ACS FOOD SCIENCE & TECHNOLOGY

pubs.acs.org/acsfoodscitech

Article

Effect of Diverse Cooking Treatments on Enrofloxacin Residues and Its Metabolites in Chicken Tissues by LC-MS

Anna Tomás-Gascó, Kenneth Nazir, Paola Esposito, Cristina Minguillón, and Dolores Barrón*

Cite This: *ACS Food Sci. Technol.* 2022, 2, 1639−1649

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Although safety delays are established in the introduction into the market of the products derived from medicated animals, residues of drugs and their metabolites may remain in the edible meat and reach the food chain. In this context, the aim of this work is to evaluate the effect of common domestic cooking procedures, such as boiling (100 °C) and grilling (250 °C), on the fate of enrofloxacin (ENR) residues and its metabolites, present in liver and muscle tissues of chicken previously medicated with enrofloxacin. Although it is generally accepted a thermal degradation for enrofloxacin when cooking, a decrease in content, unaffected or even increased content is observed depending on the considered metabolite. This latter observation can be the result of either the actual thermal degradation of a structurally close precursor or an artifact resulting from the thermal modification of the matrix (muscle or liver). Nevertheless, it is clear that their global content is considerably low with respect to the remaining content of the administered ENR.

KEYWORDS: *enrofloxacin metabolites, chicken meat, effect of domestic cooking, QuEChERS optimization*

1. INTRODUCTION

Antibiotics are compounds of natural or synthetic origin widely used in human and veterinary medicine to inhibit the proliferation of bacteria. Although their prophylactic use or as a growth promoters in animals destined to human consumption is not allowed in the European Union,[1,2] antibiotics may also be administered with this aim. The most serious effect on human health is the transfer of resistant bacterial strains from animals to humans. The treated animals may, or not, metabolize efficiently and quickly veterinary drugs. Safety delays in the introduction into the market of the products derived from treated animals are established[3,4] (AEMPS, 2010; Commission Regulation No 2019/6, 2019). Even so, residues may remain in the edible portion of animals.[5] The presence of such compounds in food, although minimal, may involve a potential health risk for consumers. These compounds, often poorly studied, may be the origin of allergy and toxicity problems and also of bacterial resistance.[6−9]

To ensure consumer health and prevent the potential risk that such antibiotic treatments may involve for humans, the EU Regulation 37/2010 established a maximum residue limit (MRL) in animal products destined to human consumption.[10] However, MRLs apply only to the amount of originally active substances in raw food, without systematically considering their metabolites. In the case of regulated quinolones, only the main metabolite of enrofloxacin, ciprofloxacin, is considered. Other metabolites and compounds that may result from the processing of meat before eating are not included in this regulation.[11] Probably the difficulty in standardizing cooking procedures and also the diversity of compounds derived from original drugs, the evaluation of the risk that they may involve, and its minor concentration in food, justifies that, in general terms, drug residues are only determined in raw meat.

Because of the assumed thermal lability of drugs and their metabolites, cooking may reduce their content in raw meat thus reducing the risk for some compounds. However, this does not guarantee the complete elimination or degradation of the antibiotic residues present in animal tissues.[5,12−16] In addition, depending on the temperature applied, this process may result in the formation of degradation products or transformation products of unknown identity and toxicity.[17]

With only a few exceptions, meat is usually (thermally) cooked to change texture, to enrich taste, to improve digestibility, and to increase security by inhibiting the growth of microorganisms. The time and temperature of the cooking process play an important role in the characteristics of cooked meat. Different methods, such as boiling, grilling, roasting and frying among others, are applied to cook meat.[15,17−20]

The simplest method, grilling meat, uses a direct heat source and applies temperatures up to 260 °C. The resulting meat has similar characteristics to those obtained by roasting.[17] Another widely used procedure is boiling, which involves the treatment with water and 100 °C. Nevertheless, regardless of the heat treatment applied, it can cause drug residue loss through evaporation, codistillation, or thermal degradation.[5]

Received: August 2, 2022
Revised: August 30, 2022
Accepted: September 6, 2022
Published: September 19, 2022

In previous works, several metabolites and transformation products from enrofloxacin in raw chicken tissues were identified and their structures were proposed. Some of these metabolites were excreted after 4 days of withdrawal period, but some of them are still persistent in tissues after this time.[21,22] Since meat is usually cooked before consumption, information on the effects of diverse thermal treatments on drug residues and metabolites is required to estimate the exposure to these compounds to further evaluate risk of toxicity.

To date, few studies have investigated the thermal stability of antibiotics. Regarding quinolones, under domestic cooking or commercial pasteurization (milk), studies that consider the effect of temperature on drug residues are scarce.[11,17,18,23−27] Nevertheless, only Junza[23,24] considered the effect of temperature on metabolites and transformation products.

In this context, the aim of this work is to evaluate the effect of common domestic cooking procedures, such as boiling and grilling, on the fate of enrofloxacin residues and its metabolites present in the liver and muscle tissues of chickens previously medicated with enrofloxacin.

2. EXPERIMENTAL PROCEDURES

2.1. Chemicals and Reagents. The standards purchased were enrofloxacin (ENR) from Sigma-Aldrich (St. Louis, MO, USA) and ciprofloxacin (CIP) from Ipsen Pharma (Barcelona, Spain).

The following reagents and solvents were used during the sample treatment process. Glacial acetic acid (HAcO) was purchased from Scharlau Sharlab (Barcelona, Spain), acetonitrile (MeCN) from VWR Chemicals (Radnor, PA, USA), and methanol (MeOH) and sodium hydroxide (NaOH) from Panreac (Barcelona, Spain). Anhydrous citric acid (HCit), sodium dihydrogen phosphate monohydrate ($NaH_2PO_4{\cdot}H_2O$) and formic acid (FA) were provided by Merck (Darmstadt, Germany). In addition, anhydrous magnesium sulfate (MgSO4) was obtained from Sigma-Aldrich (St. Louis, MO, USA), C18 End-capped from Agilent Technologies (Santa Clara, CA, USA) and primary-secondary amine (PSA) 40−60 μm was purchased from Scharlau Sharlab (Barcelona, Spain). All reagents were of analytical grade, unless otherwise indicated.

Extraction cartridges OASIS HLB 3 cm^3 (60 mg), supplied by Waters (Milford, MA, USA), were used to perform the SPE technique. In addition, centrifugal filter units Ultrafree-MC-GV Durapore-PVDF 0.22 μm from Merck Millipore were used to filter the extract before injecting the sample into the LC-MS/MS system.

Ultrapure water was generated by a Milli-Q purification system from Evoqua (Pittsburgh, PA, USA).

2.2. Preparation of Standards, Stock Solutions, and QuEChERS. Individual stock solutions of quinolones were prepared at a concentration of 250 $\mu g\ mL^{-1}$ by dissolving them in 50 mM HAcO aqueous solution.

Individual standard solutions of ENR and CIP were prepared at 0.5 and 5 $\mu g\ mL^{-1}$ for its use in muscle tissues and at 0.25 and 2.5 $\mu g\ mL^{-1}$ for use in liver tissues. Working solutions of ENR at 25 and 100 $\mu g\ kg^{-1}$ (1/4 x MRL and MRL, respectively) in tissues were prepared to evaluate the reproducibility of the method.

The QuEChERS used in this study were homemade. The QuEChERS mixture used for muscle treatment contained 333 mg of $MgSO_4$ and 200 mg of C18. The mixture used for liver treatment contained 750 mg of $MgSO_4$ and 200 mg of C18.

Buffer solution used in the QuEChERS procedure consisted of an aqueous HCit solution (0.1 M) adjusted with a solution of NaOH (2 M) at either pH 4.5 (muscle tissue) or pH 5.5 (liver).

A buffer solution of sodium dihydrogen phosphate monohydrate (0.1 M) adjusted at pH 10 by adding a solution of NaOH (2 M) was used in the SPE extraction of the aqueous phase used in boiling samples.

2.3. LC-MS/MS Instrumentation and Conditions. In all cases liquid chromatography separation was carried out on a Symmetry C8 column (50 × 2.1 mm, 5 μm) supplied by Waters (Milford, MA, USA). The flow rate was adjusted to 0.3 mL/min and the injection volume was 10 μL in all analyses. The mobile phase consisted of a binary solvent system: solvent A, water with 0.1% HCOOH and solvent B, MeCN with 0.1% HCOOH.

