



Influence of Some Cooking Procedures on Quality Criteria and Some Veterinary Drugs Residues in Chicken Wings Meals

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ABSTRACT

Background: Veterinary drug residues in poultry products pose significant public health hazards such as cancer, mutagenicity, reproduction challenges and hypersensitivity reactions. Therefore, this study aimed to investigate the occurrence of some antibiotics and zeranol hormone residues in fresh chicken wings, and also study the effect of heat treatments on the concentration of these residues. **Methods:** Sixty samples of chicken wings were collected from different local markets in Cairo and Giza governorates, Egypt, and were divided into two groups (30 samples for each group) for antibiotics and hormonal residues investigations by high performance liquid chromatography (HPLC) and Enzyme-linked Immune Sorbent Assay (ELISA), respectively. The study was extended to include some cooking methods (boiling, deep frying, and grilling) as types of heat treatments for the reduction of these residues in chicken wings meals and their effects on cooking characteristics, pH, and texture profile. **Results:** The results revealed that 13.3% of samples were contaminated with ciprofloxacin, tetracycline, and amoxicillin antibiotics residues; however, zeranol hormone was detected in 73.7% of samples. Furthermore, boiling was the most effective method in the reduction of all residues concentrations. Cooking loss, hardness, springiness, cohesiveness, gumminess, and resilience in the examined samples significantly decreased by boiling compared to other two methods. **Conclusion:** Cooking methods can successfully reduce the concentration of veterinary drugs in chicken wings meals and boiling method was more effective in reducing their concentration.

Introduction

Veterinary drugs, including antibiotics and growth hormones, are generally used for therapeutic and prophylactic purposes, as well as enhancement of growth and feed efficiency in poultry production, improving healthy fresh chicken products. However, their residues are

considered a public health concern in all countries, inducing potential health hazards for consumers such as allergies, nephropathy, bone marrow toxicity, mutagenic effects, reproductive disorders, hepatotoxicity, teratogenicity, and carcinogenicity disorders of the intestinal flora (Ayalew *et al.*,

2022). Furthermore, antibiotics in poultry production may be the key to antimicrobial resistance (Krysiak *et al.*, 2021).

Hormonal substances are used as growth promoters and fertility regulation and enhancement in food-producing animals (Hirpessa *et al.*, 2020). Hormonal substances are either the naturally occurring steroids: estradiol-17 β , progesterone, and testosterone, or synthetic compounds such as zeranol, which act as anabolic agents and have a physiological effect as that of sex steroids increasing nitrogen retention and protein deposition (Qaid and Abdoun, 2022). Different studies have reported the possible hormonal carcinogenicity, genotoxicity, and interference with the natural physiological function, and Zeranol can accumulate in human food, causing adverse effects on human health as hormonal imbalance resulting in reproductive problems and even breast, prostate, or colon cancer (Senthil *et al.*, 2018).

Chicken wings are among the most popular poultry meals for home and food service consumption and a successful example of economically poultry meals due to their low price and an excellent source of many important nutrients including protein (17.6%) and fat (14.9%) (Cano *et al.*, 2022). Cooking as a method of heat treatment has been considered a very critical step in the preparation of food products resulting in changes in organoleptic properties, nutritional value, and consumer acceptance (Nawaz *et al.*, 2021), as well as the concentration of chicken tissue contents including drug residues. This effect depends on the type of cooking. Therefore, the aim of this study was mainly to evaluate the effect of some cooking processes on some quality characteristics as well as antibiotics and hormone residues in chicken wings meals.

Materials and Methods

This study was planned to determine the residues of some veterinary drugs in chicken wings followed by detection of their limits after cooking treatments (boiling, frying, and grilling). The experiment was designed as follows:

Samples collection

A total of sixty chicken samples (fresh broilers) were collected from different markets in different regions of Cairo and Giza governorates. Sampling was done on the basis that the two wings of the same bird were considered as one sample. Samples were put in plastic bags and transferred to the laboratory in an ice box and were divided into two groups (30 samples for each group) for antibiotics and hormonal residues.

Antibiotics detection and characterization

Microbial inhibition tests (qualitative evaluation): Microbial inhibition assays are routine methods for screening food items for residues of antibiotics. These tests are the initial step for detection and characterization of antibiotics. The one-plate screening method (OPS) was used. Based on the principle that the appearance of an inhibition zone around a sample indicates the presence of antimicrobial residues. The size of this zone correlates with the concentration of the residues in the sample. Followed by characterization of the detected antibiotic residues by seven plates technic (Pikkemaat, 2009)

Quantification of antibiotic residues: Antibiotic residues in positive samples were quantified using high performance liquid chromatography (HPLC) according to (Heitzman, 1994).

