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Incidence, stability and risk assessment for sulfonamides and tetracyclines in aqua-cultured Nile Tilapia fish of Egypt

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ABSTRACT

The current study was conducted to determine sulfonamides (SAs) and tetracyclines (TCs) residuals in farmed Nile Tilapia fish (*Orechromis niloticus*) using the solid phase extraction (SPE) technique and high performance liquid chromatography with diode array detection (HPLC-DAD). As well, to assess the potential health risk due to the consumption of contaminated fish following its household thermal processing. Tilapia samples were collected from four governorates in Egypt; El-Fayoum, Giza, Cairo, and Alexandria. The results showed that 56.3 % (27 out of 48 samples) of fish samples were free of antibiotics, while 10.4 % and 33.3 % of samples were contaminated by SAs and TCs, respectively. Besides, oxytetracycline (OTC) showed the highest detected concentrations ranged from 52.8 to 658.5 (μg/kg), followed by chlortetracycline (OTC) (35.89–109.76 μg/kg), and tetracycline (TC) (68.8–96.7 μg/kg). While the detected SAs were between 32.89 μg/kg (sulfamethazine: SMT) and 136.43 μg/kg (sulfadimethoxine: SDM). As well, sulfamethoxazole (SMX) showed an average concentration of 52.41 μg/kg. Notably, only 7 samples (out of 21 positive samples) had residual levels exceeded the permissible limits. The study also concluded that freezing fish at −18 °C for one week had no significant effect on the stability of SAs and TCs. As well, SAs showed more stability than TCs against the thermal processing for fish. Indeed, the stability of SAs and TCs antibiotics was arranged in a descending order, shown as follows: SMT > SDM > SMX > CTC > TC > OTC. Eventually, no potential risk to the Egyptian population was found from the consumption of the contaminated fish samples by SAs and TCs.

1. Introduction

Fish poses an important source of the animal protein worldwide as well as in Egypt. Globally, it represents about 17 % of protein intake as reported by FAO [1]. Similarly, in Egypt; fresh fish is a traditional diet and a cheap source of animal protein. Aquaculture industry is considered the fastest growing industry in some countries such as in African countries. Egypt has the largest aquaculture industry in Africa. Currently, Egypt is the tenth largest producer in the world and the second largest producer of tilapia after China [2]. The majority of the Egyptian fish production is obtained from aquaculture (77.4 %), while the remaining 22.6 % is obtained from the capture fisheries as reported by the Egyptian Ministry of Agriculture and Land Reclamation [3].

Nile Tilapia is a freshwater fish found to be one of the first species that have been cultured in the world and considered the most consumable fish in Egypt [4]. Tilapia represents about 65.2 % from the total fish production in Egypt in the year 2015 with 989,606 tons; about 88.47 % (875,513 tons) from them are produced from aquaculture while the other 11.53 % (114,093 tons) are obtained from the natural sources as reported in the statistics of fish production prepared by the General Authority for Fish Resources Development [3].

The production of Nile Tilapia in aquaculture systems such as intensive systems in tanks and cages are rapidly developed. These methods are characterized by high stockpile density and size, extensive use of formulated feed that contains antibiotics, antifungal, and other pharmaceutical preparations [5,2]. Fish bacterial diseases are one of the most vital problems facing the aquaculture industry in Egypt [4]. Therefore, antibiotics can intentionally be added to aquaculture either as prophylactic or as therapeutic to control fish diseases. Also, there are un-intended routes of aquaculture contamination by antibiotics such as the application of chicken manure to aquacultures, to increase the plankton [6] and the usage of antibiotic-contaminated water supply in aquaculture. However, the misuse of antibiotics in aquaculture and food-producing animals can lead to the occurrence of high concentration of antibiotic residues or their metabolites in animal products [6–8] including fish [5]. These antimicrobial residues if present in concentrations above the established maximum residue limits, or if used without appropriate authorization based on scientific assessment of the

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benefit and risk of the treatment, can present a hazard in products from aquaculture [9]. In this regard, antibiotics, as hazardous materials, should be applied under the directions of professionals in farms to ensure their withdrawal to the safe limits [10]. Additionally, strict legislations should be implemented to minimize the misuse of antibiotics [11].

Tetracyclines (TCs) are the most widely used antibiotics to prevent or treat bacterial diseases and promoting growth in the farming animals [5]. Also, sulfonamides (SAs) and other antibiotics; such as penicillins and several iodophore, are the most common contaminating antibiotics in animal feed [5]. Darwish et al. [11] concluded that TCs are the most predominantly prescribed antibiotics in Africa. Monterio et al., [12] recorded the detection of antibiotics OTC, TC and FF in Nile tilapia muscles, and their presence was directly related to the control of bacterial disease in cage farms. Also, different types of antibiotics; chloramphenicol and nitrofuran, were detected in the captured Tilapia fish from the fresh water of the river Nile [13]. However, to our knowledge, in Egypt, there is a lack of studies focusing on the occurrence of SAs and TCs residues in Tilapia fish in order to assess the potential risk to public health through the consumption of Tilapia fish.

Therefore, the present research was designed: 1) to evaluate the compliance of the Egyptian Tilapia fish producers to the international guidelines of antibiotic application in aquacultures, 2) to study the resistance of SA's and TC's to common household thermal treatments for fish, and 3) to assess the potential risk to public health due to the aggregate exposure to the detected levels of SAs and TCs in Tilapia fish either thermally treated or not.

2. Materials and methods

2.1. Chemicals

Chemicals, solvents and reagents were of analytical and HPLC grades. Solid phase extraction (SPE) cartridges (Oasis HLB SPE cartridge, 200 mg/6 mL) were obtained from Waters Oasis Co. (Milford, USA). Reference materials of antibiotics (sulfamethazine, sulfamethoxazole, sulfadimethoxine, tetracycline, oxytetracycline, and chlortetracycline) were purchased from Sigma-Aldrish Co (St Louis, MO, USA).

2.2. Sampling

Forty-eight random samples of Nile Tilapia fish (*Orechromis niloticus*) (weight average: 200 − 250 g) were collected from various local markets that represent four Egyptian Governorates (Alexandria, Cairo, Giza, and El-Faiyum, which include more than 25 % of the Egyptian population). Every governorate was represented by 3 sites with 4 samples in each site. The selected sites for Cairo governorate were Helwan, Al-Maadi and Al–Shuruq, while Alexandria governorate included Al-Ajami, Al-Manshieh and Abu-Qir. Al-Omraniyah, Dokki and Atfih represented Giza governorate as well as Sinnures, Ibshway and Etsa were selected from El-Faiyum governorate. Samples were collected at the summer season of 2019. Samples were transferred from the market to the lab into an ice-box, then washed under tab water and subjected to the extraction process.

