

Article

Effect of Various Thermal Treatments on Erythromycin Residues and Degradation Products in Turbot Fish Meat: Implications for Food Safety

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Abstract

Erythromycin, a widely utilized antibiotic in aquaculture, poses potential food safety risks through residues in fish products. However, research on the effects of thermal processing on its degradation remains limited. This study developed a sensitive detection method using UHPLC-Q-Exactive HF-X to quantify erythromycin and its degradation products, investigating influences of various thermal treatments and predicting additional degradants. Turbot meat samples spiked with erythromycin were processed via roasting, microwaving, deep frying, and boiling. Post-treatment degradants were identified, with potential metabolites forecasted through structural analysis. The results indicated that erythromycin rapidly degrades under all thermal treatments, with boiling and roasting promoting substantial formation of dehydration products (anhydroerythromycin A and erythromycin A enol ether). The content of N-demethylerythromycin A remained consistently low and stable (<6.67 ng/g). Additionally, five additional erythromycin degradants were screened out. The degradation pathways and product profiles of erythromycin varied depending on the thermal treatment, indicating that thermal processing does not eliminate erythromycin residues but rather transforms them into other substances. This study revealed the specific degradation pattern of erythromycin during thermal processing of fish meat, providing scientific evidence for identifying which harmful substances require priority monitoring in processed fish products, thereby enhancing the level of food safety assessment.

Keywords: thermal treatments; erythromycin; erythromycin degradation products; turbot; LC-MS



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1. Introduction

Aquaculture is a vital source of protein for humanity and plays a crucial role in global food production. It supplies over 50% of the world's seafood. With the growing demand for high-quality marine protein, aquaculture has experienced rapid expansion [1,2]. However, while its high-density and intensive farming model increases production, it may also heighten the risk of disease outbreaks and exert certain pressures on the farming environment [3–5]. Thus, antibiotics remain essential in aquaculture, given their efficacy in curbing bacterial infections and restraining excessive algal proliferation [6,7].

The regulatory frameworks governing antibiotic use in aquaculture vary across different regions globally. In the European Union, Regulation (EU) 2019/6 [8] stipulate that antibiotics are restricted to veterinary prescription for therapeutic purposes, prohibiting

their use for prevention or growth promotion [9,10]. The U.S. Food and Drug Administration regulate them as veterinary drugs, requiring strict adherence to label concentrations and enforcing withdrawal periods to minimize residues [11]. In China, the Ministry of Agriculture and Rural Affairs sets maximum residue limits and prohibits certain antibiotics like chloramphenicol for use in food-producing animals [12,13].

Antibiotics in aquaculture are primarily used to treat bacterial infections, but prophylactic or transfective administration (typically as feed additives in high-density farming systems to prevent disease) remains common despite increasingly stringent restrictions on such practices [4]. Administration methods include oral dosing (via medicated feed), bath treatments (for group therapy), or injections (for individual fish) [14,15]. Since 2022, regions including the European Union and Norway have banned the prophylactic use of antibiotics. Norway has achieved near-zero antibiotic use through vaccination [16,17].

Erythromycin is a macrolide antibiotic containing 14 carbon atoms, composed of 10 asymmetric centers, L-clarithromycin, and D-deoxyribose. Its antibacterial spectrum is slightly broader than that of penicillin [18,19]. Owing to its efficacy against most Gram-positive bacteria, certain atypical pathogens, and algal photosynthetic proteins, it has been widely adopted in aquaculture [20,21]. Despite its significant benefits, the issue of erythromycin residues in aquatic products has raised major concerns about food safety. These residues can accumulate and propagate along the food web, posing potential health risks to consumers, including allergic reactions and gut microbiota dysbiosis [6,22]. Furthermore, antibiotic residues exacerbate the health threat posed by antimicrobial resistance, thereby increasing the difficulty of treating bacterial infections in both humans and animals [23,24].

Currently, there is increased awareness of erythromycin residues in aquatic products, but less attention to thermally treated aquatic products [25]. Common thermal treatments for aquatic products include boiling, deep frying, microwaving, and roasting, all of which involve the application of heat [26]. Erythromycin is sensitive to acid, light, and heat. Under high temperature, it readily degrades into other toxic products, such as dehydrated erythromycin A and erythromycin A enol ether. Importantly, these degradation products may retain biological activity or toxicity and are considered a primary cause of gastrointestinal adverse reactions in humans [27]. Therefore, detecting the levels of erythromycin in thermally treated aquatic foods is crucial. Some studies have been conducted on detecting erythromycin in food. Duan et al. [28] established a method for determining erythromycin in milk. Wang et al. [29] identified erythromycin in chicken liver microsomes using LC-MS. Zhao et al. [30] determined the levels of erythromycin in honey. Other studies have focused on the residual effects of antibiotics during thermal processing of food. Salaramoli et al. investigated the effects of microwave and boiling treatments on telithromycin residues in chicken meatballs [31], while Zorraquino et al. reported the impact of heat treatment on the antimicrobial activity of macrolide and lincosamide antibiotics in milk [32]. However, previous studies have examined fewer thermal processing methods and conducted limited investigations into the degradation pathways and potential degradation products of antibiotics during food thermal processing. Some studies have focused solely on detecting primary harmful substances while overlooking the possibility that these primary substances may produce other substances during processing. Furthermore, literature on aquatic products is scarce, with primary focus concentrated on the toxicological studies of antibiotics in aquatic products. Research on the effects of various thermal treatments on the degradation of erythromycin in aquatic products remains limited, and analyses of degradation pathways and potential toxic metabolites are insufficient.

This investigation developed an analytical approach utilizing UHPLC-Q-Exactive HF-X for quantifying erythromycin and its degradants in heat-processed fish meat. We explored how thermal treatment durations affected erythromycin and its degradation

products, while also forecasting other possible byproducts from erythromycin breakdown. Erythromycin-spiked fish samples underwent four typical thermal processes: roasting for 25 min, microwaving for 180 s, deep frying for 150 s, and boiling for 15 min. Post-treatment degradants were characterized, with further metabolites anticipated via structural examination and mass spectrometric data. This study demonstrates the effects of different thermal treatments on erythromycin and its degradation products in fish meat. The conversion rates of erythromycin degradation products varied with different thermal treatments, indicating that the undesirable by-products requiring prioritized attention in food safety practices differ depending on the thermal treatments employed. This study will provide a scientific foundation for monitoring erythromycin and its degradation products in aquatic food products.

2. Materials and Methods

2.1. Material and Chemicals

Live farmed turbot was sourced from Qianhe mart (Dalian, Liaoning, China) and commercially processed (killing and gutting) by professional staff at the mart. The processed fish were then transported to our laboratory via cold chain for subsequent experiments. Acetonitrile, methanol, isopropanol, n-hexane and formic acid were acquired from Spectrum Chemical (Gardena, CA, USA). Sodium chloride and anhydrous magnesium sulfate were purchased from Tianjin Kermel Chemical Reagent (Tianjin, China). Erythromycin, N-demethylerythromycin A, Erythromycin A enol ether, Anhydroerythromycin A and ^{13}C , d_3 -Erythromycin were obtained from Sigma-Aldrich (Madison, WI, USA) and Aladdin Reagent (Shanghai, China).

2.2. Preparation for Standard Solutions

Standard solutions were prepared as follows: 4 mg each of erythromycin, erythromycin A enol ether, anhydroerythromycin A, and N-demethylerythromycin A standards were accurately weighed, solubilized in methanol and brought to a total volume of 4 mL to yield 1.0 mg/mL standard stock solutions.

Additionally, 1 mg of the internal standard was weighed and dissolved in 1 mL of methanol to prepare a 1.0 mg/mL internal standard stock solution, which was stored frozen and protected from light in a refrigerator.

2.3. Preparation of Thermal Treatment Samples

Turbot was chosen as the model species because of its significant economic value, widespread consumption in China and Europe, common preparation into fish mince and fish balls for consumption, and frequent exposure to antibiotics in intensive aquaculture systems. The turbot meat was minced into a mince to obtain a positive control sample. Erythromycin solution was incorporated into the mince to attain a uniform concentration of 1000 ng erythromycin per gram of mince.

The positive fish meat was divided into four groups, each comprising 20 portions. Each portion was weighed at $5.0 \text{ g} \pm 0.1 \text{ g}$ and shaped into fish balls. For each thermal treatment, 20 samples were selected and divided into 5-time groups, with each group containing 4 parallel samples.

The boiling temperature was maintained at 100°C for durations of 3, 5, 7, 10, and 15 min. The roasting temperature was maintained at 200°C for durations of 5, 10, 15, 20, and 25 min. The deep-frying temperature was maintained at 175°C for durations of 30, 60, 90, 120, and 150 s and the microwaving was set at 800 W for durations of 60, 90, 120, 150, and 180 s.

This study did not analyze negative control samples (fish balls without added erythromycin) because the focus was on the degradation behavior of erythromycin. The overall experimental methodology was based on the assumption that no pre-existing contamination was present. Subsequent studies should incorporate negative controls to establish a baseline.

2.4. Sample Processing Prior to Analysis

Following thermal treatment, the samples were aliquoted into 50-mL centrifuge tubes, to which 50 μ L of ^{13}C , d_3 -Erythromycin (1 $\mu\text{g}/\text{mL}$) and 20 mL of 50% (*v/v*) acetonitrile in water were added. The mixtures were homogenized at 8000 rpm for 30 s and then sonicated for 15 min. Subsequently, 5 mL of n-hexane, 4 g of anhydrous magnesium sulfate, and 0.5 g of sodium chloride were introduced. The resulting mixtures were vortexed for 2 min and centrifuged at $3500\times g$ for 10 min at 4 $^{\circ}\text{C}$. Following centrifugation, the acetonitrile layer was collected and evaporated to complete dryness using a high-speed vacuum concentrator (Copenhagen, Labogene, Denmark). The residue was reconstituted in 1 mL of a 50% (*v/v*) acetonitrile aqueous solution. This solution was centrifuged at $20,000\times g$ for 10 min at 4 $^{\circ}\text{C}$, and the resulting supernatant was collected for LC-MS analysis. All determinations were performed in quadruplicate.

2.5. Ultra-High Performance Liquid Chromatography-Mass Spectrometry Instrument Conditions and Optimization

The content of erythromycin degradation products was determined using UHPLC-Q-Exactive HF-X Interventionary (Thermo Fisher, Germering, Germany). Separation was performed on an Acquity UPLC BEH C18 column (2.1 $\times$ 100 mm, 1.7 μm). The mobile phase A was 0.1% formic acid in water, and the mobile phase B acetonitrile solution. The separated conditions were as follows: 80% B, 0–0.1 min; 85–5% B, 0.1–4 min; 5% B, 4–8 min; 5–80% B, 8–9 min; 80% B, 9–12 min. The injection volume was 1 μL .