Samples preparation: The samples were homogenized and frozen till the time of analysis at -20 °C in the dark.

Detection of ciprofloxacin

Chemicals: Methanol (HPLC grade) (Fischer Scientific, U K.); Deionized water or HPLC grade water; Oxalic acid (Fluka Nisht Direkt, Germany); 5% (w/v) of trichloro-acetic acid (TCA); Citric acid monohydrate; disodium hydrogen phosphate dehydrate; Ethylene-diamine tetraacetic acid disodium salt McIlvaine Buffer; Dichloromethane, Hexane, Acetone, and Petroleum ether were obtained from Fischer scientific (Leicestershire, UK); A monohydrous.

Calibration standards: Calibration standards were prepared using concentrations of 100, 500, 1000, 5000, 10000 μ g/l of Ciprofloxacin in eluent

and spiked samples with same concentration.

Liquid chromatography operating conditions: Injection volume of 25 μ l; flow rate of 1 ml/min; UV at wavelength of 280 nm; column C18 Teknorama (250 \times 4.6mm; 5 μ m) column temperature, 50 $^{\circ}$ C; LC mobile phase isocratic mode 50ml/l acetic acid : acetonitrile methanol (900:50:50) (Kamberi *et al.*, 1998).

Detection of oxytetracycline

Chemicals: Standard Merck Oxytetracycline HCl was obtained from Pfizer Inc./Turkey. Certified grade citric acid was obtained from Merck Inc. Methanol, acetonitrile, and nitric acid were obtained from Carlo Erba and 0.45 μ m Nylon filters were obtained from Waters Assoc. Other chemicals were analytical or HPLC grade.

Preparation of stock and working standard solutions: Calibration standards were prepared using concentrations of 50, 100, 200, 500, and 1000 μ g/L of OTC in eluent.

Sample extraction: Two grams of sample were homogenized in a blender for 2 min and then 0.1 g citric acid was added. One milliliter of nitric acid (30%), 4 ml of methanol and 1 ml of deionized water were added to the mixture. The suspension with solid particles was put in a vortex for good mixing, kept in an ultrasonic bath for 15 min and then centrifuged for 10 min at 5300 rpm. After filtering through a 0.45 μ m nylon filter, 20 μ l of solution was injected into HPLC for analysis (Şenyuva *et al.*, 2000).

Chromatographic conditions: Isocratic separation was achieved using a Hypersil BDS C18 (5 mm, 250 \times 4 mm) column supplied by HP (Germany). The mobile phase consisting of distilled water (pH=2.1 with H₂SO₄): Acetonitrile, 85:15(v/v), was pumped at a flow rate of 1.5 ml/min. The analytes were detected at 360 nm wavelength using a setting of 0.01 A.U.F.S. The injected volume was 20 μ l and chromatography was performed at 24 $^{\circ}$ C.

Detection of amoxicillin (El barawy et al., 2011)

Chemicals: All chemicals and reagent used were of HPLC grade or analytical grade. Amoxicillin trihydrate, sodium hydroxide, hydrochloric acid, and potassium dihydrogenphosphate were obtained from Sigma (St. Louis, MO, USA). Methanol and other solvents were supplied by J.T. Baker (Phillipsburg, NJ, USA). Deionized water was obtained from Milli-Q Plus analytical deionization system.

Preparation of stock and working standard solutions: Stock solution of amoxicillin (100 μ g/ml) was prepared in volumetric flask (Pyrex, Iwaki Glass Co., Tokyo, Japan). By dissolving 10 mg in 100 ml deionized water, pH levels were adjusted to improve their solubility. Afterward, the stock solution of amoxicillin was diluted to make standard solutions of 50, 100, 250, 500 and 1000 μ g/l, then they were filtered through MFS syringe filters (0.2 μ m, Advantee MFD, Inc., Japan).

Sample extraction: One gram of homogenized muscle samples was accurately weighted into a 20 mL centrifuge tube and extraction solution, and 0.1 MKH₂P₀₄ buffer (pH 2.5) was added with vortex mixing for 5 min. Then mixture was centrifuged at 7,000 rpm for 5 min (Kubota 6900, Tokyo, Japan). The upper layer was transferred to a clean tube, and the precipitate was extracted repeatedly twice. The supernatants were collected and the aqueous extract was placed onto the Bond-Elut column.

