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Research Article

Impacts of Different Types of Cooking and Freezing on Antibiotic Residues in Chicken Meat

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Abstract

Nowadays Antibiotics are applying for control of infectious diseases in chickens digestive system; incorrect use of these drugs deposits some residue in product. Impacts of different types of cooking and freezing on antibiotic residues in chicken meat were done by determination the residues of ciprofloxacin and oxytetracycline in experimentally treated chickens' muscles after cooking treatments (Boiling, Microwave, Roasting) and freezing of the chicken muscles. The method of determination of the residues using HPLC. The author found that quinolones are very stable during thermal procedures even by heating to ultra-high temperatures had nearly no effects on degradation. The reduction in antibiotic residue concentrations during boiling and frying was due to migration of the antibiotic residues from the chicken meat slices to cooking medium (water and oil). The effect of freezing for 6 months on Ciprofloxacin and Oxytetracycline as the reduction was 62.62% for Ciprofloxacin and for Oxytetracycline was 2.05%. The effect of freezing for 12 months on Ciprofloxacin and Oxytetracycline, the first one completely disappeared while the second was reduced by 32.38%. The authors concluded that the frequent use of antibiotics may result in drug residues that can be found at different concentration levels in products of animal origin; which mandate to ensure the proper withdrawal periods before slaughtering and marketing.

Keywords: Antibiotic residues; Chicken meat; HPLC

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Introduction

Chicken meat is a very popular food commodity around the world, and its consumption has increased over the last decades in many countries. The veterinary drugs are inevitably used for therapeutic or disease-preventive reasons in parallel with promoting growth [1]. The use of antimicrobial agents for growth promotion purposes in farm animals was prescribed in the mid-1950s. Since then tetracycline, chloramphenicol, and procaine penicillin (sub-therapeutic doses) supplemented feeds were extensively employed in the poultry industry to promote growth and egg production [2]. This wide spread use of antibiotics in poultry industry resulted in the presence of residuals in foodstuffs leading to a potential health hazards for consumers which include; carcinogenicity, mutagenicity, bone marrow toxicity and allergy [3], as well as appearance of a resistant strains of pathogenic bacteria [4].

Occurrence of antibacterial residues in animal originated foodstuffs could pose consumers health risk. Antibiotic residues in food are potential threat to direct toxicity in human and their low levels would result in death of intestinal flora, cause disease and the possible development of resistant strains which cause failure of antibiotic therapy in clinical situations. However, the principal hazardous effect is likely to develop the resistance of bacteria following the ingestion of sub-therapeutic doses of antimicrobials. The resistance could be transferred from nonpathogenic microorganisms to pathogenic ones, which would then no longer respond to normal drug treatment [5].

For protecting human from exposure of any veterinary residues, a withdrawal time has been determined. The withdrawal time is defined as the time interval from administration of a drug to animal until slaughter to assure that drug residues in meat are below maximum residue limit [6,7]. Antibiotic residues are procedurally measured on uncooked meat, but most chicken meat undergoes further processing prior to consumption (thermal or food additive treatments or both) for the purpose of increasing palatability and shelf-life. Since most types of foods are cooked before consumption, more information about the effect of cooking on residues are required to give a more accurate estimate of consumer exposure to these chemicals and any breakdown products. However, few studies have been carried out on the occurrence of the antibiotic residues in cooked foods and the stability of these compounds following heat treatment [8,9]. The residues of a range of veterinary drugs have varying degrees of stability during cooking and, therefore, the cooking influences the level of risk posed by such residues [10]. For many years several studies were conducted for evaluating whether antibiotic residues can be destroyed by cooking procedures, pasteurization, or canning processes [11-13]. Residues of a range of antibiotics have varying degrees of stability during cooking and, therefore, the cooking influences the level of risk posed by such residues [14]. Since the most of food producing animals are always cooked before consumption and the variations in antibiotics levels in the tissue are dependent on the type of cooking [8], more findings about the effect of cooking on antibiotic residues are needed to accurately determine consumer exposure to these drugs.