Mass spectrometry detection employed an electrospray ionization source in positive ion mode (ESI^+) with full-spectrum analysis and targeted monitoring scanning. Source parameters were as follows: sheath gas flow rate: 40 psi; auxiliary gas flow: 10 argon; auxiliary gas heater temperature: 320 $^{\circ}\text{C}$; purge gas flow: 2 psi; spray voltage: +4.0 kV; capillary temperature: 320 $^{\circ}\text{C}$. ESI parameters for target compounds are listed in Table 1.

Table 1. Main mass spectrometry parameters of erythromycin and its degradation products. Asterisks (*) indicate the quantifier ions selected for quantitative analysis.

Compound Name	Parent Ion (<i>m/z</i>)	Fragment Ion (<i>m/z</i>)	Collision Energy/eV
Erythromycin	734.468	158.117 * 576.372	15
Erythromycin A enol ether	716.458	158.117 * 558.363	16
Anhydroerythromycin A	716.458	158.118 * 522.342 558.364	16
N-demethylethromycin A	720.452	562.358 * 144.102	20
^{13}C , d_3 -Erythromycin	738.48	162.139 * 580.394	15

2.6. Method Validation

2.6.1. Linear Range

Mixed standard solutions at different concentrations were detected. The calibration curve was plotted with the ratio of peak areas between the analyte and internal standard on the y -axis, and standard concentrations of 0.1, 0.5, 1, 5, 10, 50, 100, 200, and 500 ng/mL on the x -axis. The correlation coefficient (R^2) for the analyte was calculated. ^{13}C , d_3 -Erythromycin was selected as the corrective internal standard.

2.6.2. Limit of Detections and Limit of Quantifications (LODs and LOQs)

The diluted and mixed standard solution was tested. The concentrations at which each standard achieved a signal-to-noise ratio of 3 and 10 were recorded, representing the instrument's LODs and LOQs for each substance, expressed in ng/mL.

2.6.3. Recovery Rate

A total of $3 \text{ g} \pm 0.1 \text{ g}$ of turbot meat was accurately weighed, then mixed with the internal standard solution and four concentration levels of standard solutions: ultra-low, low, medium, and high. The final concentrations of the standards were 50, 125, 250, and 400 ng/mL, respectively. Proceed with extraction according to step 2.4.

2.6.4. Intra-Day and Inter-Day Precision

Samples were prepared by adding 10 μL , 20 μL , or 50 μL of the spiked working solution to blank fish meat samples. The relative standard deviation (RSD) of erythromycin and its degradation products obtained from fish meat samples spiked with the standard substance, when subjected to pretreatment and assay on the same day, represents the within-day precision. The RSD values of erythromycin and its degradation products obtained from the same fish meat samples over two days represent the between-day precision.

2.7. Quantitative Analysis of Erythromycin Degradation Products Without Standard Samples

Semi-quantification of the five erythromycin degradation products lacking reference standards was conducted using an internal standard, with the calculation method as follows:

$$C_1 = \frac{A}{a} \times C_2 \quad (1)$$

where C_1 is the target substance concentration, A is the target substance peak area, a is the internal standard peak area and C_2 is the internal standard concentration.

2.8. Statistical Analysis

Quantitative data analysis employed Xcalibur software 4.3. Non-targeted detection data were analyzed using MS-DIAL 3.96. Statistical analysis was performed using SPSS 20.0. A one-way analysis of variance (ANOVA) was conducted for each time point to compare differences among the four heat treatment methods. Differences were considered statistically significant when the ANOVA results were significant ($p \leq 0.05$). Different letters in the figures indicate significant differences between groups. All data are expressed as mean $\pm$ standard deviation ($n = 4$).

3. Results and Discussion

3.1. Method Validation Results

3.1.1. Optimization of LC-MS Conditions

UHPLC-Q-Exactive Orbitrap MS offers advantages in detecting relatively low-abundance fragment ions and serves as a powerful tool for analyzing complex compound

systems, having been widely adopted for compound identification and screening [33]. The Acquity UPLC CSH-C18 column provided excellent separation of erythromycin and its degradation products (as shown in Figure 1). This method employed 0.1% formic acid aqueous solution and acetonitrile as the mobile phase. In positive ion mode, formic acid promotes compound ionization, enabling all analytes to achieve stable retention times [34].

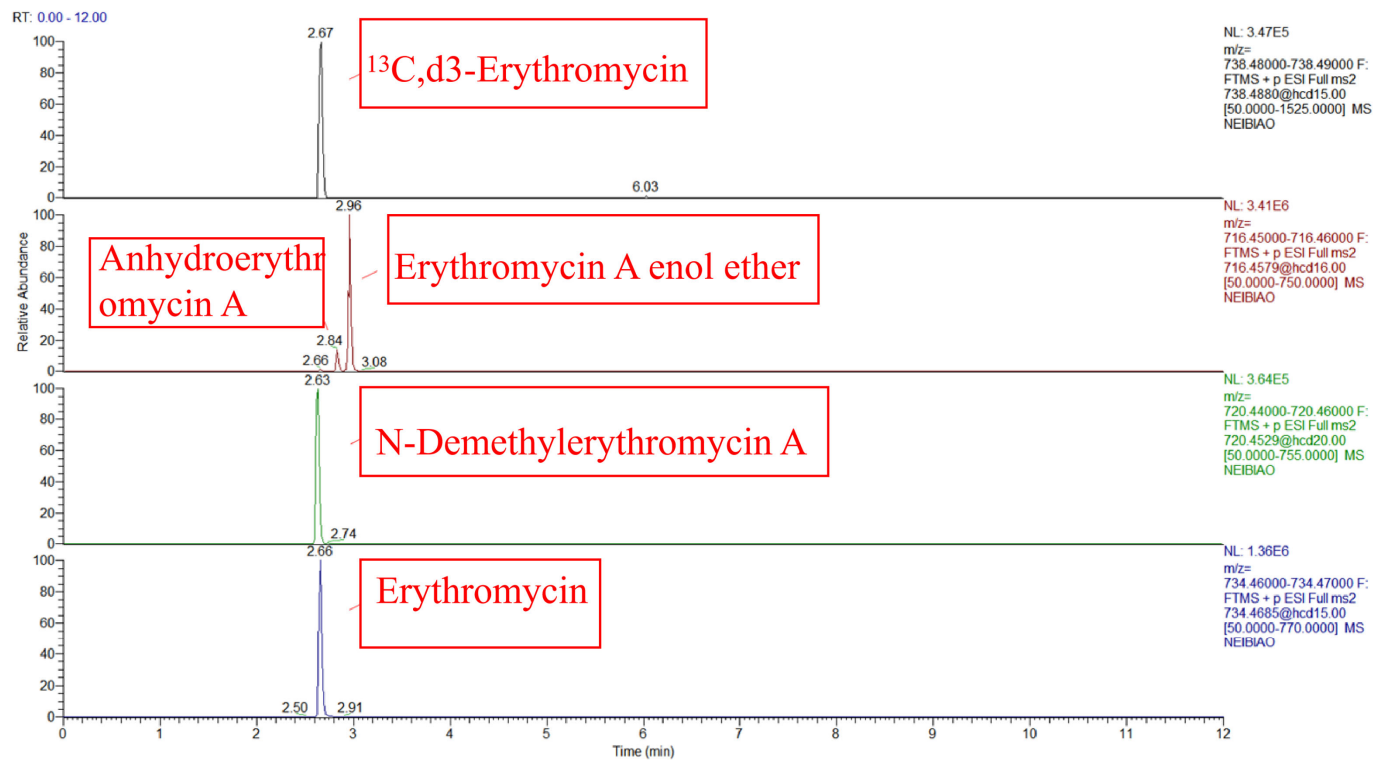


Figure 1. Chromatogram.

3.1.2. Standard Curve, LOD and LQD

The linear range, correlation coefficient (R^2), linear equation, LOD, and LOQ for the four analytes are shown in Table 2. The analyte showed good linearity across the specified concentration range ($R > 0.99$).

Table 2. LODs, LOQs, linear range, standard curve, correlation coefficient and precision results.

Results	Erythromycin A	N-Demethylerythromycin A	Anhydroerythromycin A	Erythromycin A Enol Ether
LODs (ng g ⁻¹)	3	3	3	0.5
LOQs (ng g ⁻¹)	5	5	5	1
Linear Range	1–500	1–500	1–500	0.1–500
Standard Curve	$y = 0.0187266 + 0.00988151x$	$y = 0.0187266 + 0.00988151x$	$y = 0.0187266 + 0.00988151x$	$y = 0.0187266 + 0.00988151x$
Correlation Coefficient (R^2)	0.9985	0.9929	0.9918	0.9978
Precision Results				
Intra-day	9.4	9.0	7.2	5.7
Inter-day	6.7	8.4	7.8	9.4

The LOD and LOQ values showed little variation across the different analytes. The LOD and LOQ for erythromycin A, dehydrated erythromycin A, and demethylated erythromycin A ranged between 3 ng/g and 5 ng/g, consistent with previous research findings [35]. The LOD and LOQ for erythromycin A enol ether were 0.5 ng/g and 1 ng/g, respectively, with RSDs ranging from 5.7% to 9.4%. Intra-day and inter-day precision fell within acceptable ranges. Therefore, this method is suitable for the trace detection of erythromycin A and its degradation products.

3.1.3. Recovery Rate and Precision

Recovery evaluation was conducted at four levels (ultra-low, low, medium, and high). The recovery rates for Erythromycin A at ultra-low and low levels were 72.21% and 79.37%, respectively, while the recovery rate for Demethyl-Erythromycin A at the medium level was 78.54%. Under the remaining conditions, the recovery rates of the analytes ranged from 99% to 130%, with $RSD \leq 16.48\%$ (Table 3). We observed that recovery rates significantly exceeded 120% only when the standard concentration was at ultra-low and low levels, which may be related to the matrix effect of fish meat. Both the accuracy and precision of the method met the requirements for residue analysis testing [36].

Table 3. Recovery of target analytes in turbot.