2.3. Extraction of SAs and TCs

The extraction method was developed from two previous studies by Pleasance et al. [14] and Evaggelopoulou and Samanidou [15]. Five mL of TCA (10 %) was added to 10 *g* sample of minced fish muscles and homogenized for 1 min. Then 35 mL of citrate buffer (0.3 *M*, pH 4.0) was added to the mixture and homogenized for 5 min. The homogenized mixture was decanted into a 50 mL Falcon tube and centrifuged for 20 min at 5000 rpm. The sample residue was re-extracted using 35 mL acetone following the same procedures of the first extraction. Acetone extract was then poured on a 100 mL rotary flask prior to be evaporated, at 38 °C under vacuum, to dryness. The dried residue of acetone was well dissolved using the citrate buffer extract (~30 mL) with the help of ultrasonic waves and transferred to a 150 mL separating funnel prior to be defatted by liquid/liquid extraction using n-hexane. One gram of NaCl and 30 mL n-hexane were added to the citrate buffer extract and the mixture was manually shaken for 1 min, then the mixture was left for few minutes for separation. The mixture of citrate buffer extract and n-hexane can be centrifuged (5000 rpm, 15 min.), to break the emulsion if existed. The citrate buffer extract was loaded onto the SPE cartridge (activated by 5 mL methanol HPLC grade + 5 mL HCl 0.5 N + 5 mL de-ionized water). The SPE was then washed using 5 mL methanol in water (5%) + 5 mL de-ionized water. After that, the SPE cartridge was air-dried under reduced pressure. The target extract was eluted using 10 mL of methanol HPLC grade which was evaporated at 38 °C to complete dryness. The dried residue was then dissolved in 500 μL of methanol (HPLC grade) and subjected to analysis by HPLC.

2.4. Determination of SAs and TCs

SAs and TCs were determined by HPLC-DAD following the method of Mostafa et al. [16]. Chromatographic separation was achieved using a reversed-phase C_{18} analytical column (150 × 4.6 mm i.d., Phenomenex®, 5 μm particle size). The separation was conducted using a gradient elution between 0.1 % formic acid in water (as mobile phase A) and acetonitrile (as mobile phase B). The separation program started with 90 % mobile phase A which was then gradient up to 80 % within 8 min, then gradually decreased to 60 % over 6 min and was then isocratically held for 8 min. Finally, the column was equilibrated by the initial mobile phase composition for 10 min before each analysis. The flow rate of mobile phase was adjusted at 1.2 mL/min during all phases of the gradient run. The column temperature was maintained at 25 °C and the injection volume was 20 μL. Quantitation was achieved with PDA detection at 280 and 365 (nm) for SAs and TCs, respectively.

2.5. Analytical method evaluation (in-house validation)

One hundred grams of fresh fish meat and free from antibiotics was spiked with antibiotics as 200 μg/kg for each antibiotic. The spiked sample was left for one hour at room temperature (for the optimal merge and distribution of antibiotic in the muscles) then subjected to the extraction procedures. Three replicates (n = 3) were used for the study and analyzed during the same day. Recovery ratio was calculated according to the EU directive No. 2002/657/EC [17] using the following equation:

$$\text{Recovery}(\%) = \left(\frac{\text{Measured content}}{\text{Fortification level}}\right) \times 100$$

Precision was estimated by calculating the relative standard deviation percent (RSD%) for each drug recovery replicates. Quantification limits of antibiotics in fish flesh were estimated as 10 times the signal-to-noise ratio. Linearity was determined from matrix-matched calibrations created from five different concentrations.

2.6. Stability evaluation for SAs and TCs in frozen and cooked fish

Fresh samples of big sized Tilapia fish (1 kg) were used in this study. Where a number of cohesive slices were separated from the fish samples with an average weight of 50 − 100 grams. Each slice was accurately weighed and injected with a proper amount of antibiotics standard solution to acquire a final concentration of 1 mg/kg. The injected slices were left for one hour, at room temperature, for the optimal antibiotics distribution and merge within the fish tissue. For the freezing treatment, samples were kept in polyethylene bag and stored in a deep freezer set at −18 °C for one week. For the frying and grilling processes,

Table 1
Evaluation parameters for SAs and TCs analytical method.

Antibiotics		Regression equation	Correlation coefficients (r^2)	QL*(μg/kg fish)	Recovery (%) ± SD	RSD**(%)
SAs	SMT	y = 1.8498x - 68.556	0.9998	15.00	73.96 ± 3.34	4.50
	SMX	y = 3.7175x - 678.88	0.9996	10.00	72.66 ± 2.22	3.06
	SDM	y = 4.1088x - 1697.6	0.9989	07.00	75.22 ± 4.11	5.46
TCs	OTC	y = 1.7151x - 384.28	0.9941	13.50	88.70 ± 2.49	2.81
	TC	y = 2.0014x - 636.77	0.9963	15.00	80.89 ± 2.65	3.28
	CTC	y = 1.483x - 646.94	0.9978	26.00	83.86 ± 3.71	4.43

* QL: Quantification limit.
** RSD: Relative standard deviation.

the fish samples were exposed to the two processes until the well done; frying in corn oil at 190 °C for 7 min and grilling on a metal baking tray at 180 °C for 10 min. Each treatment was done in 3 replicates (n = 3). Following the 3 treatments (freezing, frying and grilling), samples were directly subjected to the antibiotic extraction process before quantitative analysis.

2.7. Risk assessment for the exposure to SAs and TCs in fish

2.7.1. Exposure assessment

Exposure assessment was built on the mean detected concentrations of antibiotics in Tilapia fish. As well, the estimated daily intake (EDI, μg) of SAS and TCs from fish consumption was calculated as follows:

$$EDI = \frac{Cf \times Occ}{BW}$$

Where: *Cf* is the daily fish consumption for the Egyptian population (g/person), *Occ* is the occurrence of antibiotic in fish muscles expressed as (ng contamination mean/g fish) and *BW* is the mean body weight for an adult consumer (70 kg). The daily consumption from aqua-cultured Tilapia fish for the Egyptian population (55.23 g/day) was considered from the reported data by Wally [18]. The daily consumption from other food items, where SAs and TCs might be found, are: cattle meat (29.89 g), poultry meat (29.31 g), fresh milk (180 g), eggs (19.8 g) and other fish kinds (42.65 g) [18]**, [**the database of **CAPMAS** (Central Agency for Public Mobilization and Statistics: www.capmas.gov.eg)**]** .

