) for TCs were recommended by many agencies. The joint FAO/WHO Expert Committee on Food Additives[(7)] recommended 200 μg/kg and 600 μg/kg for chicken tissues and liver, respectively, expressed as the sum of TCs. In addition, the EU had set the MRLs of TCs to be 100 μg/kg and 300 μg/kg for chicken tissues and liver, respectively[(8)]. However, TC levels above the MRLs have been reported in chicken products in many countries such as Mexico[(9)], Belgium[(10)], Saudi Arabia[(11)], Bulgaria[(12)] and Egypt[(13)]. Such residues in the chicken meat may pose a potentially serious health threat to the consumer.

Most foods of animal origin undergo further processing

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Journal of Food and Drug Analysis, Vol. 21, No. 1, 2013

prior to consumption for the purpose of increasing palatability and shelf life. The risk assessment of TC residues should consider thermal (cooking) effects. The thermal death time (TDT) method had been successfully used to determine the kinetics of heat labile microorganisms, nutrients, and food quality factors following a thermal process[14]. With the TDT method, the decimal reduction time (*D*-values: a heating time that results in 90% destruction of quality factor concentration) is useful for generating kinetic data suitable for use in calculating the retention of nutrients and contaminants during a thermal process[15,16].

The thermal stability of TC residues in animal food products was considered by many researchers. Some of them mentioned that TCs in animal tissues were more stable under heat treatment and were not totally inactivated by cooking conditions or heat processing[17-20]. On the other hand, some studies indicated that TCs in animal tissues were more sensitive to heat treatment and were totally destroyed by treatment at 100°C for 30 min or at higher temperature for shorter times[20,21]. On the contrary, Al-Ghamdi *et al.*[11] stated that OTC increased on boiling, and Kuhne *et al.*[22] found that the concentrations of TCs in heat-treated (100°C) meat and bone meals were higher than those found before the heat treatment. Therefore, this work was conducted to report the effects of various ordinary cooking procedures (boiling, roasting and microwaving) on TC residues (OTC, TTC, CTC and DOC) in chicken meat and to determine the cooking time required for each cooking procedure to make the cooked sample safer for consumption.

MATERIALS AND METHODS

I. *Samples*

Chicken meat samples containing high levels of TC residues derived from survey conducted on the Giza open market[13] were selected to study the thermostability of TCs during actual cooking procedures. According to that survey, no sample has more than one TC, as well as each sample has own TC level. So, twelve breast samples and twelve thigh samples were selected. Each three samples have the same TC with different levels. For each chicken sample, meat was separated from bone (deboning), chopped and re-analyzed to obtain the initial (0-time) TC levels. For each chopped chicken meat sample, three portions (100 g each) were placed into heat-safe plastic bags and cooked as described in cooking procedures.

II. *Chemicals and Reagents*

Standards of OTC, TTC, CTC and DOC were supplied by Sigma (St Louis, MO, USA). Methanol and acetonitrile were of HPLC grade. Trichloroacetic acid, citric acid monohydrate, sodium citrate trihydrate and oxalic acid were of analytical grade. Water was purified before use by a Milli-Q system (Millipore, Bedford, MA, USA).

III. *Cooking Procedures*

(I) *Boiling*

Three portions (100 g each) of chicken thigh samples were placed into a strainer (heat-safe plastic bags), immersed in boiling water (about one liter; boiling water was added during boiling time to keep the volume of water) until apparently done, well-done and extra-done for the specified time (20, 30 and 40 min, respectively), one sample was removed at each time, allowed to cool at room temperature, minced and blended with the resultant juice and analyzed in triplicate. The same procedure was applied to chicken breast samples (100 g).

(II) *Roasting*

Three portions (100 g each) of chicken thigh samples were placed into a strainer (heat-safe plastic bag), placed on a metal tray and cooked to done, well done and extra done in the center of an electric oven (J. P. Selecta, S. A. (Spain), w: 2000, v: 230, Hz: 50, serial No: 0361031) at 180°C for the specified time (40, 60 and 80 min, respectively), one sample was removed at each time, allowed to cool at room temperature, minced and blended with the resultant juice and analyzed in triplicate. The same procedure was applied to chicken breast samples (100 g).