Extraction with Bond-Elut certify columns: A3-ml (500 mg) Bond-ElutC18 certify disposable extraction column was activated with 4 ml of methanol followed by 1 ml of 0.005 MKH₂P₀₄ buffer (pH 6.8). At this stage, care was taken to prevent air from reaching the stationary phase. The sample extraction was loaded onto the column and the excess was collected as waste. The column was then washed with 1 ml of deionized water and dried in a vacuum state for 20 min. Subsequently, elution was then carried out with 1 ml H₂O: methanol (60:40) and decanted into a 10-ml Quickfit glass tube. The extract was evaporated under a gentle stream of nitrogen at 50 $^{\circ}$ C. The dry residue of Bond-Elut eluate was dissolved in 1 ml deionized water (pH 9.0). This was filtered through a MFS

syringe filter (0.2-µm, AdvantecMFD, Inc., Japan). A 20-µl aliquot was transferred to injection vial and HPLC analysis was performed.

Chromatographic conditions: The LC separation was performed at a flow rate of 1 ml/min on a Mightysil RP-18 GP 250-4.6 mm 1>5 µm column (Kanto Chemical, Tokyo). The injection volume was 20 µl. Mobile phase consisted of 0.005 M KH₂PO₄ buffer with pH 6.8-MeOH (90:10, v/v), operating at 210 nm and was filtered through 0.2 µm membrane filters and degassed under vacuum prior use.

Detection of zeranol hormone residues (Mor et al., 2011)

Screening of zeranol residues were carried out using commercial ELISA kits and samples extraction and estimation were performed based on the manufacturer procedure described by the manual ELISA kits.

Reagents: Dilution buffer; Conjugate solution; Antibody solution; Rinsing buffer; Substrate solution.

Sample preparation: Skin was removed from the samples and was grounded; One gram of ground sample was homogenized with one ml of acetonitrile in a centrifugal; Screw cap vial, then mixed vigorously (vortex); The tube was shaken by hand for 15 min; Centrifuge for 10 min/2500 rpm; One milliliter of the supernatant was transferred into a glass tube and evaporated to dryness at 50 °C; The residue was reconstituted with 100 µl of 100% methanol and vortex for 1 min; Fifty microliter of dried residue was diluted with 450 µl dilution buffer; An aliquot of 50 µl was used as a sample in the ELISA.

Test procedures: The test procedures were done according to the chart enclosed in the kits (Europrxima, Amhm, the Netherlands). The results were calculated by following equation:

Percentage maximal absorbance = (OD sample/OD standard) × 100

To obtain the zeranol content in tissue samples the calculated zeranol concentration has to be multiplied by a factor 5.

Control of antibiotic and hormone residues hazards using different cooking processes

Three samples that had higher antibiotics or hormone residues concentration were prepared for different cooking procedures and the negative samples were prepared to evaluate cooking characteristics, pH, and texture profile.

Boiling: Chicken wings samples were cooked in sufficient amounts of boiling water to cover it at ratio 2:1 (water: chicken) for 17 min

Deep frying: Frying in a pan contains sunflower oil at 180–200 °C for 9 min till complete cooking.

Grilling: Samples were grilled for 10 min on manual grill until complete cooking and then transferred to the laboratory for the following tests.

Evaluation of cooking characteristics, pH, and texture profile analysis (TPA)

Each sample was weighted before and after cooking process to extract the moisture content (%), cooking loss (%), and cooking yield (%) and pH levels were measured according to the ACOA (Chemists Association Official Analytical, 1990). The value of moisture retention (representing the amount of moisture retained in cooked samples per 100 g of the raw sample) was calculated (El-Magoli et al., 1996). TPA was carried out using Brookfield CT3 instrument (Brookfield Engineering Laboratories, Inc., MA02346-1031, USA) (Abdel-Aal and Rabalski, 2012). After removing the bone from the sample. The following TPA characteristics were determined: hardness, springiness, gumminess, cohesiveness, and resilience as described in the operating instruction manual.

Data analysis

Statistical analysis was performed using SPSS One-Way ANOVA, version 22 (IBM Corp.). Data were treated as a complete randomization design (Steel and Torrie, 1960). Multiple comparisons were carried out applying Duncan test. The significance level was *P*-value < 0.05.