As the chicken cut-up meat are extensively consumed, therefore, concerned authorities should take an active part in controlling such products by imposing legal regulations and specified chemical and bacteriological standards to ensure a maximum safety to consumers [15]. There is lack of information on the effect of freezing with time on the concentration of antimicrobial residues in foods of animal origin. Although freezing is a form of preservation method of meat by inhibiting the growth of microorganisms, the fate of antimicrobial residues concentration when frozen with time is mysterious.

The aim of this work was planned to ensure food safety and protect public health by a reliable screening analysis to determine the residuals level of oxytetracycline and ciprofloxacin as common veterinary antibiotics in chicken meat after different cooking methods and after freezing by using HPLC.

Material and Methods

This study was planned to determine the residues of ciprofloxacin and oxytetracycline in experimentally treated chickens' muscles after cooking treatments (Boiling, Microwave, and Roasting) and freezing of the chicken muscles. The method of determination of the residues using HPLC. The experiment was designed as follow:

Chicken and drug administration

Forty-five broiler chickens were randomly divided into 2 groups. Every 15 were housed in identified cage. Two groups were separately dosed with ciprofloxacin (Ciprofloxacin® 10%, Al-Nasr) and oxytetracycline (Oxytetracycline® 20%, Adwia) via I/V and the intramuscular route. The chickens were reared to one day old and slaughtered at traditional slaughterhouse.

Sample preparation

The chicken carcasses were divided into six parts (represented by breast and thigh); one part was left raw as control and three parts were subjected to different cooking treatments; boiling, roasting and microwave. The last two parts were subjected to freezing, one for 6 months and the other for 12 months.

Treatments

Cooking by boiling: Slices of chicken samples "thigh and breast" were placed in boiling water and boiled for 15-30 minutes for muscles.

Cooking by roasting: Slices of chicken samples "thigh and breast" were roasted at 60-65°C for 15-30 minutes for muscles.

Cooking by microwaving: Slices of chicken samples "thigh and breast" were micro waved at 800W with one spoonful of sunflower oil for 15-20 minutes for muscles. The cooked meat had a "well done" appearance on the outside.

Freezing treatments

Slices of chicken "thigh and breast" were preserved in deep freezer at -20 °C for 6 months and for 12 months. All for mentioned samples were subjected to determination of ciprofloxacin and oxytetracycline by HPLC as follow.

Quantification of antibiotic residues

Antibiotic residue detection and quantification were performed using high performance liquid chromatography (HPLC). An Agilent 1200 HPLC system (Germany) consisting of a quaternary pump G1311A, a vacuum degasser G1322A, an automatic injector G1329A with sample tray G1330A, a column thermostat G1316A, a fluorescence detector G1321A, a multiple wave detector G1365B and integration software (ChemStation G2170AA and G2180AA) was used. Analytical column type Agilent C18; 5 µm, 250 x4.6 mm (catalogue no. 1111-3500) was obtained from Agilent Technologies (Germany). Isocratic separation was achieved using an Agilent C18 (5mm, 250x4.6mm).

Calibration standards: Standards were prepared using concentrations of 0.5, 1 and 5.0mg/L of Oxytetracycline (Sigma), Ciprofloxacin (Sigma) in fluent and spiked samples with same concentration. These standards and spikes were prepared from the daily prepared stock solution and treated as indicated for each.

Ciprofloxacin residues: Ciprofloxacin residues were determined as described by Verdon et al. [16].

Oxytetracycline residues: Oxytetracycline residues were determined according to Senyuva et al. [17].

Statistical analysis

The statistical analysis for this study was performed using [18].