(III) *Microwaving*

Three portions (100 g each) of chicken thigh samples were placed in a microwave bag and cooked in a Gold-star Microwave Oven (Model ER-535 MD) (2450 MHz) at six power level. The samples were withdrawn after 10, 15 and 20 min (for apparently done, well-done and extra-done, respectively), one sample was removed at each time, allowed to cool at room temperature, minced and blended with the resultant juice and analyzed in triplicate. The same procedure was applied to chicken breast samples (100 g).

IV. *Analytical Method*

The determination of TC residues was achieved according to Shalaby *et al.*[23] using HPLC-DAD. The HPLC-DAD system consisted of a HP 1100 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an auto sampler, quaternary pump 4 and a diode array detector. A reversed-phase Nucleosil 100 C18 analytical column (250 mm × 4.6 mm i.d., 5 μm, Germany) was used. Briefly, TCs in samples were extracted by citrate buffer and trichloroacetic acid, followed by sonication and centrifugation. Purification was performed on SPE (Strata C18-E cartridge) using 10 mL of 0.01 M methanolic oxalic acid for the elution of TCs. Separation of TCs was conducted on a Nucleosil 100 C18 analytical column by multistep gradient elution, and monitored at the wavelength of 351 nm.

V. *Analytical Quality Assurance*

The analytical quality assurance of the TC residues method was performed using cooked chicken meat by spiking TCs into a negative-TCs cooked chicken thigh and breast samples at a level of 100 ng/g, to meet the MRLs of EU[8]. Spiked samples were left to stand at 4°C for 1 h before analysis to establish the accuracy and precision, limits of detection (LOD), limits of quantification (LOQ) and sensitivity of the method. The LOD is considered to be the quantity yielding a detector response approximately equal to thrice the background noise. The LOQ is the lowest amount that can be analyzed within acceptable precision and accuracy at signal to noise ratio of 10. Each sample was analyzed in triplicate and the mean was considered without correction for recovery.

VI. *Thermostability Parameters*

The traditional model based on first-order kinetics was used to describe degradation curves[24]. Degradation curves were obtained for each cooking procedure. The pattern was expressed as an equation: {$y = k\ 10^{mx}$, where, y was the tetracycline residues (ng/g) in a sample after cooking, k was a constant equal to the tetracycline residues (ng/g) in a sample at 0-time, m was the slope of the regression line, and x was the cooking time (min)}. Decimal reduction times, *i. e. D*-value (minutes of heating required to drop the antibiotic concentration by 10-fold or one $\log_{10}$ cycle) were calculated from the slope of the portion of the linear section of the degradation curves. The coefficients of determination (R^2), 95% confidence limits, and statistical differences ($p = 0.01$) were calculated by GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA).

VII. *Statistical Analysis*

The ANOVA test was performed for statistical analysis using Assistant computer programs[25]. A p value of less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

The method performance characteristics were determined by single-laboratory validation using cooked chicken meat and the averages of three replicates (Table 1). The limits of detection (LOD) recorded for both chicken thigh and breast were similar, averaging 4.4, 5, 13 and 10 ng/g for OTC, TTC, CTC and DOC, respectively. The corresponding LOQ values recorded were 10, 13, 27 and 22 ng/g. The average recoveries from cooked chicken thigh were 90, 71, 83 and 88% for OTC, TTC, CTC and DOC, respectively. The corresponding values obtained for the cooked chicken breast were 91, 71, 83 and 89%. Similar results were previously given by Shalaby *et al.*[23] and Salama *et al.*[13]. The obtained recoveries were within the AOAC acceptable range for trace analysis, 60 to 115%[26], and met both European Union regulation 2002/657/EC and the regulation set by the Codex Alimentarius Commission. The relative standard deviations (RSD) were lower than 10% which complied with the requirement of the Codex Alimentarius Commission. The response of the DAD was linear and highly correlated ($r^2 = 0.9995$) within the range of 50 to 5000 ng/injection and each TC agent had its own linear equation as previously stated by Shalaby *et al.*[23]. The sensitivities of the method, which correlated directly to the detector used, were 1.44, 1.90, 0.95 and 1.23 ng for OTC, TTC, CTC and DC, respectively. Moreover, the method used revealed a satisfactory separation of the studied TCs with better selectivity and reduced peak width and retention time, which were similar to those stated in previous studies[13,23]. Generally, the method was satisfactory for use in the present study and the results were not corrected for recovery.