Results

Antibiotics detection and characterization

As shown in **Figure 1**, the microbial inhibition test of examined samples depends on the appearance of an inhibition zone around the

sample which indicates the presence of antimicrobial residues and its size depends on the residue concentrations. Accordingly, 13.3% of samples were contaminated with antibiotics residues. **Table 1** summarizes results of the incidence and concentrations of some antibiotics residues in the contaminated samples, out of which one sample was exposed to co-occurrence of multidrug residues. In total, 13.33% of samples was positive. Ciprofloxacin residue was detected in 75% of the contaminated samples with a mean value of 142.13 ± 63.60 $\mu\text{g/kg}$ above the Maximum Residue Limit (MRL). The recommended limit was 100 $\mu\text{g/kg}$ (EC, 2010). Oxytetracycline residues were identified in 6.6% of total examined samples (50% of the contaminated samples) with a mean concentration of 128.15 ± 8.27 $\mu\text{g/kg}$ that does not exceed the permissible level (200 $\mu\text{g/kg}$) (Dix, 1986). The lowest detection rate of antibiotics residues was observed for amoxicillin antibiotic (3.3%) with a concentration of 60 $\mu\text{g/kg}$ that exceeds the recommended MRL (50 $\mu\text{g/kg}$) (Moreno and Lanusse, 2017).



Figure 1. Microbial inhibition test.

Detection of zeranol hormone

According to **Table 2**, zeranol hormone residues were detected in 73.7% of examined samples with a mean concentration value of 1.098 ± 0.30 ng/g , which is considered near to the MRL (2 ng/g) according to codex Alimentarius commission (O'Brien and Mitchell, 2018).

Table 1. Results of antibiotics residues in the contaminated chicken samples (n=30).

Positive Samples (13.33%)		
Detected antibiotics	Percent	Mean \pm SD
Ciprofloxacin	75	142.13 ± 63.6
Oxytetracycline	50	128.15 ± 8.27
Amoxicillin	25	60.00 ± 00.00

Table 2. Results of positive samples of Zeranol hormone residues (ng/g) in examined chicken samples (n =30).

N	%	Minimum	Maximum	Mean \pm SD
22	73.7	0.62	1.80	1.09 ± 0.30

Effect of different cooking procedures on antibiotics and zeranol hormone residues

The efficiency of heat treatment methods is shown in **Tables (3 and 4)** and **Figures (2, 3, 4, and 5)**. The reduction efficiency of treatments revealed the effect of cooking methods on detected antibiotics residues. The results showed that the concentration of ciprofloxacin residues was affected by all cooking methods as it was reduced by 33.86, 30.16 and 15.59% by boiling, frying, and grilling methods, respectively. However, its reduction (%) was the lowest among other antibiotics. Regarding the effects of heat treatments on oxytetracycline residues, they were reduced by 84.15, 74.15, and 55.83% by boiling, frying, and grilling, respectively, while amoxicillin concentration showed a high reduction (88.01, 82.01, and 63.16% by boiling, frying, and grilling, respectively). On the other hand, **Table 4** reveals that boiling significantly diminished the concentration of zeranol hormone residues ($P < 0.05$) more than frying and grilling methods.

Effect of different cooking procedures on cooking characteristics and physical properties of samples

Results in **Table 5** showed that pH, cooking loss, moisture retention, and cooking yield of control (uncooked) samples were significantly lower than the other treated samples with different

cooking methods. By boiling, cooking yield, moisture content, and moisture retention were significantly ($P<0.05$) higher than those recorded after frying or grilling methods.

Table 3. Effect of different cooking procedures on residues of some antibiotics in chicken wings samples.

Treatment	Ciprofloxacin		Oxytetracilin		Amoxicillin	
	ppb	Reduction(%)	ppb	Reduction(%)	ppb	Reduction(%)
Before treatment	142.13±63.60 ^s	00.00±0.00 ^d	128.15±8.27 ^a	00.00±0.00 ^d	60.80±0.00 ^a	00.00±0.00 ^c
After boiling	94.00±44.14 ^c	33.86±3.20 ^a	20.31±2.40 ^d	84.15±2.91 ^a	7.29±0.00 ^d	88.01±0.00 ^a
After frying	99.27±41.85 ^c	30.16±2.08 ^b	31.13±6.12 ^c	74.15±6.36 ^a	10.94±0.00 ^c	82.01±0.00 ^a
After grilling	119.97±59.65 ^b	15.59±4.28 ^c	56.60±2.26 ^b	55.83±2.69 ^c	22.40±0.00 ^b	63.16±0.00 ^b

Notes: a, b, and c indicate significant difference ($P < 0.05$) between any two mean values within the same column using Duncan test; Data presented as Mean±SD..