Results and Discussion

The residues of a range of veterinary drugs have varying degrees of stability during cooking and, therefore, the cooking influences the level of risk posed by such residues [10]. Table 1 presented the effect of boiling on ciprofloxacin residues in chicken muscles, as it was found before boiling 388ug/kg, and after boiling 301ug/kg, with reduction percentage as 22.42. Higher results were recorded by Lolo et al. [8]; Khan et al. [19], lower results were recorded by Fathyet al. [20]. Results presented in table 2 showed that Oxytetracycline was dramatically reduced by boiling from 244ug/kg to 53.84ug/kg with 77.93% of reduction. Higher results were recorded by Salama et al. [21]; Shalaby et al. [22]; Abou-raya et al. [23], lower results were recorded by Lolo et al. [7]; Heshmati et al. [8]; Vivienne et al. [24]. Data presented in table 3 and 4 showed the effect of microwave treatment on Ciprofloxacin and Oxytetracycline residues, respectively. As the reduction % was 35.57 and 81.48 for both antibiotics, respectively. Higher results were recorded by Roca et al. [25]; Faten et al. [26], lower results were recorded by Fathyet al. [20]. Table 5 and 6 showed the effect of roasting on both Ciprofloxacin and Oxytetracycline, and the results recorded reduction percentage as 17.01 and 37.41, respectively. Higher results were recorded by by Lolo et al. [7]; Heshmati et al. [8]; Vivienne et al. [24], lower results were recorded by Salama et al. [21]; Shalaby et al. [22]; Abou-raya et al. [23]. The results in tables 1,3,5 were greatly similar to those obtained by Roca et al. [25] who stated that quinolones are very stable during thermal procedures even by heating to ultra-high temperatures had nearly no effects on degradation. On other words these results disagreed with those reported by Fathy et al. [20], who found that ciprofloxacin residues could be degraded by heat treatment.

Ciprofloxacin Residues Before Boiling ug/kg	Ciprofloxacin Residues After Boiling ug/kg	Percent of Reduction of Ciprofloxacin %
388	301	22.42%

Table 1: Effect of boiling on ciprofloxacin residues in chicken muscles.

Oxytetracycline Residues Before Boiling ug/kg	Oxytetracycline Residues After Boiling ug/kg	Percent of Reduction %
244	53.84	77.93%

Table 2: Effect of boiling treatment on oxytetracycline residues in chicken muscles.

Ciprofloxacin Residues Before Microwave Treatment ug/kg	Ciprofloxacin Residues After Microwave Treatment ug/kg	Percent of Reduction of Ciprofloxacin %
388	250	35.57%

Table 3: Effect of Microwave treatments on reduction of ciprofloxacin residues in chicken muscles.

Oxytetracycline Residues Before Microwave Treatment ug/kg	Oxytetracycline Residues After Microwave Treatment ug/kg	Percent of Reduction of Oxytetracycline %
244	45.2	81.48%

Table 4: Effect of microwave treatment on reduction of oxytetracycline residues in chicken muscle.

Ciprofloxacin Residues Before Roasting Treatment ug/kg	Ciprofloxacin Residues After Roasting Treatment ug/kg	Percent of Reduction of Ciprofloxacin Residues %
388	322	17.01%

Table 5: Effect of roasting treatment on reduction of ciprofloxacin residues in chicken muscles.

Oxytetracycline Residues Before Roasting Treatment ug/kg	Oxytetracycline Residues After Roasting Treatment ug/kg	Percent of Reduction of Oxytetracycline %
244	91.29	37.41%

Table 6: Effect of roasting treatment on reduction of oxytetracycline residues in chicken muscles.

These results were differed with those achieved by Khan et al. [19], that they found that ciprofloxacin residues in chicken tissues were degrade by boiling by 68.18 %.These results were agreed with those obtained by Lolo et al. [8], who decided that enrofloxacin residues in chicken tissues didn't degrade by grilling. The current results were disagreed with those obtained by Fathy et al. [20], who decided that ciprofloxacin amounts in chicken tissues could be degraded by grilling from 5999.3ppb to 1956.61ppb. In the same lines the present results were in an acceptance with those reported by Khan et al. [19], who found that ciprofloxacin residues in chicken tissues degrade after grilling by 33.34%. The achieved results were agreed with those obtained by Lolo et al. [8], who decided that enrofloxacin residues didn't degrade by roasting. Whereas our results were in differs with those results declared by Khan et al. [19], who found that ciprofloxacin residues in chicken tissues could be degraded by roasting processes by 68.76%. From the point of view these results were in accordance with those reported by Khan et al. [19], who deduced that microwaving can degrade ciprofloxacin residues. Our current results were disagreed with those achieved by Javadi [9], who revealed that highly detectable residual amount of enrofloxacin residues in chicken tissues were appeared after microwaving. The reduction in antibiotic residue concentrations during boiling and frying was due to migration of the antibiotic residues from the chicken meat slices to cooking medium (water and oil). Based on a study of Lolo et al. [8], concerning the effect of different cooking processes (microwaving, roasting, boiling, grilling and frying) on enrofloxacin residues in the breast and whole leg of chicken, there was a reduction in concentration and the lost amount of residue from the chicken meat found in water or exudates and the amount of residue increased with roasting and grilling because the low moisture content of the treated meat caused an apparent concentration of the quinolone residue.