2.7.2. Risk characterization

The assigned maximum residue limits (MRLs) for SAs and TCs in fish muscles are 100 μg/kg for every single antibiotic according to the European Union [19]. Also, the MRLs for SAs and TCs in other food items (cattle meat, poultry meat, fresh milk and eggs), where they might be found, are 100 μg/kg except for TCs in eggs which is 200 μg/kg [19]. The acceptable daily intake (ADI, μg/kg bw/day) for SAs and TCs are respectively 3 and 5 (μg/kg bw/day) according to JECFA [20]. The risk characterization was performed following the new approaches assigned by Goumenou & Tsatsakis [21] for single chemicals and for chemical mixtures. These new parameters were the source related Hazard Quotient (HQ_S) and Hazard Index (HI_S) and the adversity specific Hazard Index (HI_A).

HQ_S was calculated according to the following equation:

$$HQs = \frac{EXP\ aggregated}{ADI}$$

$$EXP\ aggregated = \frac{EXP\ \text{from spscific food item}}{\text{Correction factor}(CF)}$$

$$CF = \frac{(\text{Consumption of specific food} \times \text{MRL in the specific food})}{\sum_i^n (\text{Consumption of food i} \times \text{MRL in the food i})}$$

Where: ***n*** refers to the number of food items where SAs and TCs might be found. The calculated *CF* value for each of SMT, SMX, SDM was 0.1796, while it was 0.1688 for each of TC, OTC and CTC. Where the numerator of the equation refers to the consumed amount from Tilapia fish multiply the MRL, while the denominator represents the SUM of the calculated values for the other 5 food items representing the whole diet (previously mentioned in the exposure assessment section). Regarding the risk characterization for a mixture of ***n*** chemicals (3 SAs and 3 TCs) in a specific food item (fish), the source related **HI_S** was calculated as follows:

$$HIs = \sum_i^n (HQs)i$$

Where, ***n*** is the number of chemicals in the mixture.

HI_A was calculated as follows:

$$HI_A = \sum_i^n HQi = \sum_i^n \frac{EXPi}{ADIi}$$

Where, we considered the sum of the 3 SAs and the sum of 3 TCs because each group members have the same mode of action.

2.8. Statistical analysis

Microsoft Office Excel (Microsoft Office 2010) was used for the calculation of the result averages, standard deviations and relative standard deviations as well as to generate the linear regressions.

3. Results and discussion

3.1. Method quality assessment

Data of Table 1 showed parameters of the in-house method validation. A linear correlation for the antibiotic concentrations against the peak area with high precision was observed when calibration lines were generated using five concentrations in the range of 20–500 (ppb). Wherein the obtained correlation coefficient (r^2) values located between 0.9941 and 0.9998.

As well, limits of quantifications (LOQ) for SAs, by the adopted HPLC-DAD, located in range of 07–15 (μg/kg). While the sensitivity of HPLC-DAD against TCs was lower than SAs, where the LOQ of TCs located between 13.50 and 26 (μg/kg). These results were in agreement with Furusawa [22], Yu et al. [23], Chung et al. [24] and Nunes et al. [25] who reported LOQ values for SAs ranged from 05 to 22.9 ppb. While the LOQ of Yu et al. [23], Abou-Raya et al. [26] and Tölgyesi et al. [27] methods ranged between 10 and 34 ppb for TCs. Generally, the presented method showed a high sensitivity that can enable the determination of lower quantities of antibiotics than the MRLs recommended in the EU directive No. 37/2010/EC [19], which equivalent to 100 μg/kg for SAs and TCs.

Additionally, recoveries of the adopted multi-residue method of extraction for SAs and TCs, at concentrations equals the MRLs values, located in the averages of 72.66–75.22(%) and 80.89–88.70(%), respectively. Notably, extraction recoveries of TCs were markedly higher than those of SAs using the adopted multi-extraction method. Specifically, the recovery ratios for SMT, SMX, SDM, TC, OTC and CTC were 73.96, 72.66, 75.22, 88.70, 78.22 and 81.19 (%), respectively.

Our results of SAs recoveries were higher than those of Pleasance et al. [14] who extracted SAs from salmon flesh using liquid extraction by acetone, however the resulted recovery ratios were poor (~60 %). The usage of Oasis SPE (HLB) following the liquid extraction, in our investigation, represented higher recoveries than those of Pleasance et al. [14]. Besides, our results of TCs recoveries were in agreement with Shalaby et al. [28] and Nguyen et al. [29] who extracted TCs using citrate buffer solution (pH 4.0) followed by a cleanup using Oasis SPE (HLB) cartridges and scored recovery average of 79.89–94.85(%). Whilst, higher recoveries were scored by Evaggelopoulou and Samanidou [15], when followed the same extraction procedures, being 95.7 %, 95.5 % and 97.3 % for OTC, TC and CTC, respectively. These differences in the recovery rates from different studies could be attributed to the different extraction methods that used by those studies. Some methods used the solvent extraction and others used the buffer extraction followed by a clean-up using SPE for the determination of either SAs or TCs. However, the present study used a combination of both the solvent and the buffer methods for better recoveries and as a multi-residue extraction purpose for both SAs and TCs.

Basically, the multi-residue method of the present investigation represents recovery values higher than the minimum ratios recommended by the EU in the directive No. 2002/657/EC [17] concerns the validation criteria of recovery test. Moreover, precision values (RSD %) of the calculated recoveries did not exceed 5.46 %, which comply EU recommendations and below 10 % as the maximum acceptable ratio for RSD% of biological samples [29].

3.2. Occurrence of SAs and TCs residuals in fish

Data of Table 2 showed that there was a low SAs incidence in the samples of the 4 governorates. Where, the samples of Alexandria governorate were completely free of SAs. While only one sample from Al-Shuruq zone (Cairo governorate) and 2 samples from Al-Dokki zone (Giza governorate) contained SMT with averages of 32.89 and 44.72 (μg/kg), respectively. As well, only 2 samples from Etsa zone (El-Fayoum governorate) contained both SMX and SDM with concentrations average of 52.41 and 136.43 (μg/kg) respectively. Meanwhile, in another Egyptian study by Rezk et al. [30], SDM was not detected in fish farms at Kafr El-Sheikh, Sharkia, El-Behera, El-Fayoum and Giza governorates. Additionally, Guidi et al. [31] did not detect any molecule from SAs in 193 samples of Nile Tilapia fish in Brazil.