An LC-ESI-QqQ system was used in the quantification of ENR in samples. An Acquity-Ultra Performance LC system equipped with a thermostatic auto sampler from Waters (Milford, MA, USA) and coupled to an API 3000 triple-quadrupole mass spectrometer from PE Sciex (Framingham, MA, USA), provided with a turbo ion spray source in positive mode. Mass spectrometry analysis were carried out on multiple reaction monitoring (MRM) and positive ionization mode with a dwell time of 200 ms. LC−MS/MS conditions were optimized by direct injection of an ENR solution at a concentration of 1 mg L^{-1}. The following parameters were optimized: capillary voltage 4500 V, nebulizer gas (N2) 10 (arbitrary units), curtain gas (N_2) 12 (arbitrary units), collision gas (N_2) 15 (arbitrary units), declustering potential (DP) 45 V, focusing potential 200 V, entrance potential 10 V. Drying gas (N_2) was heated to 400 °C and introduced at a flow rate of 4500 mL min^{-1}. MS/MS product ions were produced by collision activated dissociation (CAD) of the selected precursor ion. Two transitions were followed. The most intense transition (360 → 316 (29 V)) was used for quantification while the second (360 → 342 (29 V)) ensures identification. The system was controlled using Analyst 1.4.2 software from Applied Biosystems (SCIEX, Foster City, CA, USA).

Gradient system for LC-ESI-QqQ was programmed as follows: initially B was maintained at 2% for 2 min, from 2 to 4 min B increased to 10%, from 4 to 6 min B increased again to 15%, from 6 to 7 min B increased sharply to 60%. Finally, B decreased to 2% in 1 min and maintained at this percentage for 3 min to recover initial conditions.

An LC-LTQ-Orbitrap system was used in the characterization of metabolites. This system consisted of an Accela LC system equipped with a thermostatic auto sampler and coupled to an LTQ Orbitrap Velos mass spectrometer both from Thermo Scientific (Hemel Hempstead, UK). Mass spectrometry analyses were carried out on full-scan and product ion scan MS/MS modes with a mass range of 100−700 Da. The resolving power was 30,000 for full-scan mode and 15,000 for MS/MS events. Positive ionization mode was used in all experiments. A source voltage of 3500 V and a capillary temperature of 300 °C were applied. A high collision dissociation (HDC) energy of 30−60% was used for MS/MS experiments. The instrument was controlled using XCalibur 2.2 software from Thermo Fisher Scientific (Hemel Hempsted, UK).

The gradient elution used for LC-LTQ-Orbitrap was programmed as follows: initially B was maintained at 7% for 2 min, from 2 to 4 min B increased to 30% and then maintained at this percentage for 2 min more. Finally, B decreased again to 7% in 1 min and maintained at this percentage for 3 min.

2.4. Auxiliary Equipment. A technical balance, 440-45N from Kern Pharma (Barcelona, Spain), and an analytical balance, AM1000 from Mettler Toledo (Greifensee, Switzerland), were used in the weighing of reagents and samples. A potentiometer, micropH 2002, and a combined pH electrode, 5203, both from Crison (Barcelona, Spain), were used in the preparation of the buffer solutions.

A vortex mixer, VX-200 from Labnet International (Edison, NJ, USA), an ultra sonicator from J.P. Selecta (Barcelona, Spain), a centrifuge, MIKRO 220R from Hettich Zentrifuguen (Lauenau, Germany), and an evaporator, MiVac Quattro concentrator Duo Pump with SpeedTrap from GeneVac (Warminster, PA, USA), were used during sample treatment. The SPE procedure was performed on a vacuum manifold with disposable liners for 24 cartridges connected to a vacuum tank from Supelco (Bellefonte, PA, USA).

2.5. Origin of Samples and Animal Pharmacological Treatment. Chicken tissues from nonmedicated animals, coming from an organic farm (Cal Roio, La Nou del Berguedá, Barcelona), were used in both blank samples and in the preparation of the

https://doi.org/10.1021/acsfoodscitech.2c00232
ACS Food Sci. Technol. 2022, 2, 1639−1649

calibration curve. Chicken tissues from medicated animals were obtained from the Faculty of Veterinary Medicine of the Universitat Autonoma de Barcelona (Cerdanyola del Vallès, Barcelona). The therapeutic treatment consisted of a dose of 20 mg kg^{-1} of ENR dissolved in the drinking water for 3 days, and the animal experiment protocol was approved by the institutional ethics committee.

Three types of samples were analyzed. Two nonmedicated specimens (A1 and A2) randomly selected from the organic farm were slaughtered. Two specimens (A3 and A4) were slaughtered on the third day of the pharmacological treatment (from now on: during treatment DT samples). Another two chickens (A5 and A6) were slaughtered 2 days after the stop of the pharmacological treatment (from now on: post treatment PT samples). All animals were handled and sacrificed according to the ethical protocols of the mentioned farms. Liver and muscle tissue samples from all different chickens were ground and stored at −20 °C until sample treatment.

2.6. Cooking Procedure. Samples, consisting of 2 g of muscle or 1 g of liver formed as a "hamburger" of approximately 1 cm in diameter, were exposed at two different cooking treatments: boiling (**B**) and grilling (**G**). These tissues were also analyzed without cooking treatment (raw meat, **R**).

The grilling procedure consisted of 3 min/side in the case of muscle and 2 min/side for the liver at 250 °C. The boiling procedure consisted of a 5 min treatment in 10 mL of Milli-Q water. The mixture was filtered to separate the boiled sample (**B**) from the boiling water (**BW**). The two parts were analyzed separately.

2.7. Sample Treatment and Cleanup. To avoid interferences, two different cleanup procedures were used in the study. A QuEChERS method was used for solid samples whereas a SPE method was used to cleanup and preconcentration of BW samples.

2.7.1. SPE. The **BW** samples were cleaned using SPE Oasis HLB cartridges. Initially, 1 mL of MeOH, 1 mL of water, and 1 mL of NaH_2PO_4 buffer solution (pH 10) were passed through the cartridge to condition the sorbent. Next, a mix of 5 mL of **BW** sample and 0.5 mL of NaH_2PO_4 buffer solution, followed by 3 mL of water, were passed through the cartridge. Finally, the analytes were eluted by adding 2 mL of MeOH to the cartridge.[23,24] The solvent present was then evaporated completely, and the extract reconstituted as for the QuEChERS procedure. All experiments were made in triplicate.

2.7.2. QuEChERS. An amount of 2 g (±0.1 mg) of minced chicken muscle or 1 g (±0.1 mg) of minced chicken liver was introduced into a 50 mL capped polypropylene centrifuge tube. Analytes were extracted with a mixture of 8 mL of MeCN and 2 mL of HCit buffer solution [H_2Cit^-/ $HCit^{2-}$] (pH 4.5 for the muscle tissue and pH 5.5 for the liver tissue). Then, the tubes were sonicated (5 min) and centrifuged at 5000 rpm (5 min). The supernatant was transferred into a 15 mL capped polypropylene centrifuge tub with the optimized amount of sorbents ($MgSO_4$ and C18) (QuEChERS tube). After shaking the QuEChERS tube for 30 s, the mixture was centrifuged at 5,000 rpm (5 min). Finally, a 5 mL aliquot of the supernatant (organic phase with the analytes) was transferred into a centrifuge vial to completely remove solvent by evaporation. The obtained extract was reconstituted with 300 μL of water, vortexed for 30 s and filtered by centrifugation at 10,000 rpm (2 min). Finally, the filtered solution was transferred to LC-MS/MS vials and kept frozen (−20 °C) until analysis. All samples were analyzed in triplicate.

2.8. Optimization of QuEChERS Method. Sample treatment and cleanup by SPE was already optimized in our lab on the occasion of precedent studies.[23,24] However, this is not the case for the QuEChERS procedure. To optimize the QuEChERS extraction method, screening of experimental factors using Placket−Burman design was applied for quinolones (ENR and CIP) using blank chicken tissues (muscle and liver). Subsequently, Doehlert Design was used to find the optimum conditions of selected factors.

In the Placket−Bruman design five factors were considered and each factor was studied at two levels (low and high): $MgSO_4$ (0 and 1000 mg), C18 (0 and 600 mg), PSA (0 and 600 mg), shaking time (20 and 60 s), and pH (3 and 10). For each tissue 12 independent runs (24 in total) at all the possible combinations were performed.