Table 1. Performance characteristics of the analytical method for cooked chicken meat (n = 3)

Analyte	Matrix	LOD (ng/g)	LOQ (ng/g)	S. L. (ng/g)	R. (%)	RSD (%)
OTC	Thigh	4.4	10	100	90	4.6
TTC		5.0	13		71	8.6
CTC		13.0	27		83	6.2
DOC		10.0	22		88	5.3
OTC	Breast	4.4	10	100	91	5.4
TTC		5.0	13		71	7.2
CTC		13.0	27		83	7.5
DOC		10.0	22		89	6.6

LOD: limit of detection.
LOQ: limit of quantification.
S.L: spiked level.
R: recovery.

The use of tissues incurred with TC residues and actual cooking procedures offers a realistic perspective on the effects of cooking on residual concentrations. However, the internal temperature in the centre (calculated geometrically) of the cooked sample was monitored. The internal temperature of the meat did not rise above 100°C in any method. The highest achieved temperatures were approximately 97°C during boiling, 99°C during roasting and 98°C during microwaving. The effect of ordinary cooking procedures on TC residues in chicken meat is given in Table 2. The obtained data revealed that the reduction of TC residues in cooked samples was related to cooking procedures, cooking time and TC agents. In this concern, Javadi[27] stated that among the various factors affecting antibiotics residues after the cooking process, cooking time and temperature can play major roles in antibiotic residue reduction while cooking food.

Concerning the effect of cooking procedures, the obtained data revealed that the reduction in the OTC content of breast meat boiled for 20 min was 57.3%, compared to 100% by the microwave cooking for the same duration of time. Cooking of breast meat for 40 min by boiling and