TCs showed a frequent incidence as compared to SAs, and OTC was the most detected compound. OTC's positive samples from Cairo and Alexandria Governorates located within the permissible limits for TCs (100 μg/kg) except for one sample from Al-Agami site which increased the concentration average of Al-Agami samples to be 209.94 μg/kg. Giza and El-Fayoum Governorates had several positive samples for OTC with high concentrations. These elevated concentrations equal 1.5–6 times the MRLs values (100 μg/kg) being 151.76, 522.14 and 658.52 (μg/kg) respectively for Al-Omraniyah, Ibshway and Atfih sites. These results were in agreement with Monteiro et al. [12] who found that OTC was the most detected antibiotic in Brazilian Tilapia samples with a concentration range of 10–1.379 mg/kg, which were above the MRLs set by the EU regulation. The high detected concentrations of OTC, in the present investigation, should be taken into considerations, because it could pose a health hazard to people who consume a big amount of fish from those sites. Those samples of Giza and El-Fayoum Governorates with high OTC concentrations above the MRL could have been collected shortly following the application of OTC to control the bacterial diseases in fish [32]. Additionally, in a few cases, the fish samples could contain elevated levels of antibiotics because of the addition of antibiotic-containing materials, such as chicken manure, to aquaculture to increase the plankton [6].

On the other hand, Sinnures site from El-Fayoum Governorate showed positive samples for OTC however with a concentration range within the accepted limits. CTC was frequently detected, following OTC, in samples of Al-Maadi, Al-Shuruq, Al-Dokki and Atfih with concentration means of 48.45, 35.89, 79.36 and 109.76 (μg/kg), respectively. These above results were in accordance with Cháfer-Pericás et al. [33]; Cháfer-Pericás et al. [34] and Barman et al. [35] who detected OTC in Tilapia fish samples, however with concentrations below the MRL. Meanwhile, Guidi et al. [31] did not detect any molecule from TCs in 193 Nile Tilapia samples in Brazil. Besides, Guidi et al. [36] did not found TCs in 26 Tilapia samples, when they screened 14 antibiotics (from quinolones and TCs).

It can be concluded from the results that 56.3 % (27 out of 48) of the samples were free of SAs and TCs, while 10.4 % (5 samples) of the samples were contaminated by SAs and 33.3 % (16 samples) were contaminated by TCs (Fig. 1. a.). Nine samples (42.9 %) out of the 21 positive samples had a multi content of more than one molecule from either SAs or TCs or from both families [1 sample from Al-Shuruq site and 2 samples from Al-Dokki site contained SAs + TCs] (Table 2 and Fig. 1. b.). Notably, 7 samples (33.3 %) out of the 21 positive samples exceeded the permissible limits (Fig. 1. c.). Particularly, 6 OTC containing samples plus one SDM containing sample had concentrations above the MRLs.

3.3. Thermal stability of SAs and TCs residuals in fish

For the thermal stability of SAs and TCs under investigation, it was found that storing the fish samples frozen at −18 °C for one week had no significant effect on the stability of both SAs and TCs (Fig. 2. a.). The

Table 2
Occurrence of SAs and TCs residuals in fish samples.

Governorates	Sampling zone	Mean residue (μg/kg)					
		SMT	SMX	SDM	OTC	TC	CTC
Alexandria	Al-Ajami	< d.l.*	< d.l.	< d.l.	209.94	< d.l.	< d.l.
	Al-Manshieh	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.
	Abu-Qir	< d.l.	< d.l.	< d.l.	70.08	< d.l.	< d.l.
Cairo	Helwan	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.
	Al-Maadi	< d.l.	< d.l.	< d.l.	< d.l.	< d.l.	48.45
	Al-Shuruq	32.89	< d.l.	< d.l.	52.78	< d.l.	35.89
Giza	Al-Dokki	44.72	< d.l.	< d.l.	< d.l.	< d.l.	79.36
	Al-Omraniyah	< d.l.	< d.l.	< d.l.	151.76	< d.l.	< d.l.
	Atfih	< d.l.	< d.l.	< d.l.	658.52	96.70	109.76
El-Fayoum	Etsa	< d.l.	52.41	136.43	< d.l.	< d.l.	< d.l.
	Ibshway	< d.l.	< d.l.	< d.l.	522.14	68.87	< d.l.
	Sinnures	< d.l.	< d.l.	< d.l.	88.86	< d.l.	< d.l.
MRL (μg/kg)**		100	100	100	100	100	100

* < d.l.: below the detection limit.
** MRL: Maximum residue limits [19].

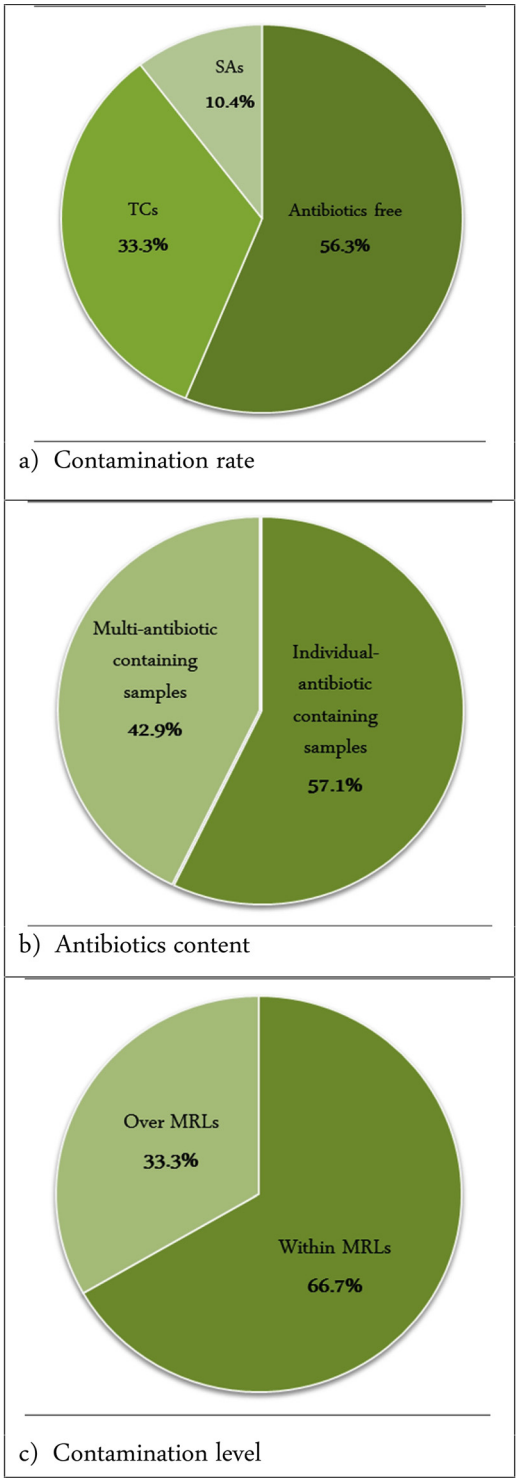

Fig. 1. Incidence of SAs and TCs in Tilapia samples.