Subsequently, in the Doehlert design the most influential factors detected above were studied at additional levels to obtain maximum information and better prediction. The studied factors for the muscle were 5 levels pH (3, 4.5, 6.5, 8.5 and 10), 7 levels $MgSO_4$ (0, 167, 333, 500, 667, 834, and 1000 mg), 7 levels for C18 (0, 100, 200, 300, 400, 500, and 600 mg), and 3 levels PSA (0, 300, and 600 mg). The studied factors for liver were 7 levels pH (3.0, 4.5, 5.5, 6.5, 7.5, 8.5 and 10.0), 5 levels $MgSO_4$ (0, 250, 500, 750, and 1000 mg), and as for muscle, 7 levels for C18 (0, 100, 200, 300, 400, 500, and 600 mg) and 3 levels PSA (0, 300, and 600 mg). For each matrix, 23 experiments were performed (46 experiments in total), and 3 of them were replicates of the central point.

2.9. Quality Parameters. To evaluate linearity of the analytical procedures, calibration curves were prepared. The curves were prepared at 6 levels of concentration within the range of 5−250 μg kg^{-1}. Also, each concentration level was prepared and assayed twice.

The precision of the QuEChERS procedure was evaluated in terms of repeatability (intraday precision, same day) and intermediate precision (interday precision, different days). Blank chicken muscle **R** was used to perform the study. The samples were prepared the same way as the ones for the calibration curve, using the procedures described above (section 2.7). The samples were spiked at 2 different concentration levels of 0.25 × MRL and MRL using a solution of ENR at a concentration of 5 μg·mL^{-1}. The interday precision was evaluated by preparing samples on 3 different days. On each of these days, 7 replicates at the mentioned levels, were prepared to evaluate the intraday precision. A total of 21 samples were prepared.

2.10. Quantification of Enrofloxacin. For each type of tissue, meat or liver, a matrix-matched calibration curve was prepared using **R** samples. These calibration curves were used in the dosing of **R**, **B**, and **G** samples. Another curve was prepared for **BW** samples. The blank **BW** sample, required to prepare the calibration curve, was obtained from boiling blank tissue in the same conditions as real samples. Calibration curves were constructed using the obtained peak area versus the known concentration within the range of 5−250 μg kg^{-1}. All samples were weighted prior to cooking procedure. The meat, either muscle or liver, and water samples were quantified using the appropriate calibration curve.

The concentration of ENR was determined in chicken muscle and liver tissues at the three cooked states considered (**R**, **G**, and **B**) and in **BW**. Results are all referred to raw weight to avoid the effect of possible losses of water during cooking.

2.11. Data Treatment. The LC-ESI-QqQ data was processed using Analyst 1.4.2 software from Applied Biosystems (Foster City, CA, USA). This software provides the chromatograms obtained in the targeted analysis. To quantify the samples, the peak areas belonging to ENR were integrated for the different tissues and cooked states (**R**, **B**, **G**, **BW**).

The LC-LTQ-Orbitrap data was processed with Compound discoverer 2.0 software from Thermo Fisher Scientific (Hemel Hempsted, UK). This software permits to perform a targeted analysis for specific compounds in many samples simultaneously. As a result, the software shows in which sample is the compound present.

To study the effect of the cooking process on the evolution of metabolites and TPs, cooked samples were compared with the raw ones. Only the compounds present in raw samples are considered. In addition, only compounds with an intensity greater than 10000 cps have been considered.

3. RESULTS AND DISCUSSION

In order to evaluate the effect of the cooking treatments on ENR and its metabolites in tissues from chicken medicated with this quinolone it is necessary to have available an optimized and validated method to extract and quantify the level of ENR in the different matrices as is explained in this section.

The linearity was established for the QuEChERS and SPE method in both muscle and liver tissues. Additionally, the

https://doi.org/10.1021/acsfoodscitech.2c00232
ACS Food Sci. Technol. 2022, 2, 1639−1649

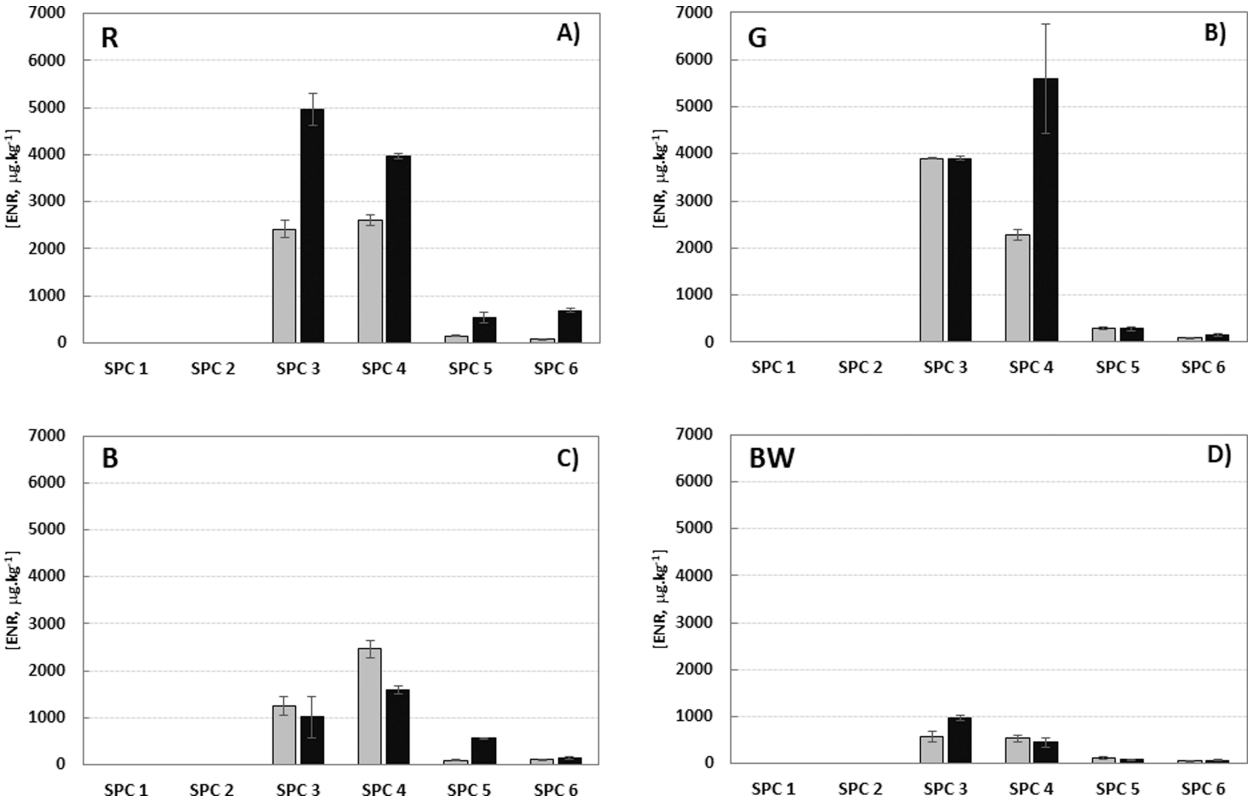


Figure 1. Enrofloxacin (ENR) concentrations in muscle (gray) and liver (black) chicken samples: raw (A), grilled (B), boiled (C), and boiled water (D).

precision was also established for the QuEChERS method in muscle tissue. In both cases, the validation of the parameters was performed using spiked raw blank samples. Linearity was determined for ENR in the range of 5−250 μg kg^{-1} with levels 5, 25, 50, 100, 150, and 250 μg kg^{-1}. Calibration curves were prepared at blank muscle and liver tissues, testing the linearity of QuEChERS method used in the treatment process. In addition, calibration curves with the **BW** of tissues, testing the linearity of the SPE method were also prepared. In all cases, linearity has been proved with a correlation coefficient greater than 0.992 in the range studied.

Precision was determined at two levels of concentration (0.25 × MRL and MRL) from 7 replicates during the same day (repeatability, intraday precision) and in 3 successive days (reproducibility, interday precision). The QuEChERS method has proved to be reproducible given that RSD values at 0.25 × MRL were 8% in both intra- and interday precision and RSD values at MRL were 5% and 6% in intra and interday precision, respectively. In all cases, the results were within the acceptance criteria for the validation of analytical methods, which is RSD ≤ 15%.[28]

3.1. QuEChERS Procedure Optimization. To optimize the QuEChERS performance, using liver and muscle chicken as a matrix and with ENR and CIP as analytes, an experimental design approach was used. Experimental factors evaluated were the amounts of $MgSO_4$, C18, and PSA, shaking time and pH. To reveal the significance of each factor, a Placket−Burman design was first applied. Subsequently, a Doehlert design was used to predict the optimum values for each factor.