The results of boiling and microwaving in another study by [9], confirmed decrease of enrofloxacin residues after cooking. Oxytetracycline levels were reduced to a minor extent by frying and roasting [4,9,27-29]. The overall loss of oxytetracycline residues was due to denaturation of protein [30]. Generally, Tetracycline (TC) residues are considered relatively unstable compounds. Temperature during cooking has the largest impact on the loss of tetracycline residues [10,11,13,23,27,28,31-35], studied the changes by microwave, boiling and roasting cooking processes on TCs including Oxytetracycline (OTC), Tetracycline (TTC), Chlorotetracycline (CTC) and Doxycycline (DOC) in chicken breast and thigh, and determined the cooking time required to make the cooked sample safer for consumption. Cooking procedure was one of the most important agents that influenced TC residues. Among various procedures studied, microwaving was the most effective one. It is obvious that the more cooking time, the greater loss of residue. During heat processing, it was identified the most stable and the most unstable TCs are doxycycline and oxytetracycline, respectively. The time required to destroy 90% of the initial TCs contents are 23.9, 53.2 and 101.6 min for microwaving, boiling and roasting, respectively. If cooking temperature and time are sufficient, we could assure significant losses of TC residues. Therefore, it was said cooking provides safety margin for products containing TCs [11,13,31,32,34]. The effect of freezing for 6 months on Ciprofloxacin and Oxytetracycline was depicted in table 7 and 8, respectively. The reduction was 62.62% for Ciprofloxacin and for Oxytetracycline was 2.05%. Higher results were recorded by Roca et al. [25]; Faten et al. [26]. Tables 9 and 10 showed the effect of freezing for 12 months on Ciprofloxacin and Oxytetracycline. The first one completely disappeared while the second was reduced by 32.38.

Ciprofloxacin Residues Before Freezing Treatment ug/kg	Ciprofloxacin Residues After Freezing Treatment ug/kg	Percent of Reduction After Freezing %
388	145	62.63%

Table 7: Effect of freezing treatment of chicken muscles (6 months at -20°C) on ciprofloxacin residues.

Oxytetracycline Residues Before Freezing Treatment ug/kg	Oxytetracycline Residues After Freezing Treatment ug/kg	Percent of Reduction %
244	239	2.05%

Table 8: Effect of freezing treatment of chicken muscles (6 months at -20°C) on Oxytetracycline residues.

Ciprofloxacin Residues Before Freezing Treatment ug/kg	Ciprofloxacin Residues After Freezing Treatment ug/kg	Percent of Reduction %
388	Not Detected	100%

Table 9: Effect of freezing treatment of chicken muscles (12 months at -20°C) on ciprofloxacin residues.

Oxytetracycline Residues Before Freezing Treatment ug/kg	Oxytetracycline Residues After Freezing Treatment ug/kg	Percent of Reduction %
244	165	32.38%

Table 10: Effect of freezing treatment of chicken muscles (12 months at -20°C) on Oxytetracycline residues.

Conclusion

The authors concluded that Poultry farmers need to be educated on the possible effects associated with use of food with antimicrobial residues. It is also necessary to establish a routine screening program for antibiotic residues by the appropriate authorities. Greater attention from a public health aspect is needed on the safety of drug residues as a result of indiscriminate use of antibiotics and the expanding general increase of chemicals and drugs in the food supply. The frequent use of antibiotics may result in drug residues that can be found at different concentration levels in products of animal origin.

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