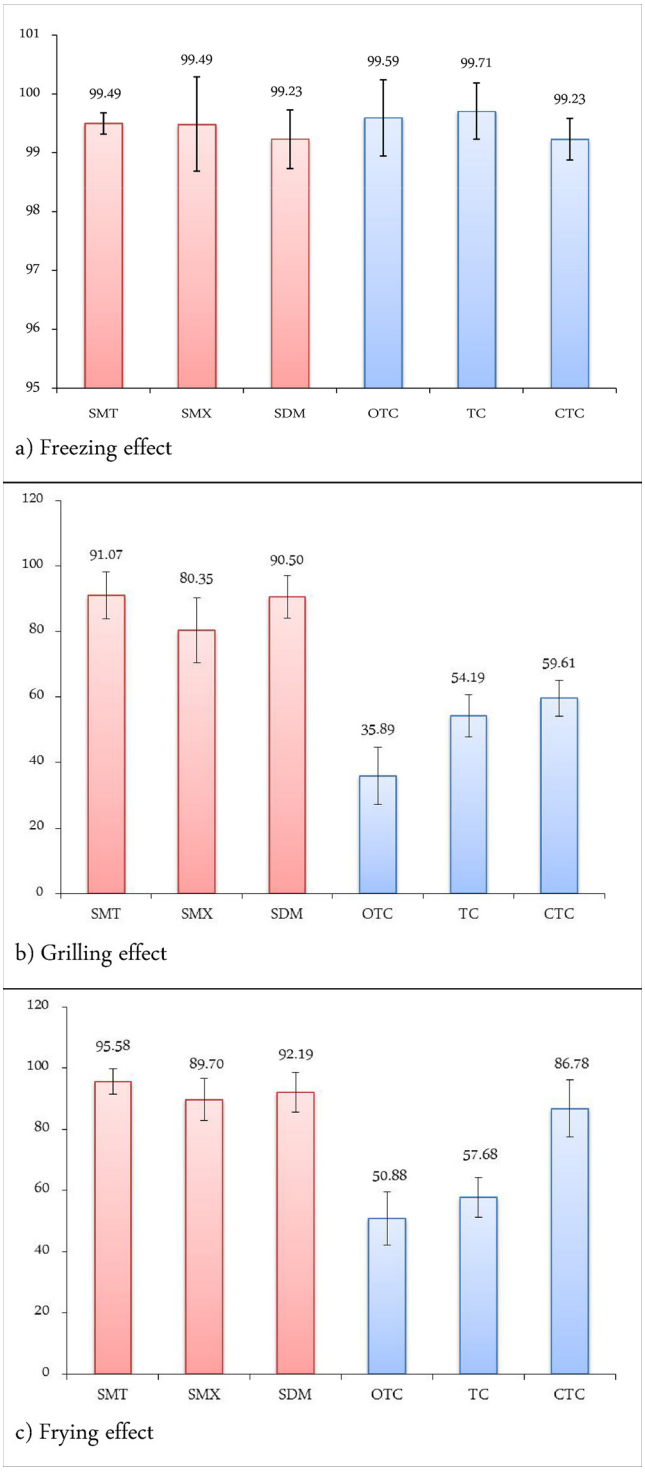

Fig. 2. Stability of SAs and TCs through; a) freezing, b) grilling and c) frying processes.

determined concentrations were between 99.23 and 99.71 (%) of the initial concentrations. These results were in agreement with Papapanagiotou et al. [37]; Gratacós-Cubarsí et al. [38]; Liman et al. [39]; Vivienne et al. [40]; Fahim et al. [41] who reported no significant reductions in SAs and TCs residues in meat and fish tissues preserved under freezing.

Grilling fish for 10 min at 180 °C had slightly affected SAs and moderately affected TCs (Fig. 2. b.). Specifically, the grilling process had reduced the initial concentration of SMT, SMX and SDM by 8.30, 19.65 and 8.50 (%). On the other hand, TCs were more affected by the grilling, as the degraded ratios were 64.11, 45.81 and 40.40 (%) respectively for OTC, TC and CTC. Our results were far from those of Furusawa and Hanabusa [42] who reported that the residues of SAs, including SMX, in the chicken muscle cooked to well done, by roasting at 170 °C for 12, were reduced by 30–40 %. However, our reduction ratios in OTC, TC and CTC levels were comparable to those obtained by Abou-Raya et al. [26] who reported degradation ratios of 72.1, 46 and 32 (%), respectively when roasted chicken meat until well-done (180 °C for 40 min). Vivienne et al. [40] roasted the birds meat containing OTC

at 200 °C for 30 min and the obtained degradation ratio was 88.79 %.

Similarly, SAs were more stable than TCs against the thermal effect of deep-frying in oil for 7 min at 190 °C. The reduction ratios of SAs ranged from 4.42 % to 10.30 % (Fig. 2. c.). While, TCs had been degraded by an average of 13.22–49.12 (%). Our results were not in agreement of Ismail-Fitry et al. [43] who reported more reduction ratios for SMT and SMX when fried chicken balls at 180 °C for 6 min being 65.89 and 58.35 (%), respectively. Also, Xu et al. [44] reported that frying fish at 190 °C for 7−10 min caused 46.1 % reduction in SDM. Meanwhile, our degradation ratios in TCs were close to those of Kitts et al. [45] and Nguyen et al. [46] who recorded degradation ratios of 57 ± 3, 46.04 and 65.74 (%) for OTC, TC and CTC as a result of the deep-frying for salmon fish and meat.

The shape and thickness of the sample have a profound influence on heat penetration and distribution, and consequently on the degradation of antibiotic residues. This could clarify the variation between studies in the results of antibiotic degradation. Briefly, it was worthy to mention that the sequence of reduction for SAs and TCs for the studied treatments was grilling > frying > freezing. Also, the stability of SAs and TCs antibiotics was arranged in a descending other, shown as follows: SMT > SDM > SMX > CTC > TC > OTC. Luckily, even though OTC was the most detected antibiotic and with elevated levels, however, it was most degraded when exposed to the thermal processing.