According to the Placket−Burman design, the amounts of C18 and $MgSO_4$ were the most relevant factors for samples in a muscle matrix. When liver tissue was the matrix, the amount of C18 and pH were the most influential factors. Shaking time was found to have no influence in the results for both matrix and was set at 30 s for all the experiments.

The most significant factors in each matrix were studied at more levels to obtain a better prediction in the Doehlert design. For muscle matrix, the number of levels and ranges covered were 7 levels for the amount of C18 (0−600 mg), 7 levels for $MgSO_4$ (0−1000 mg), 5 levels of pH (3−10), and 3 levels PSA (0−600 mg). For liver matrix, 7 levels pH (3−10) and 5 levels MgSO4 (0−1000 mg) and, for muscle, 7 levels for C18 (0−600 mg) and 3 levels PSA (0−600 mg) were considered. The optimum values determined for muscle matrix were 4.5 pH, 333 mg of $MgSO_4$, and 200 mg of C18, while those for liver matrix were 5.5 pH, 750 mg of $MgSO_4$, and 200 mg of C18. PSA was determined to be not influential in the results obtained, and therefore, it was not included in the final QuEChERS composition.

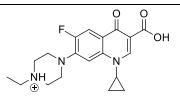
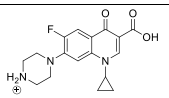
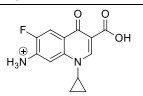
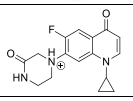
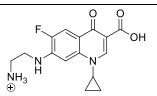
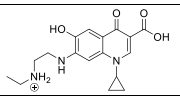
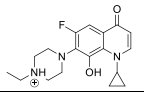
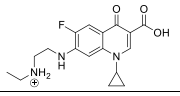
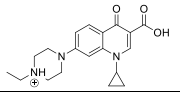
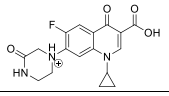
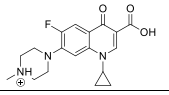
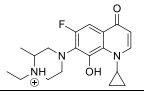
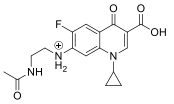
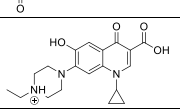
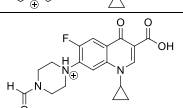
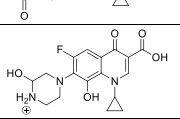
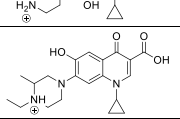
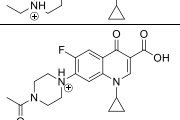
3.2. Quantification Results. The determination of ENR concentrations was carried out by LC-ESI-QqQ following the method described in the section 2.3. The quantification was performed in the two tissues in **R**, **G**, and **B** using the adequate calibration curve (section 2.9). **BW** was also analyzed. Three independent replicates were analyzed for each animal and tissue.

The results of the ENR quantification are graphically represented in Figure 1. The values obtained for the two tissues were compared for each state **R**, **G**, **B**, and **BW**.

As shown in Figure 1, it is confirmed that the antibiotic is not present in specimens (SPC) 1 and 2 in none of the tissues. These samples come from nonmedicated animals and are used as a blank tissue. Specimens 3 and 4, coming from animals medicated for 3 days, present levels of ENR higher than 2000

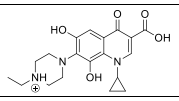
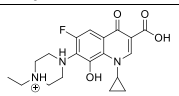
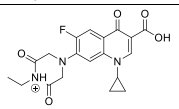
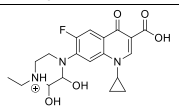
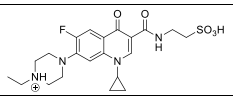
https://doi.org/10.1021/acsfoodscitech.2c00232
ACS Food Sci. Technol. 2022, 2, 1639−1649

Table 1. Qualitative Analysis of ENR and Metabolites

					MUSCLE				LIVER			
	m/z	Formula	Structure	Error (ppm)	R	G	B	BW	R	G	B	BW
ENR	360.1731	$[C_{19}H_{23}FN_3O_3]^+$		-0.7	√	√	√	√	√	√	√	√
CIP	332.1414	$[C_{17}H_{19}FN_3O_3]^+$		-2.7	√	√	√	√	√	√	√	√
M01	263.0828	$[C_{13}H_{12}FN_2O_3]^+$		-0.8	√	√	√	√	√	√	√	√
M02	302.1301	$[C_{16}H_{17}FN_3O_2]^+$		-0.7	√	√	√	√	√	√	√	√
M03	306.1248	$[C_{15}H_{17}FN_3O_3]^+$		0.0	√	√	√	√	√	√	√	√
M05	332.1609	$[C_{17}H_{22}N_3O_4]^+$		-2.2	√	√	√	√	√	√	√	--
M06	332.1756	$[C_{18}H_{23}FN_3O_2]^+$		3.9	√	√	√	--	--	--	--	--
M07	334.1565	$[C_{17}H_{21}N_3O_3]^+$		-1.2	√	√	√	√	√	√	√	√
M08	342.1804	$[C_{19}H_{24}N_3O_3]^+$		2.3	--	--	--	--	√	--	√	--
M09	346.1192	$[C_{17}H_{17}FN_3O_4]^+$		1,7	--	--	--	--	√	√	√	√
M10	346.1563	$[C_{18}H_{21}FN_3O_3]^+$		-0.6	√	√	√	√	√	√	√	--
M11	346.1917	$[C_{19}H_{25}FN_3O_2]^+$		2,3	--	--	--	--	√	√	√	√
M13	348.1350	$[C_{17}H_{19}FN_3O_4]^+$		1.1	--	--	--	--	√	√	√	√
M14	358.1765	$[C_{19}H_{24}N_3O_4]^+$		-1-1	√	√	√	√	√	√	√	√
M15	360.1354	$[C_{18}H_{19}FN_3O_4]^+$		-0.8	√	√	√	√	√	√	√	√
M18	364.1296	$[C_{17}H_{19}FN_3O_5]^+$		1.9	--	--	--	--	√	√	√	√
M20	372.1931	$[C_{20}H_{26}N_3O_4]^+$		-3.5	√	√	√	--	--	--	--	--
M22	374.1503	$[C_{19}H_{21}FN_3O_4]^+$		2.1	--	--	--	--	√	√	√	√

https://doi.org/10.1021/acsfoodscitech.2c00232
ACS Food Sci. Technol. 2022, 2, 1639−1649

Table 1. continued

					MUSCLE				LIVER			
	m/z	Formula	Structure	Error (ppm)	R	G	B	BW	R	G	B	BW
M24	374.1713	$[C_{19}H_{24}N_3O_5]^+$		-0.8	√	√	√	√	√	√	√	√
M25	376.1670	$[C_{19}H_{24}N_3O_5]^+$		-0.8	√	√	√	√	--	--	--	--
M28	388.1324	$[C_{19}H_{19}FN_3O_5]^+$		-5.4	√	√	√	--	--	--	--	--
M29	392.1613	$[C_{19}H_{23}FN_3O_5]^+$		0.8	--	--	--	--	√	√	√	√
M30	467.1761	$[C_{21}H_{28}FN_4O_5S]^+$		-0.4	--	--	--	--	√	√	√	√