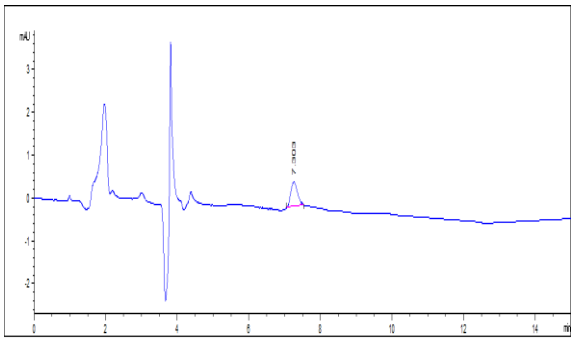
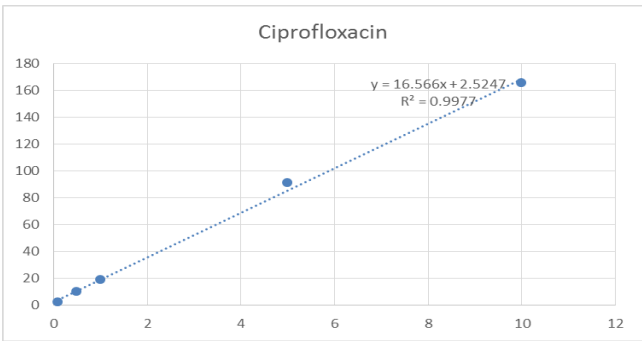


Figure 2. Chromatogram of ciprofloxacin residues in chicken samples.

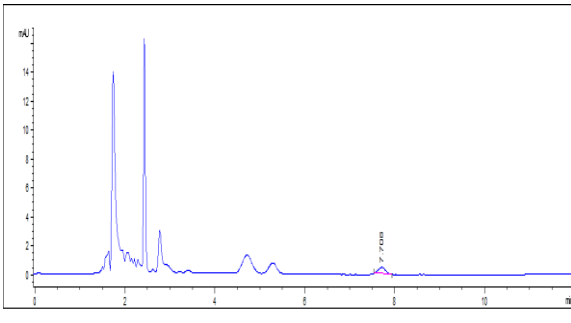
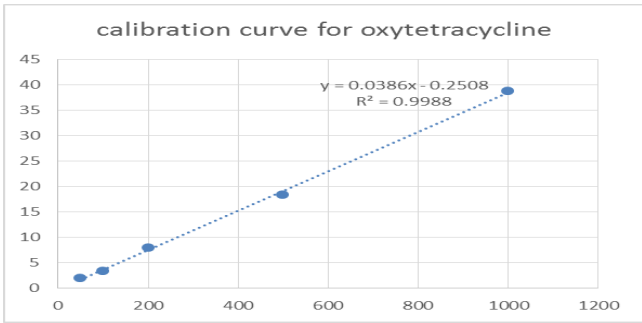


Figure 3. Chromatogram of oxytetracycline residues in chicken samples.

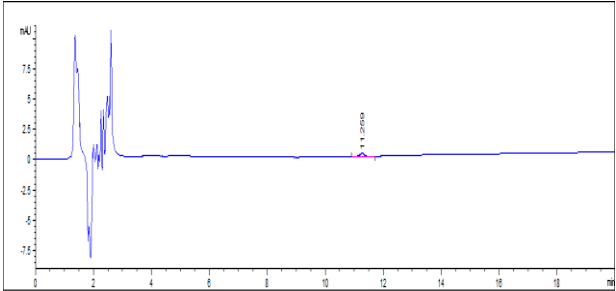
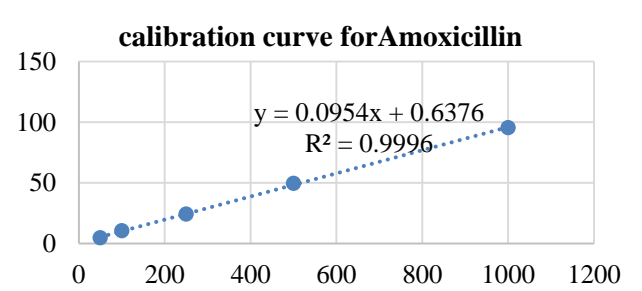


Figure 4. Chromatogram of Amoxicillin residues in chicken samples.