Results		Erythromycin A	N-Demethylerythromycin A	Anhydroerythromycin A	Erythromycin A Enol Ether
Ultra-Low	Recovery Rate (%)	72.21	123.68	126.66	115.14
	Relative Standard Deviation (RSD%)	0.80	13.07	6.08	0.95
Low	Recovery Rate (%)	79.37	104.97	127.32	117.69
	Relative Standard Deviation (RSD%)	7.14	13.99	1.97	3.25
Medium	Recovery Rate (%)	120.67	78.54	120.22	118.60
	Relative Standard Deviation (RSD%)	1.90	4.17	16.48	9.75
High	Recovery Rate (%)	101.71	117.94	99.27	111.46
	Relative Standard Deviation (RSD%)	8.50	11.28	12.05	8.74

3.2. Effects of Thermal Treatment Time on Erythromycin and Its Major Degradation Products

3.2.1. Effect of Boiling Time on Drug Residue Levels in Turbot

As shown in Figure 2A, erythromycin content decreased rapidly during 0–3 min, while erythromycin A enol ether and anhydroerythromycin A content increased significantly to 105.27 ng/g and 119.50 ng/g, respectively. From 3 to 7 min, changes in all compound concentrations were minimal. During boiling from 7 to 15 min, erythromycin exhibited a pronounced decline, reaching a final concentration of 318.94 ng/g, while anhydroerythromycin A and erythromycin A enol ether increased to 127.02 ng/g and 152.72 ng/g, respectively. N-demethylerythromycin A showed minimal variation throughout the heating process, with concentrations consistently below 6.32 ng/g. Cai et al. [37] observed that high-temperature treatment of erythromycin fermentation products reduces erythromycin content, consistent with our findings.

3.2.2. Effect of Roasting Time on Drug Residue Levels in Turbot

As shown in panels A and B of Figure 2, the changing trends of the compounds were similar following roasting and boiling treatments. As roasting time increased, erythromycin content decreased significantly. Anhydroerythromycin A and erythromycin A enol ether showed the greatest increase in content between 15 and 25 min of roasting. At 25 min, anhydroerythromycin A content reached 2.6 times the initial level, while erythromycin A enol ether content attained a maximum of 203.74 ng/g. N-demethylerythromycin A sharply increased from 0 to 5 min, peaking at 11.15 ng/g. Roasting inherently involves water evaporation, concomitant with the reduction in erythromycin concentration, the levels of its two dehydration products (erythromycin A enol ether and anhydroerythromycin A) steadily increased.

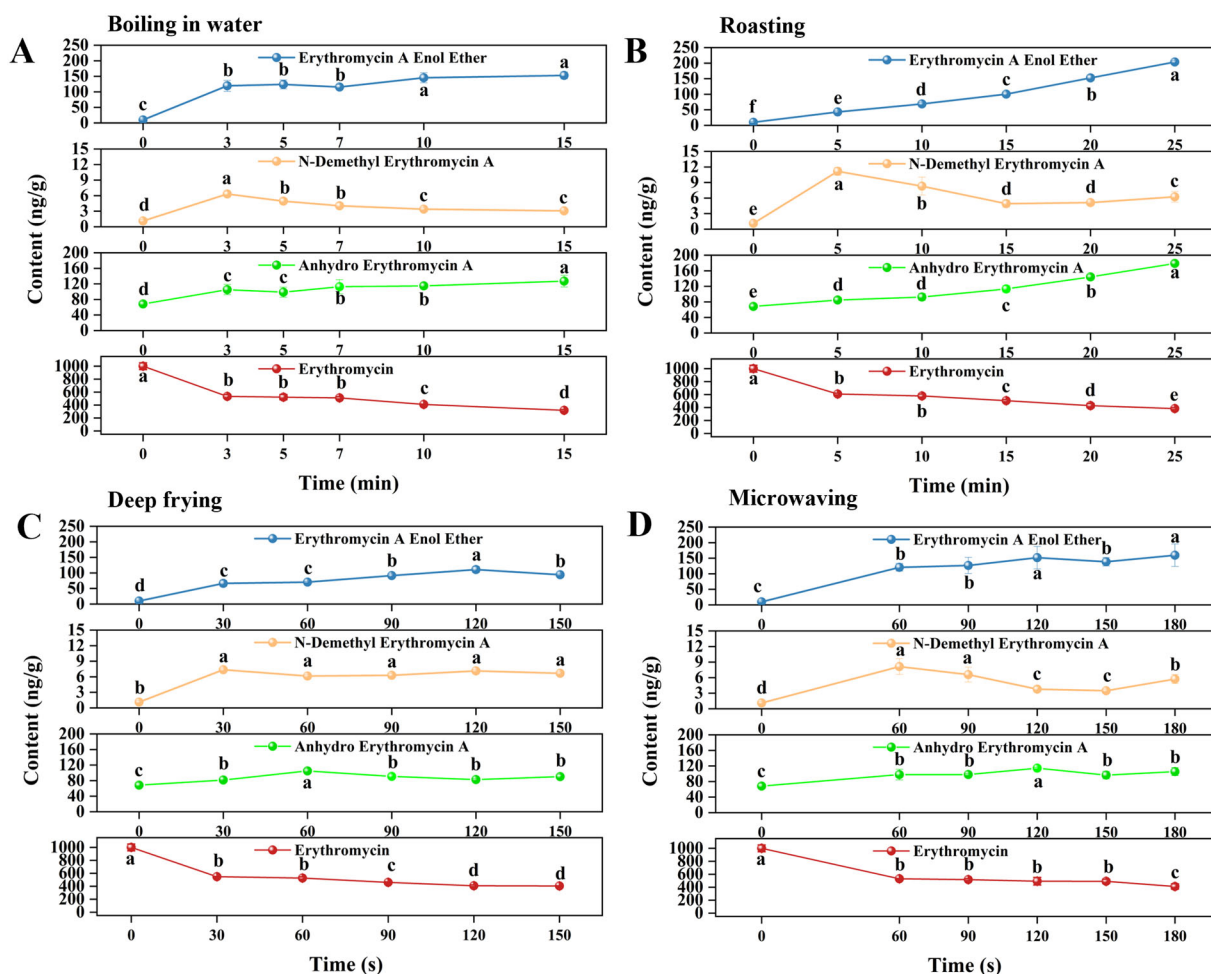


Figure 2. (A) Changes in the content of erythromycin and its primary degradation products in fish balls at different boiling times in 100 °C water. (B) Changes in the content of erythromycin and its primary degradation products in fish balls at different roasting times at 200 °C. (C) Changes in the content of erythromycin and its primary degradation products in fish balls at different deep frying times at 175 °C. (D) Changes in the content of erythromycin and its primary degradation products in fish balls at different microwave times at 850 W. (Different letters, a–f, represent significant differences at $p < 0.05$.)

3.2.3. Effect of Deep Frying Time on Drug Residue Levels in Turbot

As shown in Figure 2C, erythromycin content sharply decreased from 0 to 30 s, reaching 54.9% of the initial value, possibly attributable to the lipophilicity of it [38]. The content of erythromycin A kept balanced (30–150 s). The content of anhydroerythromycin A considerably increased steadily, reached a peak at 60 s, initially increased before declining during deep frying, peaking at 104.83 ng/g at 60 s. and finally decreased (60–120 s). Erythromycin A enol ether rapidly increased from 0 to 120 s, peaking at 110.79 ng/g. N-Demethylerythromycin A kept balanced (30–150 s), with levels maintained between 6 and 8 ng/g.

3.2.4. Effect of Microwaving Time on Drug Residue Levels in Turbot

The content of erythromycin showed a gradual decrease during the 60–180 s heating process, which was attributed to the short heating duration and absence of auxiliary medium during microwaving. Anhydroerythromycin A and erythromycin A enol ether peaked at 120 s and 180 s, achieving concentrations of 114.35 ng/g and 159.71 ng/g, respectively. N-Demethylerythromycin A reached its maximum at 60 s (Figure 2D). Microwaving is frequently employed for organic reactions and plays a significant role in studying organic

synthesis and product distribution [39]. Chen et al. [40] demonstrated that microwave heating can break the glycosidic bonds of macrolide antibiotics. Erythromycin A enol ether, anhydroerythromycin A, and N-demethylerythromycin A are dehydrated, dehydrogenated, or demethylated products of erythromycin, all containing glycosidic bonds. Prolonging heating time may decompose these compounds.

3.3. Effects of Thermal Treatment on Erythromycin and Its Major Degradation Products

As shown in Figure 3, erythromycin exhibited the most significant change compared to the control group. After roasting for 25 min, its total content decreased to 38.5% of the original level, microwaving for 180 s reduced it to 41.2% of the original level, deep frying for 150 s reduced it to 40.5%, and boiling for 15 min reduced it to 31.9%. Anhydroerythromycin A levels rose markedly after roasting, peaking at 178.83 ng/g. Boiling increased its content to twice the initial level. The content of N-demethylerythromycin A showed limited variation under four thermal treatments and remained consistently below 6.67 ng/g. This may indicate that N-demethyl erythromycin A differs from the primary dehydration products and is not a direct thermally induced degradation product. From a food safety perspective, its low abundance suggests limited toxicological relevance compared to the primary dehydration products (dehydrated erythromycin A and erythromycin A enol ether). However, its stable presence could serve as a potential biomarker. The highest levels of erythromycin A enol ether were detected after microwave heating for 180 s and roasting for 25 min, at 151.89 ng/g and 203.74 ng/g respectively. The lowest level of erythromycin A enol ether formed after deep frying for 150 s was 94.29 ng/g. These results indicated that different thermal treatments not only significantly influence the extent of erythromycin degradation but also determine the primary transformation pathways [38]. Boiling and roasting treatments promoted the formation of erythromycin dehydration products (Anhydroerythromycin A and Erythromycin A enol ether). This may be attributed to prolonged exposure of fish balls to a hot and humid environment, accelerating acid-catalyzed dehydration reactions within the fish meat matrix [41]. Frying caused rapid initial loss of erythromycin itself, but the final concentration of major degradation products was low, likely due to the lipid partitioning effect in fish meat and faster surface heating rates. Microwave processing resulted in the slowest overall degradation of erythromycin, attributed to its shorter effective heating time and lack of external medium. From a food safety perspective, these processing-dependent characteristics indicate that monitoring erythromycin alone may underestimate residue risks in boiled or roasted processed fish meat. Therefore, a monitoring strategy tailored to specific processing methods for processed fish meat is recommended [42].