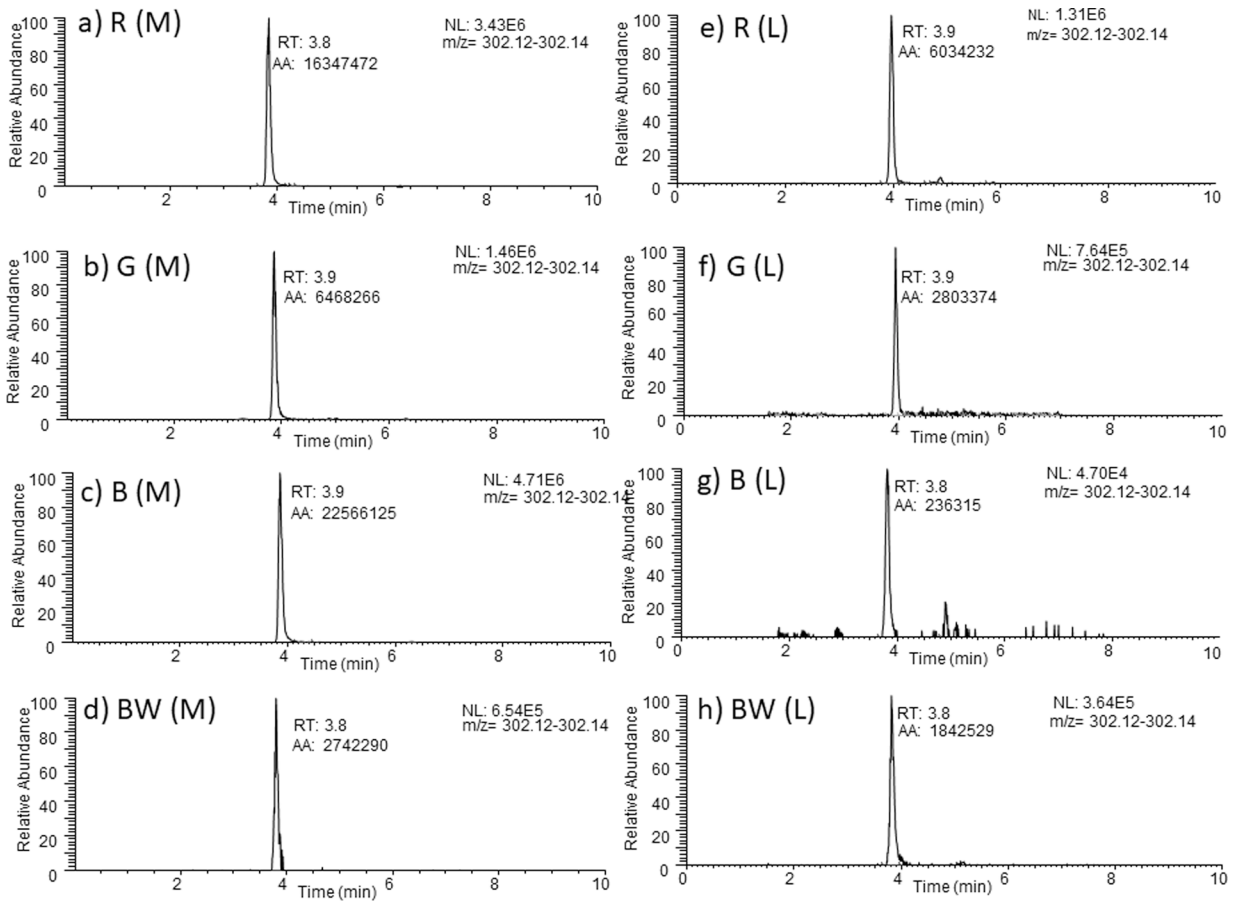


Figure 2. Chromatograms of the metabolite M02 (*m/z* 302.1301) in muscle and liver samples. Comparison among raw (a), grilled (b), and boiled muscle (c) and boiling water (d) and among raw (e), grilled (f), and boiled liver (g) and boiling water (h).

$\mu g\ kg^{-1}$ in **R** and **G**, higher than the MRL value as expected. In contrast, specimens 5 and 6 have a lower concentration of ENR, in agreement with the fact that the pharmacological treatment was stopped 2 days before slaughtering.

When Figure 1A (**R**) and Figure 1B (**G**) are compared, it can be observed that concentrations in **G** samples are slightly higher than in **R**, especially in the case of muscle tissue. This could indicate that the extraction of the antibiotics from sample can be favored by the thermal treatment. Additionally, the concentration in **B** meat (Figure 1C) is lower than in **R** samples (Figure 1A). This can be an indication of the partial transference of the antibiotic from **B** to the **BW** (Figure 1D).

3.3. Effect of Cooking on Enrofloxacin and Metabolites Content. In a previous work, the identification of metabolites from ENR in muscle, kidney, and liver tissues from pharmacologically treated chickens was studied. The studied

https://doi.org/10.1021/acsfoodscitech.2c00232
ACS Food Sci. Technol. 2022, 2, 1639−1649

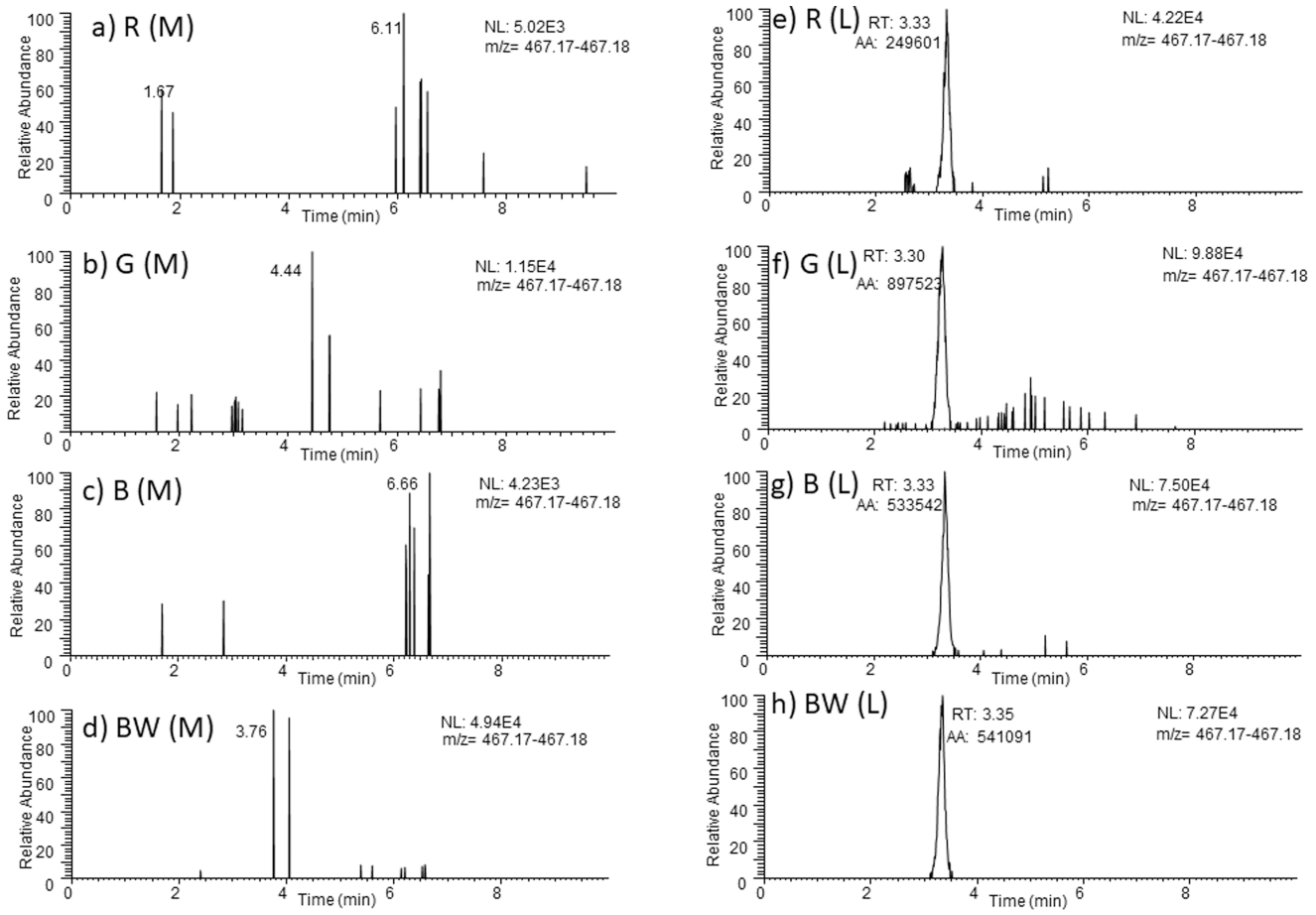


Figure 3. Chromatograms of the metabolite M30 (m/z 467.1761) in muscle and liver samples. Comparison among raw (a), grilled (b), and boiled muscle (c) and boiling water (d) and among raw (e), grilled (f), and boiled liver (g) and boiling water (h).