3.4. Safety assessment of exposure to SAs and TCs

The classic methods of risk characterization were dealing with the potential toxicity of a single stressor; however, this is not corresponding to the real-life exposure scenarios. Because, daily, the human body exposes to mixtures of stressors from various sources. The combination of stressors, e.g. toxic chemicals, can mostly cause a synergistic effect to their adverse action which allows less amount of each compound to cause damage comparing to the calculated amount when examining the toxicity of each compound in isolation [47]. So that, the present study evaluated the potential risk based on the aggregate exposure to the detected concentrations of SAs and TCs in raw and cooked Tilapia fish according to Goumenou & Tsatsakis [20]. Data of Table 3 showed the calculated HQ_S values depending on the aggregate exposure to SAs and TCs through Tilapia fish and other food items where SAs and TCs might be found. These HQ_S values ranged from 0.031 (for SMT in grilled fish) to 0.391 (for OTC in raw fish). The Hazard Index (HI_S) for SAs mixture and TCs mixture in raw, fried and grilled fish were (0.200 and 0.626), (0.184 and 0.366) and (0.177 and 0.274), respectively. Additionally, HI_A values, which evaluate the adverse effect for the groups with the same target or mode of action, ranged between 0.032 (for SAs group in grilled fish) and 0.106 (for TCs group in raw fish).

Eventually, all the measured values of HQ_S, HI_S and HI_A in raw, fried and grilled fish were < 1 for all individual or grouped antibiotics indicating no risk for the Egyptian population from SAs and TCs was found through fish consumption.

4. Conclusion

The results concluded that 56.3 % of fish samples (out of examined 48 samples) were free of SAs and TCs, while the contaminated samples by SAs and TCs posed 10.4 % and 33.3 %, respectively. Only 7 samples had residual levels exceeded the permissible limits. The study revealed that storing the fish samples frozen at −18 °C for one week showed no significant effect on the stability of both SAs and TCs. The descending order for the stability of SAs and TCs was shown as SMT > SDM > SMX > CTC > TC > OTC. Additionally, the study proved that no potential risk to public health was found from the consumption of either the cooked or non-cooked fish samples with the detected concentrations of SAs and TCs. However, it is highly recommended to consume the well done cooked fish instead of raw or semi-cooked fish. The study showed that there was no enough

Table 3
Hazard characterization according to the aggregate exposure to SAs and TCs.

Antibiotics	Mean detected conc. (μg/kg)	EDI* (μg)			EXP aggregated (μg)			ADI**(μg)	HQ_S			HI_S			HI_A		
		Raw fish	Fried fish	Grilled fish	Raw fish	Fried fish	Grilled fish		Raw fish	Fried fish	Grilled fish	Raw fish	Fried fish	Grilled fish	Raw fish	Fried fish	Grilled fish
SMT	38.81	2.14	2.05	1.95	11.16	10.67	10.17	350	0.034	0.033	0.031	0.200	0.184	0.177	0.036	0.033	0.032
SMX	52.41	2.89	2.60	2.33	15.08	13.52	12.11	350	0.046	0.041	0.037						
SDM	136.43	7.54	6.95	6.82	39.25	36.18	35.52	350	0.120	0.110	0.108						
OTC	250.58	13.84	7.04	4.97	82.01	41.73	29.43	210	0.391	0.199	0.140	0.626	0.366	0.274	0.106	0.062	0.046
TC	82.79	4.57	2.64	2.48	27.10	15.63	14.68	210	0.129	0.074	0.070						
CTC	68.37	3.78	3.28	2.25	22.38	19.42	13.34	210	0.107	0.092	0.064						

* EDI: Estimated daily intake.
** ADI: Acceptable daily intake for a 70 kg adult person per day [20].

compliance from the Egyptian Tilapia fish producers to the international legislations for permitted concentrations of SAs and TCs antibiotics in fish. Therefore, fish producers should take into their consideration the antibiotics withdrawal period before fish marketing. Also, it is highly recommended to enhance the immunity of fish by reducing the stress originated from: over-fertilizing the water greatly increases the amount of algae, which consequently reduces the amount of oxygen in water; over-feeding which leads to the choking and pollution; and the high density of fish in ponds.

Author statement

The authors certify that they have sufficiently participated in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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References