3.4. Analysis of Total Recovery Rates of Erythromycin and Its Major Degradation Products Under Different Thermal Treatment Conditions

The total recovery rates of erythromycin and its degradation products during boiling treatment ranged from 60.18% to 74.32% (Figure 4A), while those for frying treatment were 59.62% to 70.82% (Figure 4C). This may be attributed to the dissolution of some target compounds by the heating medium. From the total recovery rates of degradation products observed in roasting and microwaving, even in the absence of a heating medium, the recovery rates remained low at 68.26–77.35%. This finding suggested that during thermal treatment, erythromycin not only converted to anhydroerythromycin A, erythromycin A enol ether, and N-demethylerythromycin A but also generated additional degradation products.

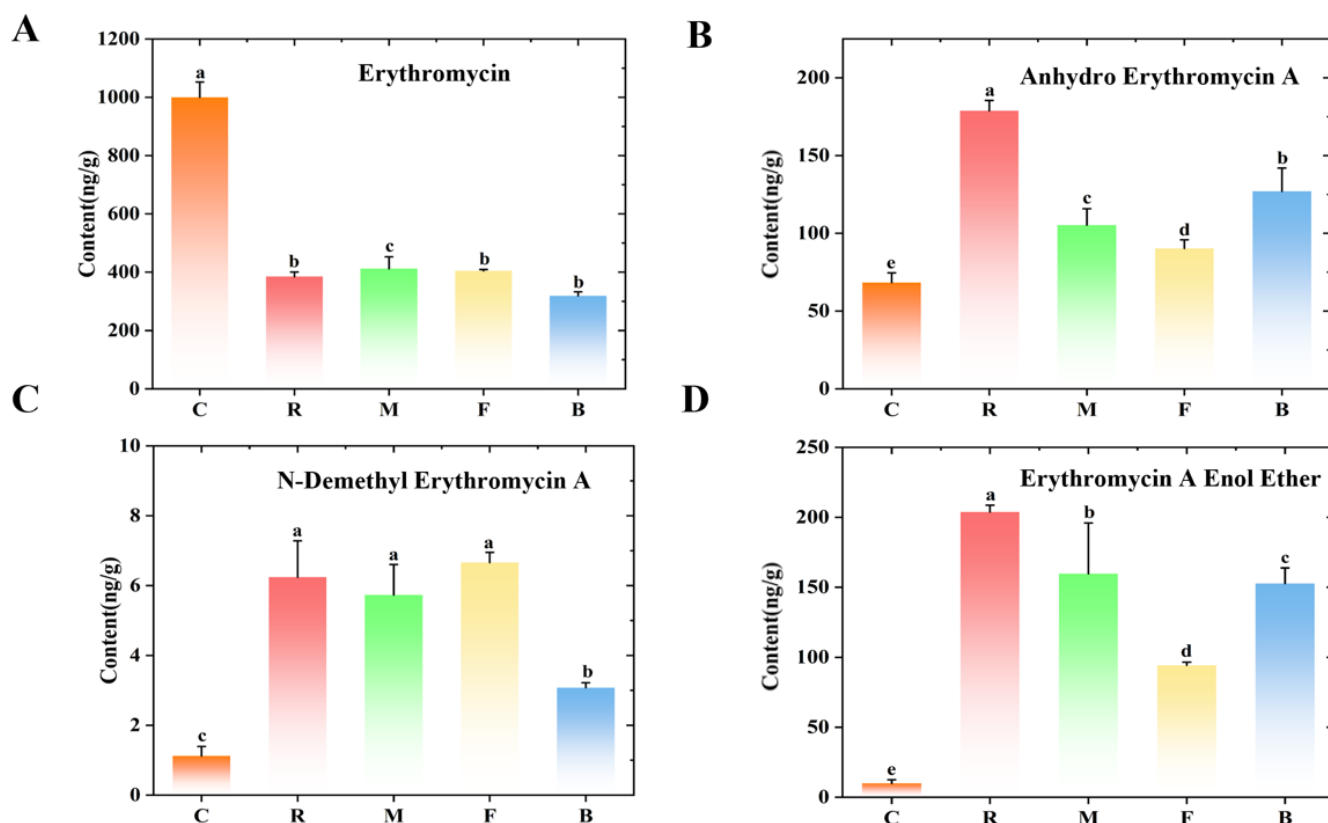


Figure 3. (A) Changes in Erythromycin content in fish balls after different thermal treatments and in unprocessed fish balls (B) Changes in Anhydroerythromycin A content in fish balls after different thermal treatments and in unprocessed fish balls (C) Changes in N-demethylerythromycin A content in fish balls after different thermal treatments and in unprocessed fish balls. (D) Changes in erythromycin A enol ether content in fish balls after different thermal treatments and in unprocessed fish balls. (C corresponds to control, R corresponds to roasting 25 min, M corresponds to microwaving 180 s, F corresponds to deep frying 150 s, B corresponds to boiling 15 min, different letters, a–e, represent significant differences at $p < 0.05$).

3.5. Analysis of Other Degradation Products of Erythromycin

3.5.1. Screening of Other Degradation Products of Erythromycin

In addition to the primary degradation products detected earlier, erythromycin undergoes further degradation during thermal processing. Potential product 1 is pseudoerythromycin A enol ether (psEAEN). Currently, predicted degradation pathways for erythromycin include acid-catalyzed degradation, dehydrogenation, demethylation, C₆ hydroxyl methylation, hydrolysis, oxidation of the methyl group at C₂, and demethoxylation of L-cladinose, among others. Possible fragmentation patterns and adduct ions of erythromycin were assessed to delineate the range of parent ions for new products, followed by verification through mass spectrometric analysis. Mass spectrometry is a standard method for studying macrolide antibiotics, matching the ion fragments of the target compound with known fragment mass numbers to identify its identity [43]. Using Xcalibur software, the precursor ion scan range was set to 716.45–716.46 m/z in the total ion chromatogram of the sample, yielding the mass spectrum of psEAEN. Verification of the parent and fragment ions revealed that the characteristic fragment ion m/z values for psEAEN—83.05, 127.07, 158.12, 558.36, and 716.45—were all identifiable in the mass spectrum (Figure 5). Examination of each sample confirmed the presence of this compound, establishing that the detection of psEAEN was not incidental. Erythromycin readily undergoes dehydration and dehydrogenation under acidic conditions to form erythromycin

A enol ether. The psEAEN arises from the cleavage of the ester bond between C₁ and C₁₄ in erythromycin A enol ether, followed by the formation of a lactone structure via the connection of the -OH groups at positions C₁ and C₁₁. This compound has been previously reported to exhibit toxicity in certain contexts [44]. However, its concentration in this study was limited, and its health implications remain unclear.

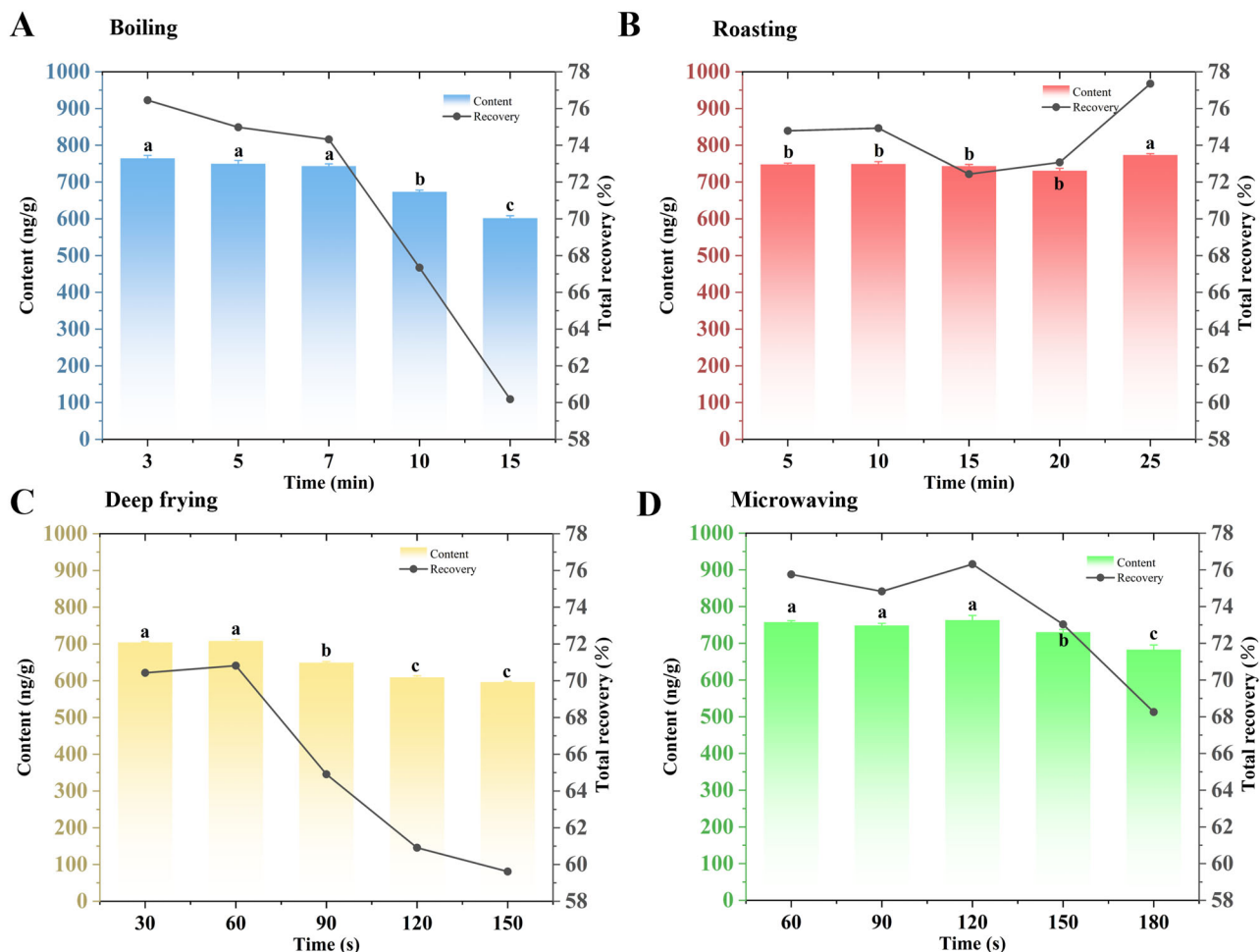


Figure 4. (A) The total recovery rate of erythromycin and its primary degradation products in fish balls during boiling. (B) The total recovery rate of erythromycin and its primary degradation products in fish balls during roasting. (C) The total recovery rate of erythromycin and its primary degradation products in fish balls during deep frying. (D) The total recovery rate of erythromycin and its primary degradation products in fish balls during microwaving. (Different letters, a–c, represent significant differences at $p < 0.05$).