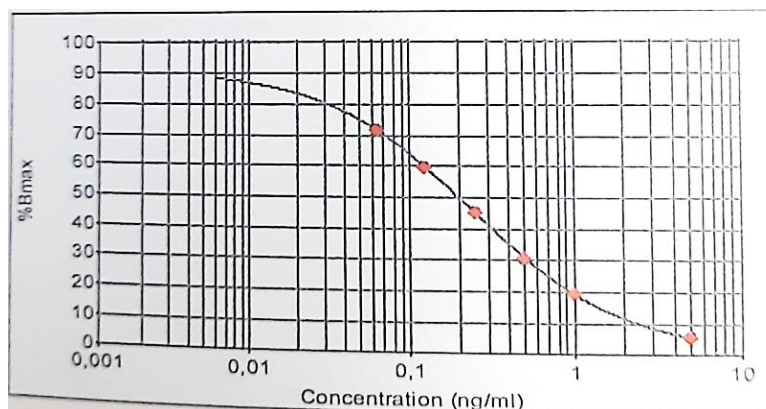


Figure 5. Standard curve of zeranole hormone in chicken samples.

Table 4. Effect of different cooking procedures on residues of zeranol hormone in chicken samples.

Treatment	ng/g	Reduction (%)
Before treatment	1.47±0.29 ^a	0.00±0.00 ^d
After boiling	0.88±0.14 ^d	40.14±2.23 ^a
After frying	1.09±0.28 ^c	25.85±6.46 ^b
After grilling	1.26±0.18 ^b	14.29±5.68 ^c

Notes: a, b, and c indicate significant difference ($P < 0.05$) between any two mean values within the same column using Duncan test; Data is presented as Mean±SD.

Cooking loss is an important quality index for evaluation of cooking methods and it is also of great economic importance and highly related to eating quality including appearance, tenderness, and juiciness of cooked food. **Table 5** shows that cooking loss in the examined samples was significantly decreased by boiling than the other two methods.

TPA is an important method for determining food quality and its directly affects consumer acceptance. Results revealed that texture properties were significantly affected by processing, as there was a significant reduction in hardness, springiness, cohesiveness, gumminess, and resilience by all cooking methods. Furthermore, the lowest hardness, springiness, cohesiveness, gumminess, and resilience were recorded in boiling treatment compared to those in the other treatments ($P < 0.05$). This may be due to its high content in moisture retention compared to other treatments.

Discussion

Chicken meat is considered an important and popular food with high nutritional value and may be

contaminated with different substances including veterinary drug residues such as antibiotics and hormones, leading to various health problems for consumer. Despite their beneficial effects in animal treatment, they can cause health problems in both animals and human. The presence of antibiotic residues in foodstuff and its transmission to the consumer may cause genotoxic, carcinogenic, immunotoxic, and endocrine effects (Toldrá and Reig, 2006).

In the present study, three different antibiotic classes (quinolones, tetracyclines, and beta-lactams) have been detected and their screening showed that ciprofloxacin (quinolones) had the highest occurrence percentage followed by oxytetracycline and amoxicillin (β -lactams) out of contaminated samples (Al Mashhadany, 2015). could detect antibiotics residues in 6.3% of chicken muscle samples according to direct microbiological method, while other studies recorded higher prevalence of antimicrobial residues (40-42%) of the examined muscle samples (Moghaddam *et al.*, 2022, Onwumere-Idolor *et al.*, 2022).

Table 5. Effects of different cooking procedures on cooking characteristics and physical properties of chicken wings samples.