[1] FAO, The State of World Fisheries and Aquaculture. Rome: 2018, (2018) http://www.fao.org/3/i9540en/i9540en.pdf.
[2] Global Agriculture Information Network (GAIN Report, 2016), The State and Development of Aquaculture in Egypt 11 USDA Foreign Agriculture Service, Cairo, June 2016.
[3] Egyptian Ministry of Agriculture and Land Reclamation (MALR, 2016), Statistics of Fish Production, Insects and Food –manufacturing. Egyptian Ministry of Agriculture and Land Reclamation, Economic Affairs Sector, December, (2016).
[4] H.A. Saleh, N.M. Sabry, M. Abd Al-Razik, F.A.S. Mohamed, M.S. Ibrahim, Pathogenicity and characterization of Streptococcosis in Egyptian Nile Tilapia (*Oreochromis niloticus*) in Kafr Elshikh governorate, Alex. J. Vet. Sci. 52 (1) (2017) 173–180, https://doi.org/10.5455/ajvs.243929.
[5] F. Canada-Canada, A.M. De La Pena, A. Espinosa-Mansilla, Analysis of antibiotics in fish samples, Anal. Bioanal. Chem. 395 (4) (2009) 987–1008, https://doi.org/10.1007/s00216-009-2872-z.
[6] S.M. Aly, A. Albutti, Antimicrobials use in aquaculture and their public health impact, J. Aquac. Res. Dev. 5 (4) (2014) 1–6, https://doi.org/10.4172/2155-9546.1000247.
[7] M.B. Ahmed, Y.H. Abo-Sree, S.M. Abdel-Fattah, N.S. Hassan, M.M. Saad, Determination of tylosin, spiramycin, and erythromycin residues in Egyptian buffaloes' meat by thin-layer chromatography-bioautography, JPC - J. Plan. Chromatogr. - Modern TLC 26 (5) (2013) 409–416, https://doi.org/10.1556/JPC.26.2013.5.4.
[8] M.B.M. Ahmed (Ed.), Antibiotic Residues in Foods of Animal Origin, LAP Lambert Academic Publishing, 2016(2016-06-21). ISBN-13: 978-3-659-90331-1. ISBN-10: 3659903310. https://www.lap-publishing.com/catalog/details//store/gb/book/978-3-659-90331-1/antibiotic-residues-in-foods-of-animal-origin.
[9] World Health Organization in Collaboration with the Food and Agriculture Organization of the United Nations and the World Organization for Animal Health, Report of a Joint FAO/OIE/WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance, Seoul, Republic of Korea, 2006 13–16 June https://apps.who.int/iris/handle/10665/133869.
[10] M.M. Saad, M.B.M. Ahmed, Necessary usage of antibiotics in animals, in: S. Savic (Ed.), Antibiotic Use in Animals, IntechOpen, Janeza Trdine 9, 51000 Rijeka, Croatia, 2018, pp. 9–23, , https://doi.org/10.5772/intechopen.71257 [Accessed: July 5, 2019].
[11] W.S. Darwish, E.A. Eldaly, M.T. El-Abbasy, Y. Ikenaka, S. Nakayama, M. Ishizuka, Antibiotic residues in food: the African scenario, Jpn. J. Vet. Res. 61 (Supplement) (2013) S13–S22 doi: http://hdl.handle.net/2115/5235.
[12] S.H. Monteiro, F. Garcia, K.S. Gozi, D.M. Romera, J.G. Francisco, G.C. Moura-Andrade, V.L. Tornisielo, Relationship between antibiotic residues and occurrence of resistant bacteria in Nile tilapia (*Oreochromis niloticus*) cultured in cage-farm, J. Environ. Health Sci., Part B 51 (12) (2016) 817–823, https://doi.org/10.1080/03601234.2016.1208457.
[13] F. Eissa, K. Ghanem, M. Al-Sisi, Occurrence and human health risks of pesticides and antibiotics in Nile tilapia along the Rosetta Nile branch, Egypt. Toxicol. Rep. (2020), https://doi.org/10.1016/j.toxrep.2020.03.004 [Article in press].
[14] S. Pleasance, P. Blay, M.A. Quilliam, G. O'hara, Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, J. Chromatogr. A 558 (1) (1991) 155–173, https://doi.org/10.1016/0021-9673(91)80121-V.
[15] E.N. Evaggelopoulou, V.F. Samanidou, Confirmatory development and validation of HPLC-DAD method for the determination of tetracyclines in gilthead seabream (S parus aurata) muscle tissue, J. Sep. Sci. 35 (10–11) (2012) 1372–1378, https://doi.org/10.1002/jssc.201100996.
[16] A.E. Mostafa, R.A.A. Salam, G.M. Hadad, I.A. Eissa, Simultaneous determination of selected veterinary antibiotics in Nile tilapia (*Orechromis niloticus*) and water samples by HPLC/UV and LC-MS/MS, RSC Adv. 7 (73) (2017) 46171–46182, https://doi.org/10.1039/c7ra08398j.
[17] Commission decision 2002/657/EC, Off. J. Eur. Union L221 (2002) 8. Available from: https://op.europa.eu/en/publication-detail/-/publication/ed928116-a955-4a84-b10a-cf7a82bad858/language-en.
[18] A. Wally, The State and Development of Aquaculture in Egypt (11/6), Global agricultural information Network report, 2016, https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=The%20State%20and%20Development%20of%20Aquaculture%20in%20Egypt%20_Cairo_Egypt_11-6-2016.pdf.
[19] Commission Regulation (EEC) No. 37/2010. on pharmacologically active substances and their classification regarding maximum residue limits in foodstuff ;s of animal origin, Off ;. J. Eur. Union 15 (2010) Available from: https://ec.europa.eu > eudralex > vol-5 > reg_2010_37 > reg_2010_37_en.
[20] Joint FAO/WHO Expert Committee on Food Additives, Residue Evaluation of Certain Veterinary Drugs, Meeting 2010-Evaluation of Data on Ractopamine Residues in Pig Tissues, FAO JECFA Monographs; Food and Agriculture Organization of the United Nations, Rome, 2010www.fao.org/docrep/012/i1618e/i1618e00.pdf.
[21] M. Goumenou, A. Tsatsakis, Proposing new approaches for the risk characterisation of single chemicals and chemical mixtures: the source related Hazard Quotient (HQ_S) and Hazard Index (HI_S) and the adversity specific Hazard Index (HI_A), Toxicol. Rep. 6 (2019) 632–636, https://doi.org/10.1016/j.toxrep.2019.06.010.
[22] N. Furusawa, Simplified determining procedure for routine residue monitoring of sulphamethazine and sulphadimethoxine in milk, J. Chromatogr. A 898 (2000) 185–191, https://doi.org/10.1016/s0021-9673(00)00843-8.
[23] H. Yu, H. Mu, Y.M. Hu, Determination of fluoroquinolones, sulfonamides, and tetracyclines multiresidues simultaneously in porcine tissue by MSPD and HPLC–DAD, J. Pharm. Anal. 2 (1) (2012) 76–81, https://doi.org/10.1016/j.jpha.2011.09.007.
[24] H.H. Chung, J.B. Lee, Y.H. Chung, K.G. Lee, Analysis of sulfonamide and quinolone antibiotic residues in Korean milk using microbial assays and high performance liquid chromatography, Food Chem. 113 (1) (2009) 297–301, https://doi.org/10.1016/j.foodchem.2008.07.021.
[25] K.S. Nunes, J.H. Vallim, M.R. Assalin, S.C. Queiroz, L.C. Paraíba, C.M. Jonsson, F.G. Reyes, Depletion study, withdrawal period calculation and bioaccumulation of sulfamethazine in tilapia (Oreochromis niloticus) treated with medicated feed, Chemosphere 197 (2018) 89–95, https://doi.org/10.1016/j.chemosphere.2018.01.030.
[26] S.H. Abou-Raya, A.R. Shalaby, N.A. Salama, W.H. Emam, F.M. Mehaya, Effect of ordinary cooking procedures on tetracycline residues in chicken meat, J. Food Drug Anal. 21 (1) (2013) 80–86, https://doi.org/10.6227/jfda.2013210110.
[27] Á. Tölgyesi, L. Tölgyesi, K. Békési, V.K. Sharma, J. Fekete, Determination of tetracyclines in pig and other meat samples using liquid chromatography coupled with diode array and tandem mass spectrometric detectors, Meat Sci. 96 (3) (2014) 1332–1339, https://doi.org/10.1016/j.meatsci.2013.11.011.
[28] A.R. Shalaby, N.A. Salama, S.H. Abou-Raya, W.H. Emam, F.M. Mehaya, Validation of HPLC method for determination of tetracycline residues in chicken meat and liver, Food Chem. 124 (4) (2011) 1660–1666, https://doi.org/10.1016/j.foodchem.2010.07.048.
[29] V. Nguyen, V. Nguyen, C. Li, G. Zhou, The degradation of oxytetracycline during thermal treatments of chicken and pig meat and the toxic effects of degradation products of oxytetracycline on rats, J. Food Sci. Technol. 52 (5) (2015) 2842–2850, https://doi.org/10.1007/s13197-014-1306-x.
[30] M.R. Rezk, M.R. Safa'a, F.I. Khattab, H.M. Marzouk, Multi-residues determination of antimicrobials in fish tissues by HPLC–ESI-MS/MS method, J. Chromatogr. B 978 (2015) 103–110, https://doi.org/10.1016/j.jchromb.2014.12.002.
[31] L.R. Guidi, F.A. Santos, A.C.S. Ribeiro, C. Fernandes, L.H. Silva, M.B.A. Gloria, A simple, fast and sensitive screening LC-ESI-MS/MS method for antibiotics in fish, Talanta 163 (2017) 85–93, https://doi.org/10.1016/j.talanta.2016.10.089.
[32] S.H. Monteiro, J.G. Francisco, T.F. Campion, R.F. Pimpinato, G.M. Andrade, F. Garcia, V.L. Tornisielo, Multiresidue antimicrobial determination in Nile tilapia (*Oreochromis niloticus*) cage farming by liquid chromatography tandem mass spectrometry, Aquaculture 447 (2015) 37–43, https://doi.org/10.1016/j.aquaculture.2015.07.002.
[33] C. Cháfer-Pericás, Á. Maquieira, R. Puchades, J. Miralles, A. Moreno, N. Pastor-Navarro, F. Espinós, Immunochemical determination of oxytetracycline in fish: comparison between enzymatic and time-resolved fluorometric assays, Anal. Chim. Acta 662 (2) (2010) 177–185, https://doi.org/10.1016/j.aca.2009.12.044.
[34] C. Cháfer-Pericás, Á. Maquieira, R. Puchades, J. Miralles, A. Moreno, Multiresidue determination of antibiotics in feed and fish samples for food safety evaluation. Comparison of immunoassay vs LC-MS-MS, Food Control 22 (6) (2011) 993–999, https://doi.org/10.1016/j.foodcont.2010.12.008.
[35] A.K.A. Barman, M.M. Hossain, M.M. Rahim, M.T. Hassan, M. Begum, Oxytetracycline residue in Tilapia, Banglad. J. Sci. Ind. Res. 53 (1) (2018) 41–46, https://doi.org/10.3329/bjsir.v53i1.35909.