Figure 6 shows the second screened erythromycin degradation product, with a mass-to-charge ratio of 738.44. This compound is predicted to be generated by the hydrolysis of erythromycin, involving the cleavage of the carbonyl double bond at C₁ and C₉ followed by H ion attachment. The characteristic fragment ion with a mass-to-charge ratio of 558.36 is derived from the parent ion after the elimination of the clathrin sugar moiety.

Product 3, as shown in Figure 7, is formed by the removal of the cladane sugar from the erythromycin parent ion, followed by the hydrolysis of the C₁ and C₉ carbonyl bonds to generate two hydroxyl groups. Its mass-to-charge ratio is 576.37. Since the weakest bond is almost always the first point of molecular fragmentation, most fragments of erythromycin result from the cleavage of the glycosidic bond.

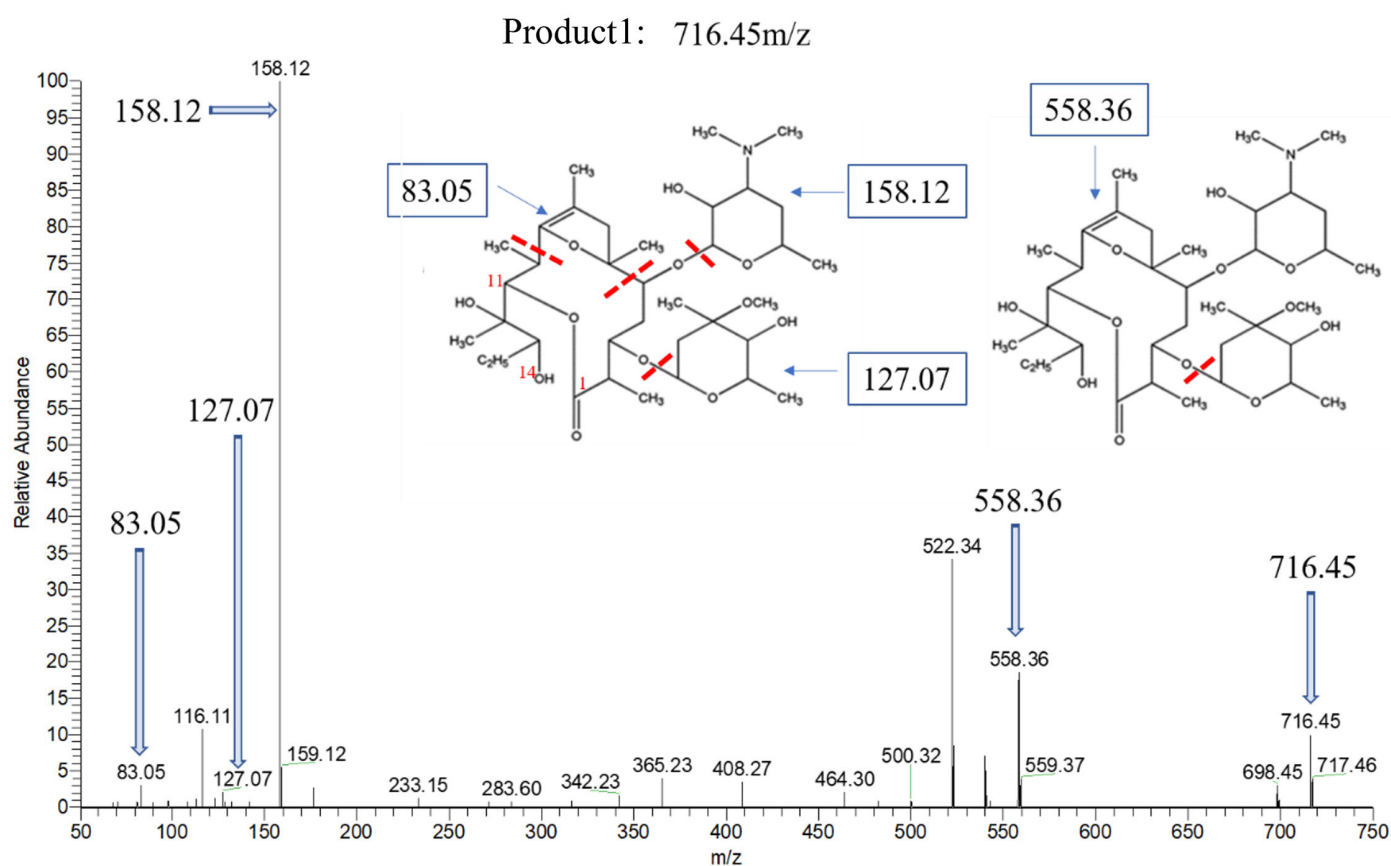


Figure 5. Mass spectrum of other degradation products of erythromycin (product 1: 716.45 m/z).

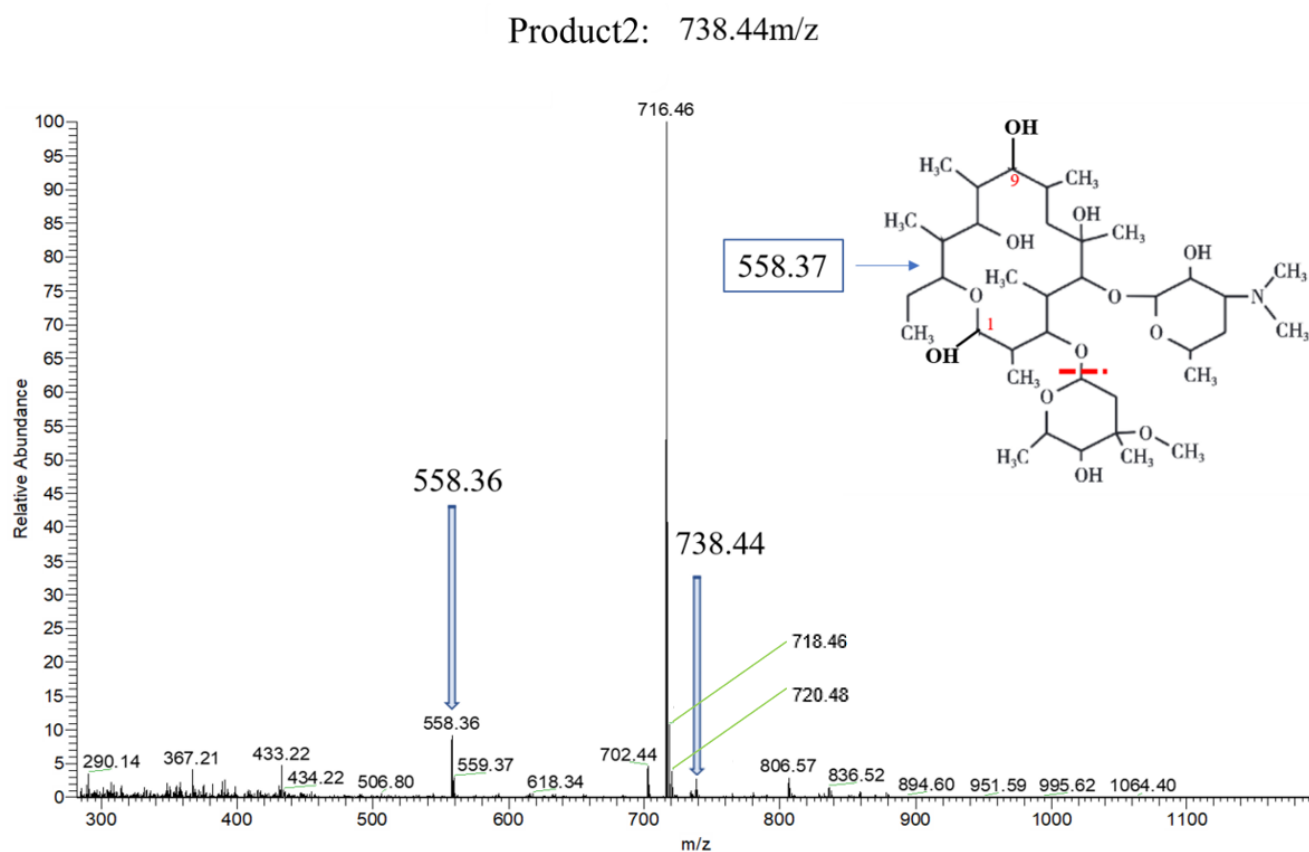


Figure 6. Mass spectrum of other degradation products of erythromycin (product 2: 738.44 m/z).

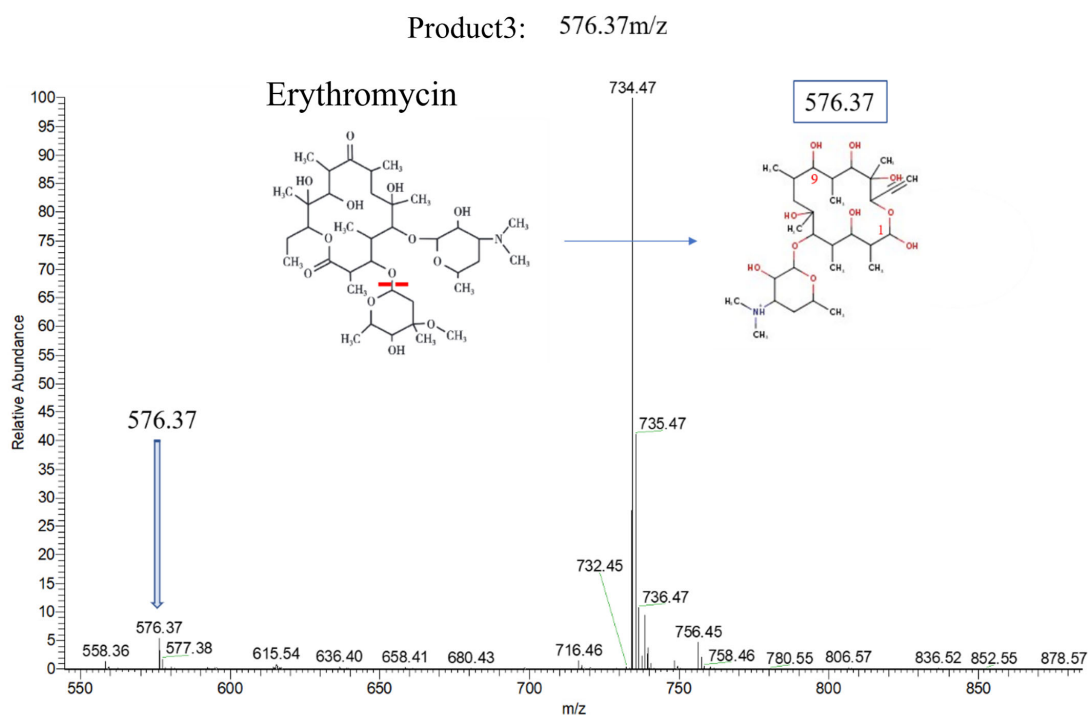


Figure 7. Mass spectrum of other degradation products of erythromycin (product 3: 576.37 m/z).