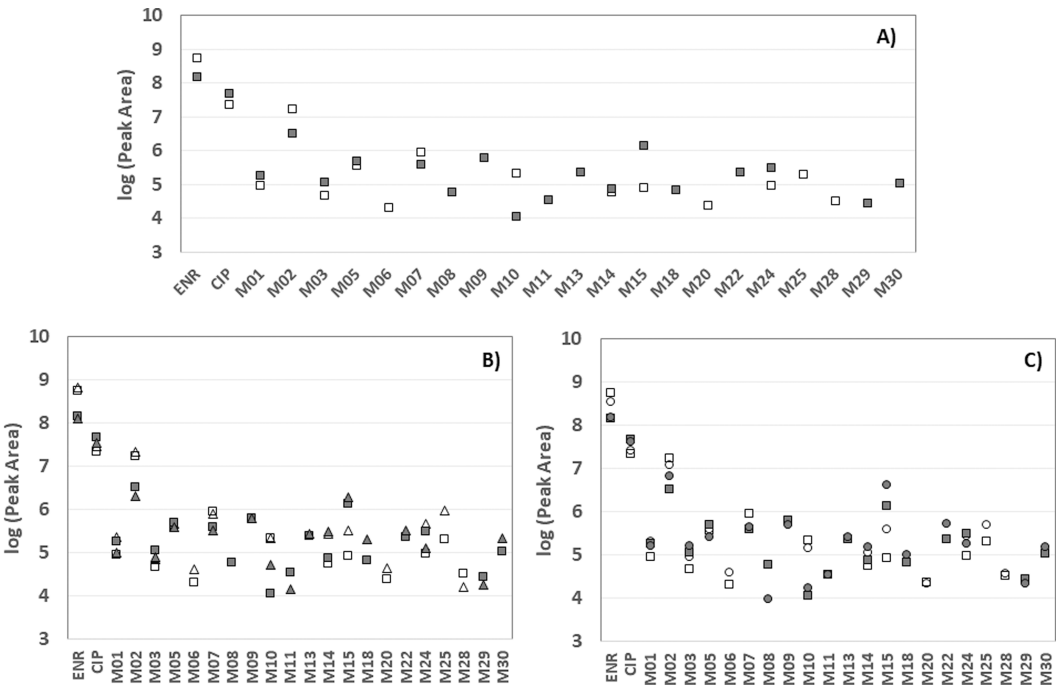


Figure 4. Comparison between signal (log of peak area) of ENR and metabolites in muscle and liver chicken samples. log(peak area) in raw samples (A): raw muscle (open boxes) and raw liver (gray filled boxes). log(peak area) in grilled samples and raw samples (B): raw muscle (open boxes), raw liver (gray filled boxes), grilled muscle (open triangles), and grilled liver (gray filled triangles). log(peak area) in boiled samples and raw samples (C): raw muscle (open boxes), raw liver (gray filled boxes), boiled muscle (open circles), and boiled liver (gray filled circles)

https://doi.org/10.1021/acsfoodscitech.2c00232
ACS Food Sci. Technol. 2022, 2, 1639−1649

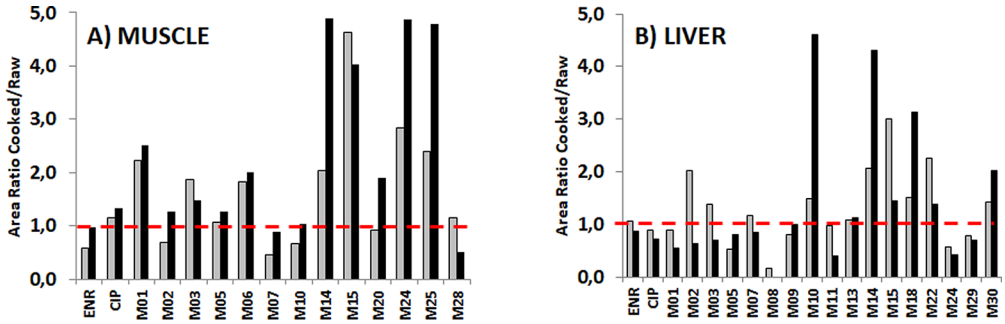


Figure 5. ENR and metabolites area ratios in cooked chicken to raw muscle samples (A) and in cooked chicken to raw liver samples (B): boiled/raw (**B**/**R**) (gray) and grilled/raw (**G**/**R**) (black).

metabolites (M01−M30) were there described in terms of m/z values and retention time and tentatively identified.[22] The same naming for compounds has been maintained for coherence reasons. The main biotransformation reactions underwent by ENR and attributed to the animal metabolism take place in either the piperazine ring or result from the transformation of the aromatic core. These compounds were determined by comparing muscle, kidney, or liver with blank tissues (samples from not medicated animals). Only compounds present in **R** samples and not in blank were considered. These metabolites were described in **R** tissues and no information about the effect of cooking on the presence of these metabolites is available.

Considering that meat is more commonly ingested in cooked form and not in **R** form, it seems interesting to study the effect that the cooking process may exert on these compounds. Tissues of two different specimens sacrificed during pharmacological treatment were analyzed using LC-LTQ-Orbitrap in full scan mode. In this study, three independent replicates are prepared and injected into LC-MS system for each tissue and cooking procedure (**B** and **G**). **R** samples, and the water used in boiled samples (**BW**) are also analyzed. A mass list, with ENR and all metabolites, was created and treated with Compound discoverer software to make a qualitative analysis of the different compounds.

Table 1 shows the molecular and structural formula[22] and the kind of sample (**R**, **B**, **G**, or **BW**, muscle or liver) in which the metabolite is present. Some compounds are present in both muscle and liver (ENR, CIP, M01-M05, M07, M10, M14, M15, and M24). Others are present in muscle but not in liver (M06, M20, M25, and M28). Finally, others are only present in liver samples (M08, M09, M11, M13, M18, M22, M29, and M30). Considering muscle samples, all the compounds studied are present in **R**, **B**, **G**, and also in **BW**, except M06, M20, and M28, that showed an increased affinity for tissues and not for **BW**. Regarding liver data, M05 and M10 are not found in **BW** while M08 is only present in **R** and **B** samples. As an example, Figure 2 shows the chromatograms for metabolite M2 (formed by the N-desethylation and decarboxylation of the oxidated form of the piperazine ring in position 3[22] in both tissues and cooked samples, while Figure 3 shows the chromatograms for metabolite M30 (formed by taurine conjugation) that is only present in liver samples.

To compare the results between **B**, **G**, and **R** samples, average areas are considered because the lack of availability of standards prevents quantification. Nevertheless, as it can be observed in Figure 4A, the areas for metabolite compounds are as a minimum of 2 orders of magnitude lower than that of ENR in **R** samples. The effect of cooking can be observed in Figures 4B and 4C. If the effect of grilling is considered (Figure 4B), while some compounds seem to be only slightly affected (M05 for instance), the action of cooking produces a decrease in the area of others (M11 or M29, for instance). Moreover, the area of a third group of compounds increases (M06, M14, M15, for instance). The same observation can be made when considering boiling as a cooking method (Figure 4C). Nevertheless, in this case, the decrease in area for some compounds is distorted by their loss to BW. In order to assess the magnitude of the modification in the area of peaks produced by the cooking process, the cooked/raw area ratio for ENR and the metabolites (ratio **B**/**R** and **G**/**R**) were calculated and plotted in Figure 5.

Three different behaviors can be observed for the two kinds of tissues studied. Some compounds (cooked/raw ratio near 1 (±0.1)) seem not affected by the cooking procedure. Some other compounds present a **B**/**R** or **G**/**R** ratio below to 1. These are compounds clearly destroyed by the cooking temperature as their concentration in cooked samples is below the value in raw meat. Finally, the remaining compounds present **B**/**R** or **G**/**R** higher than 1, which results from an increase of their concentration when cooking.

Considering muscle tissue (Figure 5A), **G**/**R** for ENR, M07 and M10 and **B**/**R** for CIP, M05, and M20 present values close to 1, which indicates the lack of effect of grilling or boiling on these compounds. Additionally, **B**/**R** for ENR, M02, M07, and M10 and **G**/**R** of M28 show ratio values lower than 1, which involves a decrease in their concentration after cooking. Lastly, M01, M03, M06, M14, M15, M24, and M25 increase the concentration in cooked samples regarding raw samples. In certain occasions, the increase in concentration after the cooking process results to be in the order of 4−5 times higher than in **R** samples (M14, M24, and M25 in **G**/**R** and M15 in both **G**/**R** and **B**/**R**).

In addition, some differences in behavior as a function of the cooking process are observed. For instance, M02 and M20 present a slight increase in **G** samples while a decrease is observed in **B** samples. Similar results were also described for ENR in chicken meat samples.[18] The levels of ENR were reduced by boiling and microwaving and increased by roasting and grilling. This was attributed to decreased moisture content in the latter that contributes to the apparent increased concentration. Nevertheless, the opposite situation is also observed. Thus, M28 has a similar concentration in **B** and **R** samples, but the increase in temperature involved by the grilling process seem to produce a significant degradation, which results in the observed decrease in concentration. CIP

https://doi.org/10.1021/acsfoodscitech.2c00232
ACS Food Sci. Technol. 2022, 2, 1639−1649

seems to be barely affected by the cooking procedure. Nevertheless, considering that ENR could degrade into CIP,[23] the observed concentration can be the result of the two factors, metabolic and thermic degradation.