[36] L.R. Guidi, F.A. Santos, A.C.S. Ribeiro, C. Fernandes, L.H. Silva, M.B.A. Gloria, Quinolones and tetracyclines in aquaculture fish by a simple and rapid LC-MS/MS method, Food Chem. 245 (2018) 1232–1238, https://doi.org/10.1016/j.foodchem.2017.11.094.

[37] E.P. Papapanagiotou, D.J. Fletouris, E.I. Psomas, Effect of various heat treatments and cold storage on sulphamethazine residues stability in incurred piglet muscle and cow milk samples, Anal. Chim. Acta 529 (1–2) (2005) 305–309, https://doi.org/10.1016/j.aca.2004.09.029.

[38] M. Gratacós-Cubarsí, A. Fernandez-García, P. Picouet, A. Valero-Pamplona, J.A. García-Regueiro, M. Castellari, Formation of tetracycline degradation products in chicken and pig meat under different thermal processing conditions, J. Agric. Food Chem. 55 (11) (2007) 4610–4616, https://doi.org/10.1021/jf070115n.

[39] B.C. Liman, M. Kanbur, G. Eraslan, E. Baydan, D.İ.N.Ç. Erdal, M. KARABACAK, Effects of various freezing and cooking processes on the residues of sulfamethazine in broiler tissues, Ankara Üniv Vet Fak Derg 62 (1) (2015) 13–16, https://doi.org/10.9775/kvfd.2013.10220.

[40] E.E. Vivienne, O.K.O. Josephine, N.J. Anaelom, Effect of temperature (cooking and freezing) on the concentration of oxytetracycline residue in experimentally induced birds, Vet. World 11 (2) (2018) 167–171, https://doi.org/10.14202/vetworld.2018.167-171.

[41] H.M. Fahim, F. Shaltout, M. El shatter, Evaluate antibiotic residues in beef and effect of cooking and freezing on it, Benha Vet Med J 36 (2) (2019) 109–116, https://doi.org/10.21608/bvmj.2019.13927.1029.

[42] N. Furusawa, R. Hanabusa, Cooking effects on sulfonamide residues in chicken thigh muscle, Food Res. Int. 35 (1) (2002) 37–42.

[43] M.R. Ismail-Fitry, S. Jinap, B. Jamilah, A.A. Saleha, Effect of different time and temperature of various cooking methods on sulfonamide residues in chicken balls, Survival and Sustainability, Springer, Berlin, Heidelberg, 2010, pp. 607–613, https://doi.org/10.1007/978-3-540-95991-5_55.

[44] D. Xu, J.M. Grizzle, W.A. Rogers, C.R. Santerre, Effect of cooking on residues of ormetoprim and sulfadimethoxine in the muscle of channel catfish, Food Res. Int. 29 (3–4) (1996) 339–344, https://doi.org/10.1016/0963-9969(96)00037-3.

[45] D.D. Kitts, C.W. Yu, R.G. Aoyama, H.M. Burt, K.M. McErlane, Oxytetracycline degradation in thermally processed farmed salmon, J. Agric. Food Chem. 40 (10) (1992) 1977–1981, https://doi.org/10.1021/jf00022a049.

[46] V. Nguyen, M. Li, M.A. Khan, C. Li, G. Zhou, Effect of cooking methods on tetracycline residues in pig meat, Afr. J. Pharm. Pharmacol. 7 (22) (2013) 1448–1454, https://doi.org/10.5897/AJPP12.454.

[47] R.N. Kostoff, M. Goumenou, A. Tsatsakis, The role of toxic stimuli combinations in determining safe exposure limits, Toxicol. Rep. 5 (2018) 1169–1172, https://doi.org/10.1016/j.toxrep.2018.10.010.