Product 4 has a parent ion at 748.48 m/z, formed by methyl substitution of the H atom on the C₆-OH position (Figure 8). The structure of this compound is analogous to that of clarithromycin, suggesting a potential similarity in their toxicological profiles. As a member of the macrolide antibiotic class, clarithromycin shares a comparable mechanism of action with erythromycin [45]. However, further research is needed to determine the toxicity of this presumed derivative at the detected concentration. Its characteristic fragment ions at 558.36 m/z and 540.35 m/z are formed by the removal of the clade sugar moiety from the parent ion, with some structural fragments also yielding methylene and hydroxyl groups.

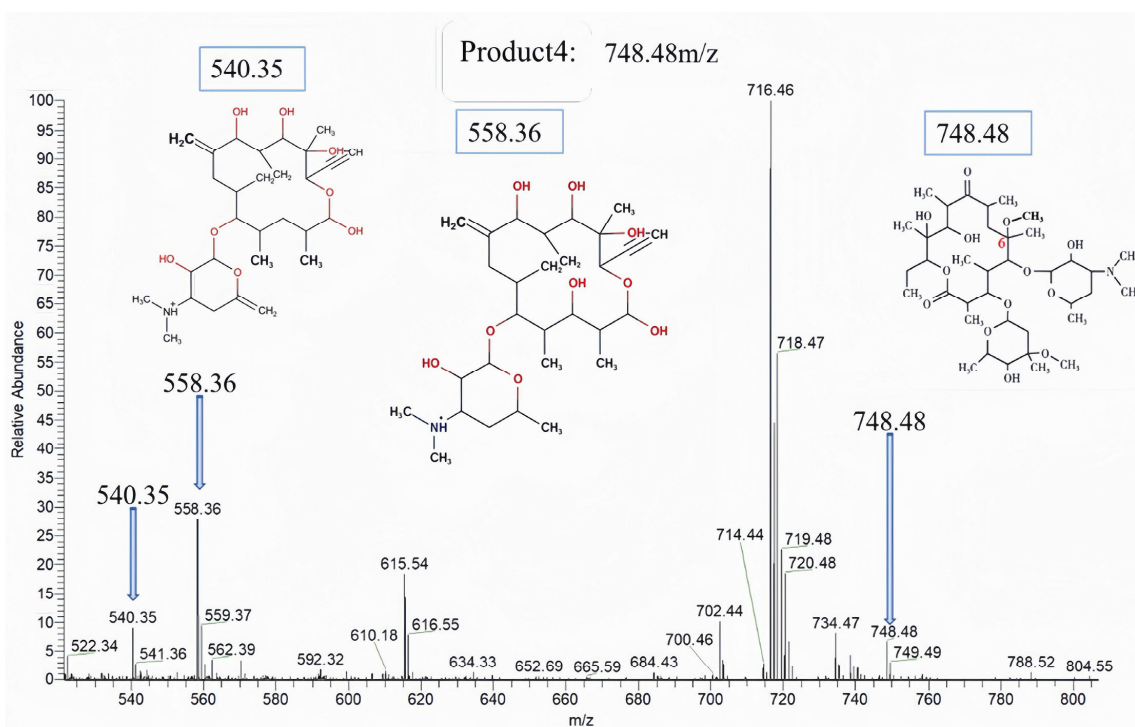


Figure 8. Mass spectrum of other degradation products of erythromycin (product 4: 748.48 m/z).

As shown in Figure 9, product 5 is formed by the elimination of cladine sugar, H_2O , and H^+ from erythromycin, with a parent ion mass-to-charge ratio of 540.35. Its characteristic fragment ion at m/z 522.34 is generated by the elimination of one molecule of H_2O from the C_5 position of the parent ion.

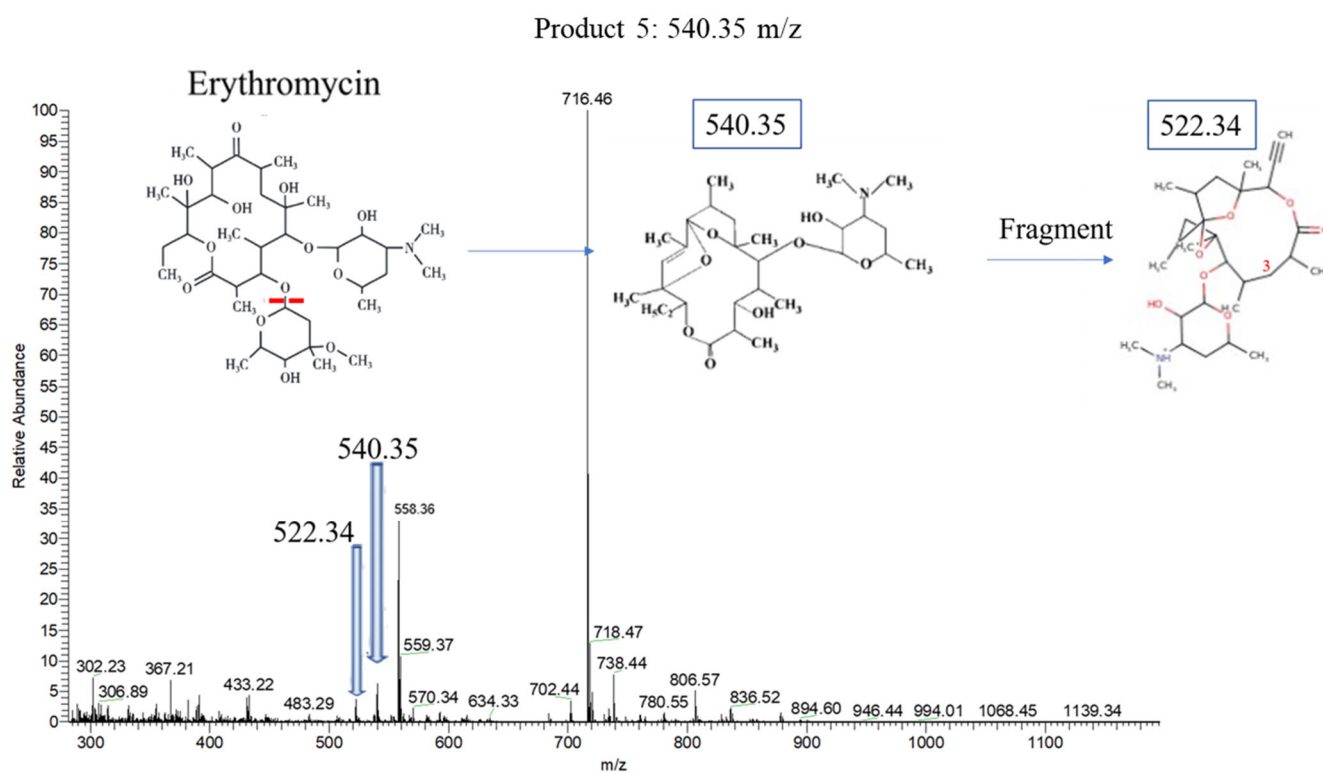


Figure 9. Mass spectrum of other degradation products of erythromycin (product 5: 540.35 m/z).

3.5.2. Analysis of the Effect of Thermal Treatment on the Content of Other Degradation Products

Product 1, psEAEN (716.45 m/z), exhibited varying residual trends under different thermal treatments. As shown in Figure 10A, deep frying caused a gradual decrease in psEAEN content, peaking at 68.98 ng/g at 30 s and declining to 51.53 ng/g by the end of frying. During boiling and roasting, psEAEN levels decreased linearly, ultimately reaching concentrations of 39.10 ng/g and 46.02 ng/g, respectively (Figure 10B,D). As shown in Figure 10C, psEAEN content consistently increased during microwave treatment from 60 to 90 s, then sharply decreased to 55.94 ng/g between 90 and 120 s. Product 3 (576.37 m/z) exhibited a similar trend to psEAEN across various thermal processing methods. The content of Product 2 (738.44 m/z) showed an increasing trend by deep frying, roasting, and boiling, with a maximum content reaching 64.96 ng/g. However, its content increased from 60 to 120 s and decreased from 120 to 180 s during microwaving. Products 4 (748.48 m/z) and 5 (540.35 m/z) showed minimal variation under various thermal treatments, with their concentrations remaining around 0.6 ng/g and 5 ng/g, respectively.