Journal of Food and Drug Analysis, Vol. 21, No. 1, 2013

Table 2. Effect of cooking procedures on tetracycline residues in chicken meat

Treatment	Time (min)	OTC		TTC		CTC		DOC	
		C (ng/g)	R (%)	C (ng/g)	R (%)	C (ng/g)	R (%)	C (ng/g)	R (%)
					Breast meat				
Boiling	0	1897 ± 35.1	0	500 ± 14.30	0	1078 ± 23.7	0	5812 ± 85.5	0
	20	810 ± 35.3	57.3	360 ± 16.7	28.0	836 ± 21.9	22.5	5145 ± 58.1	11.5
	30	501 ± 22.5	73.6	228 ± 13.4	54.4	561 ± 22.7	48.0	4203 ± 69.8	27.7
	40	162 ± 6.4	91.5	122 ± 7.9	75.6	283 ± 18.4	73.8	3382 ± 75.5	41.8
Microwave	0	1880 ± 34.3	0	500 ± 14.3	0	2148 ± 47.3	0	1190 ± 34.3	0
	10	476 ± 25.9	74.7	297 ± 11.5	40.6	1503 ± 27.9	30.0	974 ± 28.6	18.2
	15	102 ± 5.1	94.6	203 ± 10.5	59.4	868 ± 30.1	59.6	768 ± 29.7	35.4
	20	ND	100	103 ± 8.5	79.4	352 ± 17.2	83.6	634 ± 23.8	46.7
Roasting	0	1680 ± 26.5	0	500 ± 14.3	0	2148 ± 47.3	0	1216 ± 29.1	0
	40	469 ± 25.2	72.1	270 ± 7.8	46.0	1460 ± 29.2	32.0	1050 ± 22.1	13.7
	60	170 ± 14.3	89.9	150 ± 5.9	70.0	780 ± 19.5	63.7	870 ± 27.8	28.5
	80	40 ± 3.3	97.6	90 ± 3.7	82.0	310 ± 12.7	85.6	675 ± 27.0	44.5
					Thigh meat				
Boiling	0	1914 ± 38.3	0	310 ± 8.0	0	1040 ± 15.5	0	6010 ± 98.3	0
	20	929 ± 21.4	51.5	230 ± 6.9	25.8	844 ± 9.3	18.9	5165 ± 108.4	14.1
	30	547 ± 23.5	71.4	137 ± 5.2	55.8	605 ± 18.9	41.8	4250 ± 100.0	29.3
	40	294 ± 15.3	84.6	76 ± 4.0	75.5	308 ± 12.0	70.4	3360 ± 104.2	44.1
Microwave	0	1790 ± 24.5	0	500 ± 14.3	0	1196 ± 25.4	0	1202 ± 25.2	0
	10	478 ± 17.7	73.3	301 ± 9.3	39.8	736 ± 15.5	38.4	990 ± 22.8	17.6
	15	143 ± 7.6	92.0	178 ± 6.6	64.4	453 ± 16.3	62.1	805 ± 24.2	33.0
	20	18 ± 2.5	99.0	81 ± 4.6	83.8	125 ± 8.0	89.6	654 ± 20.9	45.6
Roasting	0	1650 ± 29.6	0	500 ± 14.3	0	1196 ± 25.4	0	1430 ± 32.3	0
	40	640 ± 20.5	61.2	210 ± 6.9	58.0	680 ± 19.0	43.1	1214 ± 27.9	15.1
	60	208 ± 11.0	87.4	145 ± 6.7	71.0	435 ± 20.5	63.6	1057 ± 28.5	26.1
	80	89 ± 3.8	94.6	68 ± 4.2	86.4	190 ± 10.8	84.1	826 ± 25.6	42.2

C: residual concentration of the sample.
R: reduction percentage.

roasting led to a reduction of OTC contents by 91.5 and 72.1%, respectively. Similar results with different data were given for thigh meat (Table 2). Our results revealed that cooking could lead to a reduction of TCs contents in the samples, and microwave cooking had a more pronounced effect on TCs than roasting and boiling. These findings were in agreement with those given by Ibrahim and Moats[18] who stated that cooking of lamb meat by boiling (30 min) reduced OTC by 95%, and microwave cooking (8 min) reduced the OTC content by 60%, while frying (8 min) led to 17.3% reduction in the OTC content. In addition, Al-Ghamdi *et al.*[11] stated that TTC, CTC and DOC in meat and liver were decreased after cooking by boiling. Gratacós-Cubarsí *et al.*[20] also mentioned that ordinary cooking procedures, *i. e.* microwave and boiling reduced the initial concentrations of TC residues by 56 to 82%.

With regard to cooking time, it was observed that regardless of the cooking procedures and TC agents, prolonged cooking time reduced the content of TCs significantly ($p < 0.01$). For example, the reduction of OTC in breast meat cooked by boiling for 20, 30 and 40 min were 57.3, 73.6 and 91.5%, respectively. The OTC reduction values in roasted samples for 40, 60 and 80 min were 72.1, 89.9 and 97.6%, respectively. Microwave cooking for 10, 15 and 20 min led to reduce OTC contents of breast meat by 74.7, 99.6 and 100%, respectively. Similar results with different values were recorded for TC residues in thigh meat. It was previously reported that prolonged cooking of meat might inactivate the antibiotics[17].