Treatment	Cooking characteristics and pH				
	pH	Moisture content (%)	Moisture retention (%)	Cooking loss (%)	Cooking Yield (%)
Control (uncooked)	6.41±0.01 ^d	76.30±0.01 ^a	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d
Boiling	6.48±0.01 ^b	63.73±0.01 ^b	46.12±0.03 ^a	26.99±0.01 ^c	73.10±0.06 ^a
Deep frying	6.45±0.01 ^c	63.09±0.01 ^c	37.06±0.09 ^c	41.92±0.03 ^a	58.17±0.12 ^c
Grilling	6.95±0.02 ^a	61.15±0.01 ^d	38.63±0.27 ^b	36.37±0.01 ^b	63.62±0.01 ^b
Texture profile analysis					
Treatment	Hardness (N)	Springiness (mm)	Cohesiveness	Gumminess (N)	Resilience (mm)
Control (uncooked)	4794.67±2.60 ^a	5.82±0.01 ^a	0.60±0.01 ^a	2842.00±1.53 ^a	0.19±0.01 ^a
Boiling	376.00±1.15 ^d	1.47±0.01 ^d	0.16±0.01 ^d	53.67.00±0.88 ^d	0.12±0.01 ^d
Deep frying	744.67±0.88 ^c	3.39±0.01 ^c	0.46±0.01 ^b	339.00±0.58 ^c	0.15±0.00 ^b
Grilling	1221.33±0.88 ^b	3.53±0.01 ^b	0.37±0.01 ^c	475.33±0.88 ^b	0.15±0.03 ^c

Notes: a, b, and c indicate significant difference ($P < 0.05$) between any two mean values within the same column using Duncan test; Data is presented as Mean±SD.

Quinolones and fluoroquinolones are a class of synthetic antibiotics used mainly in veterinary medicines. Fluoroquinolones are broad spectrum antibiotic used heavily in poultry production (Gouvêa *et al.*, 2015). Enrofloxacin is a synthetic fluoroquinolone and ciprofloxacin is the main metabolite of enrofloxacin. It is a bactericidal antimicrobial which inhibits the catalytic activity of bacterial DNA (Sharma *et al.*, 2009).

Similar ciprofloxacin residues mean values of 131.7±25.2 and 92.11±30.10 µg/kg were detected by Hasanen (Hasanen *et al.*, 2016) in the examined chicken breast and thigh, respectively. Hasanen recorded that enrofloxacin residues were detected in 15% of the analyzed poultry meat samples and its concentration did not exceed the suggested MRLs in any sample (Morales-Gutiérrez *et al.*, 2015) stated that even after the withdrawal period, residues of enrofloxacin and ciprofloxacin still persisted in tissues at a concentration below the MRL. Therefore, reconsidering the misuse and improper treatment regardless of insufficient withdrawal time should be a necessary measure to take.

Alaboudi detected tetracyclines residues in 12 (6.6%) chicken muscle samples and 4.4% of them exceeded the permissible level (Alaboudi, 2022). however, higher percentages (27.5% and 17.5%) in the examined chicken samples were recorded by

Jammoul (Jammoul and El Darra, 2019) and Huong (Huong *et al.*, 2020), respectively. Accumulation of tetracyclines residues in food poses public health risks such as microbial resistance, bone and, teeth staining and teratogenic effects (Hussein and Darwish, 2013).

Zeranol is a synthetic anabolic hormone obtained from the mycotoxin zeralenone that is produced from the *Fusarium graminearum* fungus. It has an estrogenic effect used as growth promoter for fattening of animals. Their residues lead to greater harmful effects such as premature sexual development in children, liver tumors, and increased fetal mortality. Two studies detected its residues in 11.43% of examined meat samples with mean concentration values of 0.1065±0.01, 0.1235±0.01, and 0.262±0.02 ppb in muscles, livers, and kidneys, respectively (Ibrahim *et al.*, 2018, Khalafalla *et al.*, 2010). However, the others (Hussein and Khalil, 2013, Sadek *et al.*, 1998) failed to find any residues of zeranol in chicken muscles. Presence of zeranol residues in chicken tissues may be due to animal administration shortly before slaughtering or being slaughtered without respect to the withdrawal time after treatment (Kart *et al.*, 2008).

Boiling, frying, and grilling are effective heat treatment methods for degrading amoxicillin, oxytetracyclin, ciprofloxacin, and zeranol hormone

residues to safe levels. Boiling could degrade amoxicillin, oxytetracycline, ciprofloxacin, and zeranol hormone residues higher than frying but grilling had low degrading effect on all antibiotics and zeranol hormone residues. Our results were in line with the study by Fathy (Fathy *et al.*, 2015), in which they degraded ciprofloxacin residues by heat treatment. Khan *et al.* (2015) found that ciprofloxacin residues in chicken tissues were decreased by boiling and roasting by 68.18% and 68.67%, respectively (Khan *et al.*, 2015). Shaltout *et al.* reported 22.42% and 17.01% reduction rate of ciprofloxacin after boiling and roasting, respectively (Shaltout *et al.*, 2019).