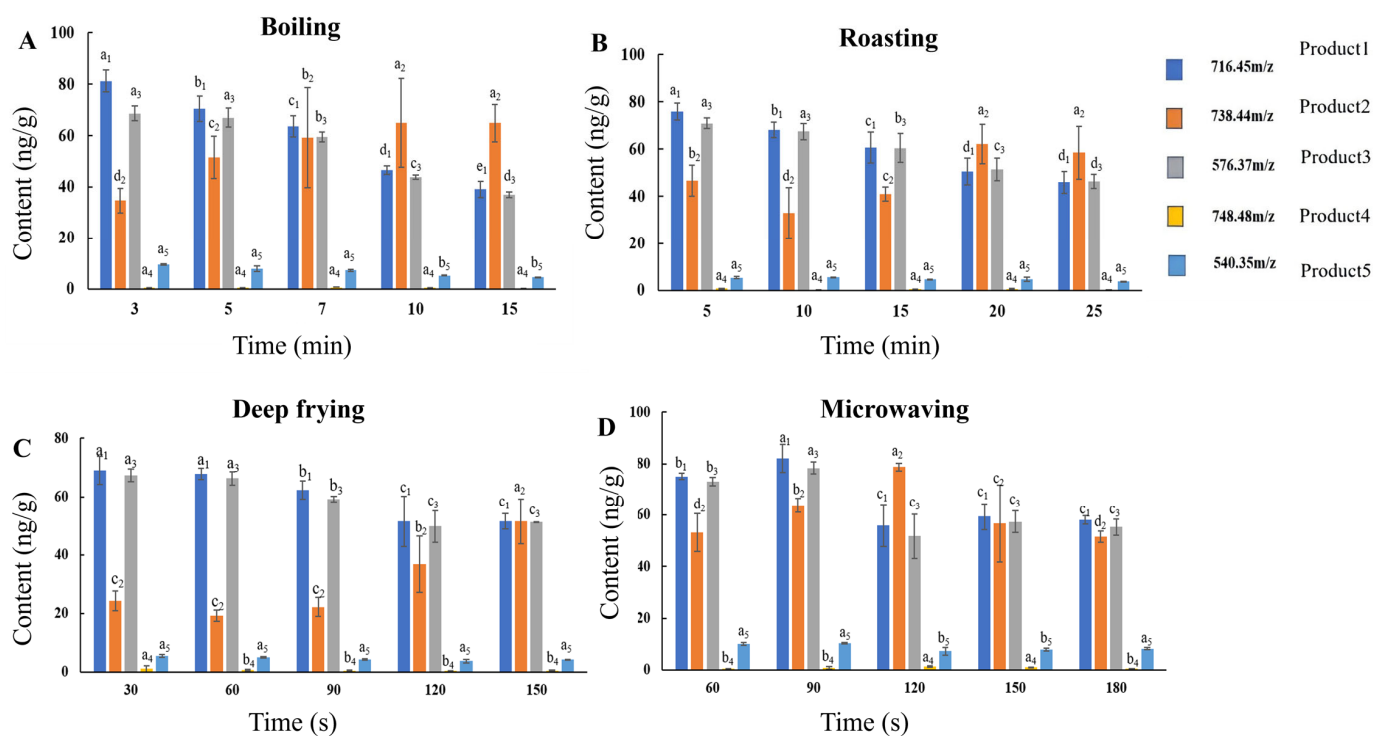


Figure 10. (A) The content of other erythromycin degradation products in fish balls during boiling. (B) The content of other erythromycin degradation products in fish balls during roasting. (C) The content of other erythromycin degradation products in fish balls during deep frying. (D) The content of other erythromycin degradation products in fish balls during microwaving. (Different letters, a–e, represent significant differences at $p < 0.05$; Different subscript numbers correspond to different product.).

3.5.3. Analysis of the Effect of Thermal Treatment on the Total Content of Erythromycin and Its Degradation Products

Analysis of the thermal treatments effects on the total content of erythromycin and its degradation products revealed that during roasting and microwaving the overall target analyte levels remained close to 1000 ng/g. This may be related to the absence of other media during heating. By roasting for 5 min, the total erythromycin content reached 947.55 ng/g, while microwaving for 90 s resulted in product levels comprising 98.3% of the initial concentration. After frying and boiling, the total levels dropped because part of the substance dissolved into the oil or water, ending with final concentrations of 754.96 ng/g and 747.48 ng/g (Figure 11). In summary, erythromycin in thermally processed foods does not disappear but remains in the food in the form of its degradation products. From a toxicological perspective, major degradation products of erythromycin such as dehydrated erythromycin A and erythromycin A enol ether may retain gastrointestinal irritation potential. Our subsequent experiments will focus on testing the residual toxicity of various harmful substances and antimicrobial resistance. From a regulatory standpoint, many countries' regulatory frameworks establish maximum residue limits (MRLs) only for the parent drug (typically 0–50 µg/kg in fish), with fewer regulations for MRLs of degradation products. This may underestimate the total residue of erythromycin in processed products. However, this mass balance is based on the parent compound plus the quantified and semi-quantified products detected by the current method. Some trace, volatile, or highly reactive degradation products may not have been captured, and due to the lack of reference standards, uncertainty exists in the semi-quantitative analysis of the other five products. In the future, we will supplement our existing research findings through other means.

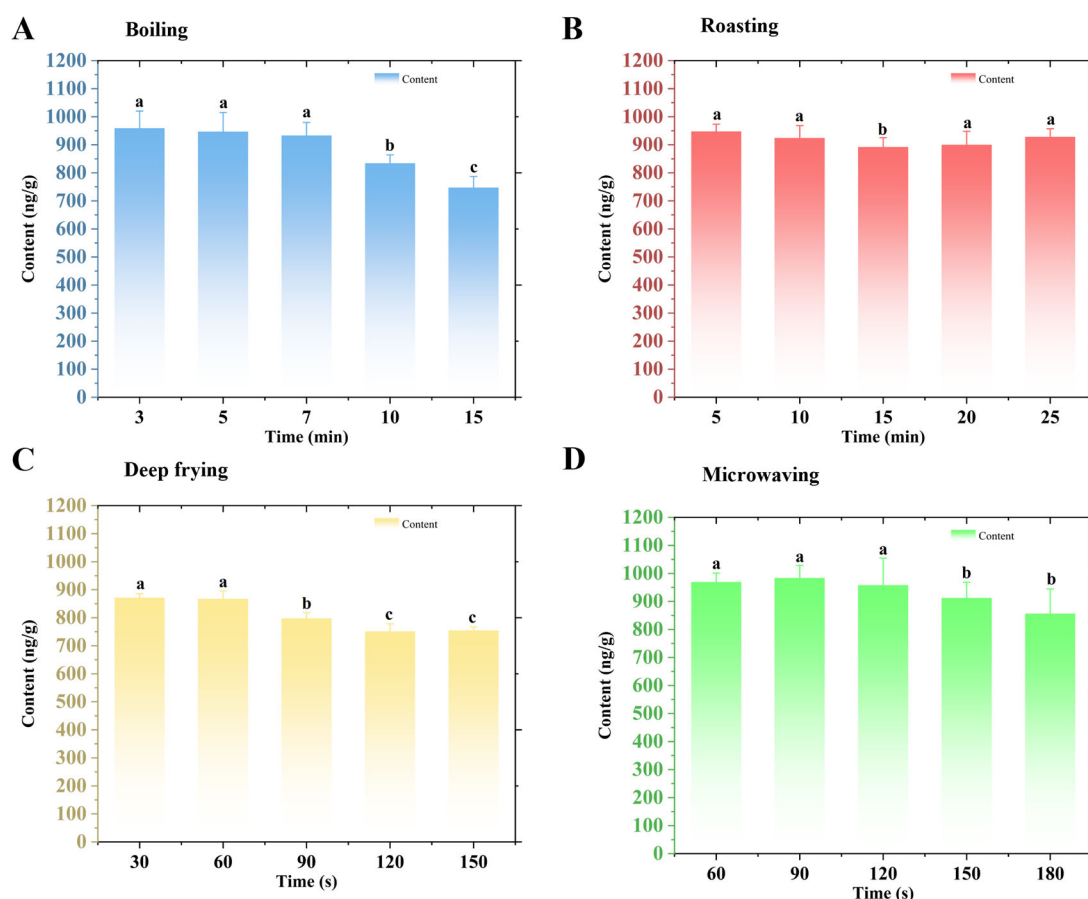


Figure 11. (A) Effect of boiling on the total content of erythromycin and degradation products in fish balls. (B) Effect of roasting on the total content of erythromycin and degradation products in fish balls. (C) Effect of deep frying on the total content of erythromycin and degradation products in fish balls. (D) Effect of microwaving on the total content of erythromycin and degradation products in fish balls. (Different letters, a–c, represent significant differences at $p < 0.05$.).

4. Conclusions

This study analyzed the residual effects of erythromycin and its degradation products in turbot meat under different thermal treatments by establishing a model. However, most results focused on quantitative analysis of erythromycin and its degradation products, with insufficient toxicological data. The erythromycin concentration selected for this study (1000 ng/g) is necessary for robust detection and identification of degradation products and pathways, but is slightly higher than actual residues. Therefore, the findings hold mechanistic significance but also carry certain limitations. The primary objective of this study was to characterize the degradation kinetics and treatment-specific transformation pathways of erythromycin in fish balls, without conducting toxicological evaluations or exposure assessments. Using isotope-labeled reverse-phase chromatography–mass spectrometry for simultaneous detection of four analytes, this method demonstrated good precision, resolution, and reproducibility. Analyzing positive fish meat samples by boiling, deep frying, microwaving, and roasting revealed that erythromycin content decreased rapidly with increasing thermal treatment duration. Among the three major degradation products of erythromycin, anhydroerythromycin A and erythromycin A enol ether showed the highest conversion yields during roasting and boiling. N-demethylerythromycin A showed no significant change during thermal processing, maintaining a concentration around 6.67 ng/g. Although all four thermal treatments reduced erythromycin concentration, the content of degradation products gradually increased. In addition to conducting quanti-

tative analysis of the primary degradation products of erythromycin, we also screened for other degradation products. By combining parent ions and characteristic fragment ions in mass spectrometry, five additional erythromycin degradation products were identified. These results indicate that erythromycin does not completely disappear during thermal processing of foods but persists in the form of degradation products. Furthermore, the degradation pathways of erythromycin differ significantly depending on the specific thermal treatments. These findings contribute to enhancing our understanding of the mechanisms underlying erythromycin conversion during food processing. These findings provide critical insights into prioritizing erythromycin degradants under specific thermal conditions, with substantial implications for food safety assessments and regulatory strategies. From toxicological and regulatory perspectives, erythromycin undergoes transformation rather than elimination during processing. The current MRLs established solely for the parent compound may fail to comprehensively evaluate the risks associated with processed seafood, and degradation products could pose transferable hazards, such as inducing adverse gastrointestinal reactions. Selecting specific degradation products for prioritized monitoring based on different thermal processing methods holds substantial significance for enhancing regulatory frameworks in food safety. Based on our findings, when processing aquatic products with erythromycin residues, the levels of anhydroerythromycin A warrant particular attention during roasting and boiling, whereas the content of erythromycin A enol ether should be prioritized for monitoring in microwaving. However, this study did not evaluate the biological relevance of individual harmful products or their potential impact on human health. The concentrations of erythromycin and its degradation products detected were not compared with known toxicological thresholds or actual consumer exposure levels. Future research should integrate exposure assessment, toxicity testing, and bioactivity evaluation to determine the practical significance of these compounds for food safety.