In relation to TC agents, it could be observed that

DOC reduction was 27.7% in chicken breast cooked by boiling for 30 min, compared to 73.6, 54.4 and 48% reduction for OTC, TTC and CTC, respectively (Table 2). The reduction of TCs obtained for chicken breast cooked by microwave for 15 min were 94.6, 59.4, 59.6 and 35.4% for OTC, TTC, CTC and DOC, respectively. The corresponding values obtained for roasted chicken breast for 60 min were 89.9, 70, 63.7 and 28.5%. Similar results with different data could be observed for chicken thigh meat as stated in the same table. From these results, it was obvious that DOC was

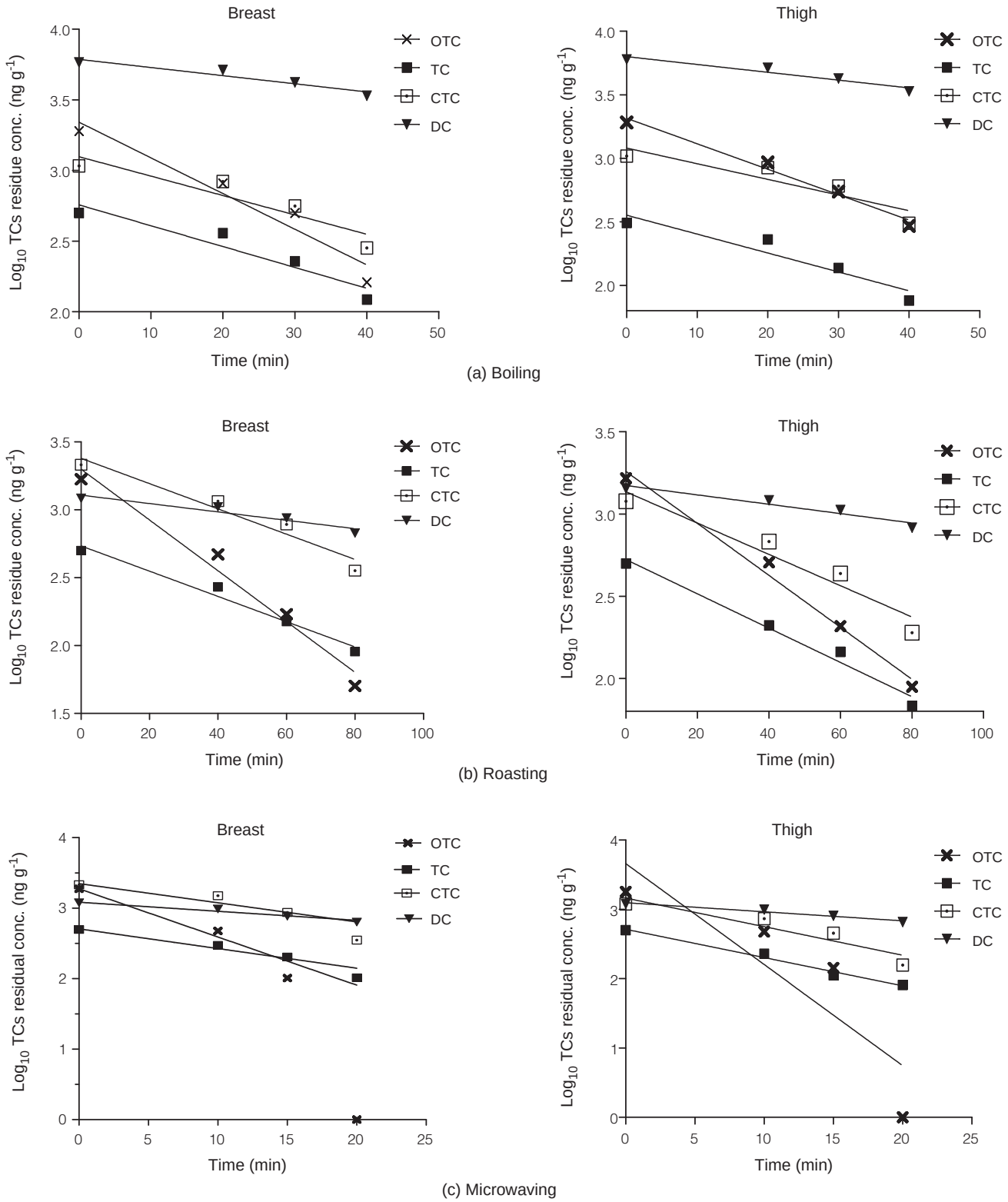

Figure 1. Degradation curves of TC residues in chicken meat during (a) boiling, (b) roasting and (c) microwaving.