These results differed with those obtained by Roca (Roca *et al.*, 2010) who stated that quinolones are very stable during heat processing even at ultra-high temperatures which agreed with the other (Hasanen *et al.*, 2016) who found that ciprofloxacin residues were stable under any cooking methods.

Vivienne *et al.* (2018) reported 53.6% and 69.6% reduction of oxytetracycline residues by roasting and boiling, respectively (Vivienne *et al.*, 2018). However, found that antibiotic concentrations were not affected significantly by heat treatments (up to 90 °C for 60 min).

Reduction in tetracycline concentrations during boiling and deep-frying was due to its transfer from meat to cooking medium (water and oil). The overall loss of tetracycline residues was due to denaturation of protein-tetracycline compounds (Nguyen *et al.*, 2013).

For the effect of cooking methods on amoxicillin, our results align with those reported by Lakew *et al.* (Lakew *et al.*, 2022), who found that amoxicillin residue was reduced by 70-87% after boiling for 30-45 minutes. They noted that there was no significant change in residue levels after boiling for more than 45 minutes. Additionally, frying at 100 °C for 530 minutes resulted in a reduction of amoxicillin residue by 81-92%. Based on the results, the effect of heat processing on the concentration of detected antibiotics residues not only depended on the method of processing but also differed according

to types of antibiotics. Boiling method had the greatest effect among the other methods and ciprofloxacin showed the lowest reduction rate compared to oxytetracycline and amoxicillin. They reported higher reduction rates in their residues concentrations under the effect of all cooking methods, which is in agreement with other study (Tian *et al.*, 2017) who stated that B-lactam and tetracycline were the most heat-sensitive antibiotics.

Similar results were obtained by Kukhtyn (Kukhtyn *et al.*, 2020) who recorded that zeranol residues were unstable during cooking and decreased during 30 min by $24.70 \pm 0.23\%$ and $32.00 \pm 0.35\%$ after 60 min and that may be attributed to melting of fat during boiling in soup with their hormonal content (Khalafalla *et al.*, 2010).

The study results demonstrated that cooking loss in the examined samples was significantly decreased by boiling compared to the other two methods. According to Kim study, meat products showed significant differences in cooking loss based on the heating method, and this difference affected the quality characteristics of the meat product (Kim *et al.*, 2001). Domínguez reported that cooking loss depends on mass transfer during thermal treatment, thus, different cooking methodologies lead to different losses (Domínguez *et al.*, 2014). Moreover, the distance between proteins becomes smaller as the cooking speed increases and more water is expelled from the meat product. On the other hand, the increase in pH for cooked samples may be attributed to the reduction of free acidic group by increasing cooking temperature (Lawrie and Ledward, 1998).

One of the main strengths of this study is its practical approach, as it investigates the effects of commonly used cooking methods on drug residues in a widely consumed chicken wings. The study also utilized reliable analytical techniques - HPLC and ELISA - for accurate detection of antibiotic and hormonal residues. Moreover, it offers relevant findings that contribute to public health awareness and food safety practices. However, the study has some limitations. The sample size was relatively limited and restricted to two governorates.

Additionally, only a limited number of drugs were investigated, and other potential residues were not included. Variations in cooking times and temperatures were also not deeply explored. Therefore, we recommend future studies on many samples in all governorates of the Arab Republic of Egypt and other countries, and the use of more cooking methods, different temperatures and different cooking times, as well as the detection of more veterinary drug residues in chicken wings and other parts of the chicken

Conclusion

Residues of veterinary drugs in food are of severe public health concern. The present study concluded lack of implementing withdrawal times of veterinary drugs and widespread misuses of anabolic zeranor hormone in chicken farms. The findings also showed an advantage of cooking as a food processing method. Generally, cooking methods can reduce the concentration of drugs in chicken wings and boiling method was more effective in reducing their concentration. Educational and awareness programs on the use of medications and strict rules should be implemented to minimize their misuse, with great care regarding withdrawal timing to prevent the development of antibiotic-resistant bacteria.

Authors' contributions

Conceptualization: Marwa, A. Sheir.; Visualization, supervision and planning the research protocol: Marwa, A. Sheir., Nesrin, S.Mohammed, and Mona, Momtaz, M. Abd-elaziz.; methodology, investigation, analysis, writing original draft preparation: Marwa, A. Sheir and Mona, Momtaz, M. Abd-elaziz involved in writing, review and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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