Figure 5B also shows the **B**/**R** and **G**/**R** corresponding to metabolites in liver tissues. As for muscle tissues, there are some compounds that show ratios around 1 (±0.1) (slightly affected by the cooking process: **B**/**R** of ENR, CIP, M01, M11, and M13 or **G**/**R** of M09 and M13). Additionally, some compounds show ratios **B**/**R** and **G**/**R** lower than 1 (CIP, M01, M05, M08, M24, and M29) which indicates a decrease of its content attributable to the cooking. In addition, some compounds show ratios higher than 1 (M10, M14, M15, M18, M22, and M30). **G**/**R** ratios are higher than **B**/**R** ratios for M10, M14, and M18, suggesting that their presence is promoted by high temperatures. Moreover, M08 is present in **B** samples but not in **G** samples, which suggest a thermolabile behavior for this compound.

When panels A and B of Figure 5 are compared, it is possible to observe an increased number of metabolites in liver samples. This is in good agreement with the role of liver in drug metabolism. The role of the matrix seems also to play a role in the behavior of the diverse compounds observed. In muscle samples, the **G**/**R** ratio is mainly larger than **B**/**R** (80% of the compounds present this behavior), while in liver samples, the **G**/**R** ratio is larger than **B**/**R** for only 32% of the compounds.

To quantify the influence of the cooking procedures in the stability of ENR and metabolites, the transformation percentages in both grilled and boiled samples are calculated according to eq 1, where A_C is the area of the compound studied after heating (**B** and **G** samples) and A_R is the area of the compound in raw samples.

In the case of boiled samples (**B**), these transformation percentages are calculated only for compounds which are not present in **BW** samples. A positive transformation percentage (T%) value (A_C is lower than A_R) indicates a decrease in the compound content produced by degradation (or its possible transfer to the cooking media in the case of **B** samples). Alternatively, a negative T% value (A_C is higher than A_R) indicates the formation of this compound due to the cooking process.

$$\text{transformation (T\%)} = \left(1 - \frac{A_C}{A_R}\right) \times 100 \quad (1)$$

Table 2 shows the calculated values of T% for residues of ENR and metabolites in muscle and liver samples. The positive T% in muscle samples range from 3% (ENR, **G**/**R**) to 50% (M28, **G**/**R**). Regarding to liver samples, there is a higher number of compounds whose concentration decreases in the cooked samples. The T% ranges in this case from 1% to 100%, being M08 one of the compounds most affected by temperature in either **B** or **G** samples (80% and 100%, respectively). The observed 12% degradation of ENR in liver samples is in accordance with literature.[25]

To our knowledge, there is very little information on the formation/transformation of antibiotic metabolites during cooking of food.[11,23,24] Considering only those found in both muscle and liver tissues, there are compounds that increase their signal when cooking procedure (**G**) is applied (T% < 0). In this case, T% ranges from 4% (M10) to more than 350% (M14, M24, and M25 in muscle samples and M10 in liver samples). Differences are also observed in the presence or absence of the considered compound depending on the kind of tissue studied. Thus, only M10, M14, and M15 are formed in both muscle and liver samples. Most compounds show an increase in concentration only in muscle tissue (CIP, M01, M02, M03, M05, and M24), while a decrease is observed in liver samples. This effect may be explained considering the formation of such compounds as an effect of the temperature applied in **B** procedure. However, the higher temperature applied in the **G** procedure may cause its degradation. The heat treatment leads to protein denaturation, water and fat loss, and change in the pH. All these factors can alter the chemical structure, modifying consequently the concentration of drug residues or their solubility.[5,17] It has been reported the efficiency of microwave heating is increased for high fat content meat, which results in the higher degradation of antibiotics than low fat tissues.[11] The influence of the matrix on the cooked treatment is also explained for ENR;[18] although, in another study, only the cooking procedure is blamed for the decrease in concentration for certain antibiotics.[13]

Table 2. Transformation Content of ENR and Metabolites during Cooking of Muscle and Liver Tissues

	muscle		liver	
	% T in boiled samples	% T in grilled samples	% T in boiled samples	% T in grilled samples
ENR		3%		12%
CIP		−34%		28%
M01		−150%		46%
M02		−26%		38%
M03		−47%		29%
M05		−27%	48%	19%
M06	−82%	−100%	not present[b]	
M07		11%		16%
M08	not present[a]		84%	100%
M09	not present[a]			1%
M10		−4%	−48%	−360%
M11	not present[a]			60%
M13	not present[a]			−13%
M14		−388%		−330%
M15		−302%		−44%
M18	not present[a]			−212%
M20	7%	−90%	not present[b]	
M22	not present[a]			−38%
M24		−387%		58%
M25		−379%	not present[b]	
M28	−16%	50%	not present[b]	
M29	not present[a]			31%
M30	not present[a]			−102%

[a]Not present in muscle tissue. [b]Not present in liver tissues.

In Table 3, the **B**/**BW** ratio in both muscle and liver tissues are presented. Values of **B**/**BW** > 1 suggest that the compound is preferably found in boiled tissue while values <1 suggest that the compound is extracted to water when the tissue is boiled. The compounds that have a **B**/**BW** close to 1 (ENR in muscle and M18 in liver) are equally distributed in meat and water.

Some compounds are present on **B** samples but not on **BW** samples. This is an indication of the low affinity of the considered compound for water. This is the case for compounds M06, M20, and M28 in muscle and M05, M08, and M10 in liver. Alternatively, compounds, such as M01, M09, M13, and M30 in liver, show values of **B**/**BW** < 1, which is an indication of their affinity for water. If an estimation of log

https://doi.org/10.1021/acsfoodscitech.2c00232
ACS Food Sci. Technol. 2022, 2, 1639−1649

Table 3. Values of Ratio between the Content of Compounds in Boil and Boil Water Samples

		muscle	liver
	log P[a]	B/BW	B/BW
ENR	−2.11	1	1.4
CIP	−3.19	2.5	2.9
M01	−4.50	0.8	0.4
M02	0.5	3.6	4.7
M03	−3.88	0.6	2.3
M05	−3.34	19	not present in BW
M06	1.77	not present in BW	c
M07	−2.59	0.5	1.6
M08	−2.28	b	not present in BW
M09	−3.75	b	0.6
M10	−2.64	3.9	not present in BW
M11	2.29	b	5.2
M13	−4.53	b	0.8
M14	−2.69	0.1	104
M15	−3.86	1.3	3.7
M18	−4.40	b	1
M20	−2.17	not present in BW	c
M22	−3.64	b	1.2
M24	−1.77	b	2.3
M25	−2.54	2.9	c
M28	−3.06	not present in BW	c
M29	−4.32	b	1.4
M30	−2.80	b	0.5

[a]Log P obtained from the Chemdraw software. [b]This compound is not detected in muscle tissues. [c]This compound is not detected in liver tissues.

P is considered (Table 3) a certain correlation between lipophilicity and the preference for remaining in tissue versus water for a given compound can be observed. However, this feature is not conclusive in the partition. Thus, compounds, such as M03, M07, and M14, have a different behavior in each tissue. The most remarkable is M14 that presents affinity for the **BW** when muscle samples are analyzed. However, when liver samples are considered the **B/BW** ratio obtained is 104, which indicates a huge preference for the tissue. Therefore, it can be assumed that the affinity of a considered compound for the tissue or for the water in which it has been boiled depends not only on the compound lipophilicity but also on the kind of tissue that contains it.

To sum up, although it is generally accepted that the possible residues of antibiotics and their metabolites undergo thermal degradation when cooking,[11] this is not always what is observed in this study. Some of the studied compounds derived from ENR increase in content after cooking. This observation can be the result of either the actual thermal degradation of a structurally close precursor or an artifact of the analytical process. Although, given the experimental procedure the loss of water by evaporation cannot be blamed, this increased content may be the result of an increased availability of the considered compound to the extraction. The effect of temperature on proteins and other cell components,[17] at which the studied compound may be linked, may favor this availability. In such case, the observation of this increase would be more related to the analytical process than to the cooking itself.

Along with this line, a direct dependence of degradation with temperature (100° in **B** and 250° in **G**) is not always observed. The kind of tissue treated, because of its different composition in proteins and lipids, plays also an important role.

Unfortunately, the evaluation of the possible consequences that the presence of the studied compounds on human health is not possible. As it is also not possible to quantify these compounds given the lack of standards. Nevertheless, it is clear that their content is considerably low respect to the remaining content of the administered ENR (Figure 4).