more heat-resistant than other TC agents, while OTC was much more heat-sensitive. Our results were in agreement with those of Hassani *et al.*[28] who stated that the thermostability of DOC was 1.3 and 3 times higher than TTC and OTC, respectively. On the contrary, Al-Ghamdi *et al.*[11] stated that OTC was more stable than TTC, CTC and DOC in meat and liver samples after cooking by boiling. The markedly variable heat stabilities within the TC group highlighted the fact that heat stability within this class could be very different despite their structural similarity.

The study was extended to generate kinetic data suitable for use in calculating retention of TC residues during cooking of chicken meat, and to estimate the time required, for each cooking method, to destroy 90% of the initial TCs amount (*D*-values). The time and temperature used in the actual cooking of meat were considered. Although heat exposure was more difficult to control in actual meat samples than in liquid media, the results would be more representative for actual cooking conditions[17]. In this concern, Kitts *et al.*[16] clarified that temperature log time were the same in both buffer and muscle systems and did not contribute significantly to the TDT curves.

As stated above, heat treatment reduced TC residues significantly ($p < 0.01$) as the time of treatment increased. This reduction was fitted to a logarithmic equation, where significant ($p < 0.01$) linear regressions were observed between the logarithm of TC residual level (ng/g) and time (min) of cooking with the coefficients of determination (r^2) ranging from 0.86 to 0.98. The obtained regression lines (Figure 1) were used to calculate *D*-values of TC residues in cooked chicken meat (Table 3). It could be observed that the general averages of *D*-values for TCs in chicken meat cooked by boiling roasting and microwaving were 53.2, 101.6 and 23.9 min, respectively. The *D*-values confirmed that microwaving is the choice procedure for destroying TC residues in chicken meat, followed by boiling while roasting comes in later order. From the same table, it could be noted that DOC was the most heat stable, while OTC was the most heat labile as evidenced by the higher and lower *D*-values, respectively.

Table 3. Decimal reduction time of TC residues in chicken meat by different heat treatments

Antibiotics	D-value (min)					
	Boiling		Roasting		Microwaving	
	B.	Th.	B.	Th.	B.	Th.
OTC	37.5	38.6	61.5	62.0	13.7	13.5
TTC	47.0	48.0	93.3	90.0	22.5	21.0
CTC	48.5	50.0	85.0	85.5	21.5	20.1
DOC	76.0	78.0	166.0	170.0	39.0	40.0
average	52.3	53.7	101.5	101.9	24.2	23.7
General Average	53.0		101.7		24.0	

B: chicken breast.
Th: chicken thigh.

Thus, longer cooking time would be required to reduce DOC content to 10% of the original value compared with those required by the other TCs. It should be stated that, some of TC breakdown products were described, but the whole degradation products have not been identified yet. However, structural degradation of the TCs was in good agreement with the reduction in antimicrobial activity, suggesting that degradation also diminished antimicrobial activity[29].

CONCLUSIONS

The present study implies that TC residues are unstable drugs that will be degraded during cooking, rendering chicken meat apparently safe for human consumption according to the permissible limit cited by the Commission Regulation (EC) No. 37/2010. It can be concluded that sufficient cooking temperature and time can have a great effect on TC residual losses and provides an additional margin of safety for consumers. This result shall help the processors and quality control officials to evaluate the presence of these antibiotics in heat-processed products rather than the raw material. This will also be useful for restaurants and housewives to prepare a safer meal.

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