AUTHOR INFORMATION

Corresponding Author

Dolores Barrón − *Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Campus de l'Alimentació de Torribera, Universitat de Barcelona, 08921 Sta Coloma de Gramenet, Barcelona, Spain; Institut de Recerca en Nutrició i Seguretat Alimentaria, Universitat de Barcelona, 08921 Barcelona, Spain*; Phone: +34934020917; Email: dolores.barron@ub.edu

Authors

Anna Tomás-Gascó − *Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Campus de l'Alimentació de Torribera, Universitat de Barcelona, 08921 Sta Coloma de Gramenet, Barcelona, Spain;* Present Address: Department of Biostatistics, Almac Group, BT282UZ Lisburn, United Kingdon

Kenneth Nazir − *Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Campus de l'Alimentació de Torribera, Universitat de Barcelona, 08921 Sta Coloma de Gramenet, Barcelona, Spain;* Present Address: Metabolomic Unit, Institute for Molecular Medicine Finland (FIMM), HiLIFE, University of Helsinki, Finland

Paola Esposito − *Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Campus de l'Alimentació de Torribera, Universitat de Barcelona, 08921 Sta Coloma de Gramenet, Barcelona, Spain;* Present Address: Controllo e Sicurezza degli Alimenti, Università di Reggio Emilia Via Amendola, 42122 671 Reggio Emilia, Italia

Cristina Minguillón − *Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Campus de l'Alimentació de Torribera, Universitat de Barcelona, 08921 Sta Coloma de Gramenet, Barcelona, Spain*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsfoodscitech.2c00232

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors are grateful to the Ministerio de Economia y Competitividad (Project CTQ2013-44077-R) for financial support.

REFERENCES

(1) Stolker, A. A. M.; Brinkman, U. A. Th. Analytical strategies for residue analysis of veterinary drugs and growth-promoting agents in food-producing animals-a review. *J. Chromatogr. A* **2005**, *1067*, 15−53.

(2) European Parliament and the Council Regulation No 1831/2003 of the 22 September 2003 on additives for use in animal nutrition. *Off. J. Eur. Comm.* **2003**, L268/29−L268/68.

(3) AEMPS: Agencia Española de Medicamentos y Productos Sanitarios. *Cómo se regulan los medicamentos y productos sanitarios en España*, 2010. http://www.aemps.gob.es/publicaciones/publica/regulacion_med-PS/home.htm.
(4) European Parliament and the Council Regulation No 2019/6 of the 11 December 2018 on veterinary medicinal products. *Off. J. Eur. Comm.* **2019**, L4/43−L4/161.
(5) Rana, M. S.; Lee, S. Y.; Kang, H. J.; Hur, S. J. Reducing veterinary drugs residues in animal products: a review. *Food Science of Animal Resources* **2019**, *39*, 687−703.
(6) Fábrega, A.; Sánchez-Céspedes, J.; Soto, S.; Vila, J. Quinolone resistance in food chain. *Int. J. Antimicr. Agents* **2008**, *31*, 307−215.
(7) Fábrega, A.; Madurga, S.; Giralt, E.; Vila, J. Mechanism of action of and resistance to quinolones. *Microbial Biotechnology* **2009**, *2*, 40−61.
(8) Marshall, B. M.; Levy, S. B. Food animals and antimicrobials: impacts on human health. *Clin. Microbiol. Rev.* **2011**, *24*, 718−733.
(9) Rusu, A.; Hancu, G.; Uivarosi, V. Fluoroquinolone pollution of food, water and soil, and bacterial resistance. *Environ. Chem. Lett.* **2015**, *13*, 21−36.
(10) Commission of the European Communities, Regulation No 37/2010 of 22 Desember 2009 on pharmacological active substances and their classification regarding maximum residue limits in foodstuff of animal origin. *Off. J. Eur. Comm* **2009**, L15/1−L15/5.
(11) Tian, L.; Khalil, S.; Bayen, S. Effect of thermal treatments on the degradation of antibiotic residues on food. *Crit. Rev. Food Sci. and Nutrition* **2017**, *57*, 3760−3770.
(12) Muaz, K.; Riaz, M.; Akhtar, S.; Park, S.; Ismail, A. Antibiotic residues in chicken meat: global prevalence, threats, and decontamination strategies: A review. *J. Food Protection* **2018**, *81*, 619−627.
(13) Khan, A. A.; Randhawa, M. A.; Butt, M. S.; Nawaz, H. Impact of various processing techniques on dissipation behaviour of antibiotic residues in poultry meat. *J. Food Processing and Preservation* **2016**, *40*, 76−82.
(14) Shaltout, F.; Shatter, M.; Sayed, N. Impacts of different types of cooking and freezing on antibiotic residues in chicken meat. *J. Food Sci. Nutrition* **2019**, *5*, 100045.
(15) Gowtham, P.; Mutthukumar, M.; Eswara Rao, B.; Kalpana, S.; Keerthika, V.; Dani Nishant, J. M. A. Impact of cooking methods of meat on antibiotic residues: review. *Pharma Innovation Journal* **2020**, *9*, 303−309.
(16) Castello Branco, L.; Rodrigues, M. V. N.; Reyes, F. G. R. Effect of food processing (fish burger preparation and frying) on residual levels of enrofloxacin and ciprofloxacin. *Food Additives & Contaminants: Part A* **2021**, *38*, 1689−1700.
(17) Sobral, M. M. C.; Cunha, S. C.; Faria, M. A.; Ferreira, I. Domestic cooking of muscle foods: impact on composition of nutrients and contaminants. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 309−333.
(18) Lolo, M.; Pedreira, S.; Miranda, J. M.; Vázquez, B. I.; Franco, C. M.; Cepeda, A.; Fente, C. Effect of cooking on enrofloxacin residues in chicken tissues. *Food Add. Cont.* **2006**, *23*, 988−993.
(19) Hassan, M.; Amin, R.; Abo Elroos, N. Enrofloxacin residues in chicken meat and giblets. *Benha Vet. Med. J.* **2019**, *36*, 175−183.
(20) Nagi, O. M.; Shawish, R. R.; Elbayoumi, Z. H. Detection of Enrofloxacin Residues in Poultry Meat and its Offal and the Effect of Boiling on these Residues. *Alex. J. Vet. Sci.* **2021**, *71*, 67−74.
(21) Morales-Gutiérrez, F. J.; Hermo, M. P.; Barbosa, J.; Barrón, D. High Resolution mass spectrometry applied to the identification of transformation products of quinolones from stability studies and new metabolites of enrofloxacin in chicken muscle tissues. *J. Pharm. Biomed. Anal.* **2014**, *92*, 165−176.
(22) Morales-Gutiérrez, F. J.; Barbosa, J.; Barrón, D. Metabolic study of enrofloxacin and metabolic profile modifications in broiler chicken tissues after drug administration. *Food Chem.* **2015**, *172*, 30−39.
(23) Junza, A.; Barbosa, S.; Codony, M. R.; Jubert, A.; Barbosa, J.; Barrón, D. Identification of metabolites and thermal transformation products of quinolones in raw cow's milk by liquid chromatography coupled to high resolution mass spectrometry. *J. Agric. Food Chem.* **2014**, *62*, 2008−2021.
(24) Junza, A.; Saurina, J.; Barrón, D.; Minguillón, C. Metabolic profile modifications in milk after enrofloxacin administration studied by liquid chromatography coupled with high resolution mass spectrometry. *J. Chromatogr. A* **2016**, *1460*, 92−99.
(25) Roca, M.; Castillo, M.; Marti, P.; Althaus, R. L.; Molina, M. P. Effect of heating on the stability of quinolones in milk. *J. Agric. Food Chem.* **2010**, *58*, 5427−5431.
(26) Javadi, A.; Mirzaie, H.; Khatibi, S. A. Effect of roasting, boiling and microwaving cooking methods on enrofloxacin residues in edible tissues of broiler. *African J. Pharm. Pharmacol.* **2011**, *5*, 214−218.
(27) Heshmati, A. Impact of Cooking Procedures on antibacterial drug residues in food: a review. *J. Food Quality and Hazards Control* **2015**, *2*, 33−37.
(28) Commission Decision of the European Communities, Decision of 12 August 2002. Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002/657/EC). *Off. J. Eur. Comm.* **2002**, L221/8−L221/21.

https://doi.org/10.1021/acsfoodscitech.2c00232
ACS Food Sci. Technol. 2022, 2, 1639−1649