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References

1. Naylor, R.L.; Hardy, R.W.; Buschmann, A.H.; Bush, S.R.; Cao, L.; Klinger, D.H.; Little, D.C.; Lubchenco, J.; Shumway, S.E.; Troell, M. A 20-year retrospective review of global aquaculture. *Nature* **2021**, *591*, 551–563. [\[CrossRef\]](#)
2. Golden, C.D.; Koehn, J.Z.; Shepon, A.; Passarelli, S.; Free, C.M.; Viana, D.F.; Matthey, H.; Eurich, J.G.; Gephart, J.A.; Fluet-Chouinard, E.; et al. Aquatic foods to nourish nations. *Nature* **2021**, *598*, 315–320. [\[CrossRef\]](#)
3. Sundberg, L.-R.; Ketola, T.; Laanto, E.; Kinnula, H.; Bamford, J.K.H.; Penttinen, R.; Mappes, J. Intensive aquaculture selects for increased virulence and interference competition in bacteria. *Proc. R. Soc. B Biol. Sci.* **2016**, *283*, 20153069. [\[CrossRef\]](#)
4. Sapkota, A.; Sapkota, A.R.; Kucharski, M.; Burke, J.; McKenzie, S.; Walker, P.; Lawrence, R. Aquaculture practices and potential human health risks: Current knowledge and future priorities. *Environ. Int.* **2008**, *34*, 1215–1226. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Maezono, M.; Nielsen, R.; Buchmann, K.; Nielsen, M. The Current State of Knowledge of the Economic Impact of Diseases in Global Aquaculture. *Rev. Aquac.* **2025**, *17*, e70039. [\[CrossRef\]](#)

6. Cabello, F.C. Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environ. Microbiol.* **2006**, *8*, 1137–1144. [[CrossRef](#)] [[PubMed](#)]
7. Watts, J.E.M.; Schreier, H.J.; Lanska, L.; Hale, M.S. The Rising Tide of Antimicrobial Resistance in Aquaculture: Sources, Sinks and Solutions. *Mar. Drugs* **2017**, *15*, 158. [[CrossRef](#)]
8. Regulation (EU) 2019/6; Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on Veterinary Medicinal Products and Repealing Directive 2001/82/EC. European Union: Brussels, Belgium, 2019.
9. Lulijwa, R.; Rupia, E.J.; Alfaro, A.C. Antibiotic use in aquaculture, policies and regulation, health and environmental risks: A review of the top 15 major producers. *Rev. Aquac.* **2020**, *12*, 640–663. [[CrossRef](#)]
10. Schar, D.; Klein, E.Y.; Laxminarayan, R.; Gilbert, M.; Van Boeckel, T.P. Global trends in antimicrobial use in aquaculture. *Sci. Rep.* **2020**, *10*, 21878. [[CrossRef](#)]
11. Henriksson, P.J.G.; Rico, A.; Troell, M.; Klinger, D.H.; Buschmann, A.H.; Saksida, S.; Chadag, M.V.; Zhang, W. Unpacking factors influencing antimicrobial use in global aquaculture and their implication for management: A review from a systems perspective. *Sustain. Sci.* **2018**, *13*, 1105–1120. [[CrossRef](#)]
12. Cabello, F.C.; Godfrey, H.P.; Tomova, A.; Ivanova, L.; Dölz, H.; Millanao, A.; Buschmann, A.H. Antimicrobial use in aquaculture re-examined: Its relevance to antimicrobial resistance and to animal and human health. *Environ. Microbiol.* **2013**, *15*, 1917–1942. [[CrossRef](#)]
13. Thornber, K.; Verner-Jeffreys, D.; Hinchliffe, S.; Rahman, M.M.; Bass, D.; Tyler, C.R. Evaluating antimicrobial resistance in the global shrimp industry. *Rev. Aquac.* **2020**, *12*, 966–986. [[CrossRef](#)]
14. Done, H.Y.; Venkatesan, A.K.; Halden, R.U. Does the Recent Growth of Aquaculture Create Antibiotic Resistance Threats Different from those Associated with Land Animal Production in Agriculture? *AAPS J.* **2015**, *17*, 513–524. [[CrossRef](#)]
15. Grave, K.; Hansen, M.K.; Kruse, H.; Bangen, M.; Kristoffersen, A.B. Prescription of antimicrobial drugs in Norwegian aquaculture with an emphasis on "new" fish species. *Prev. Vet. Med.* **2008**, *83*, 156–169. [[CrossRef](#)] [[PubMed](#)]
16. Irena, T.K.; Gakkhar, S. Modelling the dynamics of antimicrobial-resistant typhoid infection with environmental transmission. *Appl. Math. Comput.* **2021**, *401*, 126081. [[CrossRef](#)]
17. Grave, K.; Torren-Edo, J.; Mackay, D. Comparison of the sales of veterinary antibacterial agents between 10 European countries. *J. Antimicrob. Chemother.* **2010**, *65*, 2037–2040. [[CrossRef](#)]
18. Dinos, G.P. The macrolide antibiotic renaissance. *Br. J. Pharmacol.* **2017**, *174*, 2967–2983. [[CrossRef](#)] [[PubMed](#)]
19. Jelić, D.; Antolović, R. From Erythromycin to Azithromycin and New Potential Ribosome-Binding Antimicrobials. *Antibiotics* **2016**, *5*, 29. [[CrossRef](#)]
20. Wang, F.; Liu, P.; Li, J.; Xu, S.; Chen, H.; Xie, L. Effects of four antibiotics on the photosynthetic light reactions in the green alga *Chlorella pyrenoidosa*. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2024**, *281*, 109927. [[CrossRef](#)]
21. Tong, Y.; Guo, J.; Li, F.; Lai, K.P.; Mo, J. Antibiotic erythromycin in fish: Pharmacokinetics, effects, and health risks. *Environ. Pollut.* **2025**, *373*, 126203. [[CrossRef](#)]
22. Liu, X.; Steele, J.C.; Meng, X.Z. Usage, residue, and human health risk of antibiotics in Chinese aquaculture: A review. *Environ. Pollut.* **2017**, *223*, 161–169. [[CrossRef](#)] [[PubMed](#)]
23. O'Neill, J. *Tackling Drug-Resistant Infections Globally: Final Report and Recommendations*; Review on Antimicrobial Resistance; Government of the United Kingdom: London, UK, 2016.
24. Ghimpețeanu, O.M.; Pogurschi, E.N.; Popa, D.C.; Dragomir, N.; Drăgoțoiu, T.; Mihai, O.D.; Petcu, C.D. Antibiotic Use in Livestock and Residues in Food—A Public Health Threat: A Review. *Foods* **2022**, *11*, 1430. [[CrossRef](#)]
25. Ferri, G.; Lauteri, C.; Vergara, A. Antibiotic Resistance in the Finfish Aquaculture Industry: A Review. *Antibiotics* **2022**, *11*, 1574. [[CrossRef](#)]
26. Hassoun, A.; Heia, K.; Lindberg, S.-K.; Nilsen, H. Spectroscopic Techniques for Monitoring Thermal Treatments in Fish and Other Seafood: A Review of Recent Developments and Applications. *Foods* **2020**, *9*, 767. [[CrossRef](#)] [[PubMed](#)]
27. Grozinger, K.; Proudfoot, J.; Hargrave, K. Discovery and Development of Nevirapine. *Curr. Opin. Drug Discov. Dev.* **2006**, *1*, 353–363.
28. Duan, C.; Zhang, H.; Zhang, Y.; Li, Q.; Li, P.; Mari, G.M.; Eremin, S.A.; Shen, J.; Wang, Z. A Robust Homogeneous Fluorescence Polarization Immunoassay for Rapid Determination of Erythromycin in Milk. *Foods* **2023**, *12*, 1581. [[CrossRef](#)]
29. Wang, B.; Nam, S.; Kim, E.; Jeon, H.; Lee, K.; Xie, K. Identification of Erythromycin and Clarithromycin Metabolites Formed in Chicken Liver Microsomes Using Liquid Chromatography–High-Resolution Mass Spectrometry. *Foods* **2021**, *10*, 1504. [[CrossRef](#)]
30. Zhao, L.; Cao, W.; Xue, X.; Wang, M.; Wu, L.; Yu, L. Occurrence of erythromycin and its degradation products residues in honey. Validation of an analytical method. *J. Sep. Sci.* **2017**, *40*, 1353–1360. [[CrossRef](#)]
31. Salaramoli, J.; Heshmati, A.; Kamkar, A.; Hassan, J. Effect of cooking procedures on tylosin residues in chicken meatball. *J. Für Verbraucherschutz Und Leb.* **2016**, *11*, 53–60. [[CrossRef](#)]
32. Zorraquino, M.A.; Althaus, R.L.; Roca, M.; Molina, M.P. Heat Treatment Effects on the Antimicrobial Activity of Macrolide and Lincosamide Antibiotics in Milk. *J. Food Prot.* **2011**, *74*, 311–315. [[CrossRef](#)]

33. Ma, B.; Li, J.; Lou, T.; Liang, Y.; Wang, C.; Li, R.; Wang, T.; Liu, J.; Guo, Y.; Wang, Z.; et al. Comprehensive Screening and Identification of Phillyrin Metabolites in Rats Based on UHPLC-Q-Exactive Mass Spectrometry Combined with Multi-Channel Data Mining. *Int. J. Anal. Chem.* **2020**, *2020*, 8274193. [[CrossRef](#)]
34. Lorenzetti, R.; Lilla, S.; Donato, J.L.; de Nucci, G. Simultaneous quantification of GMP, AMP, cyclic GMP and cyclic AMP by liquid chromatography coupled to tandem mass spectrometry. *J. Chromatogr. B* **2007**, *859*, 37–41. [[CrossRef](#)] [[PubMed](#)]
35. Zhang, X.; Zhu, W.; Liu, Y.; Chen, L.; Yin, Y.; Huang, J.; Chen, H.; Wu, B.; Shen, C. Determination of erythromycin A and its metabolites in royal jelly by high performance liquid chromatography-tandem mass spectrometry. *J. Food Saf. Qual.* **2014**, *10*, 2994–2999.
36. Aissaoui, Y.; Jiménez-Skrzypek, G.; González-Sálamo, J.; Trabelsi-Ayadi, M.; Ghorbel-Abid, I.; Hernández-Borges, J. Determination of Multiclass Antibiotics in Fish Muscle Using a QuEChERS-UHPLC-MS/MS Method. *Foods* **2024**, *13*, 1081. [[CrossRef](#)]
37. Cai, C.; Hua, Y.; Li, H.; Li, L.; Dai, L.; Liu, H.; Dai, X. Hydrothermal treatment of erythromycin fermentation residue: Harmless performance and bioresource properties. *Resour. Conserv. Recycl.* **2020**, *161*, 104952. [[CrossRef](#)]
38. Tian, L.; Khalil, S.; Bayen, S. Effect of thermal treatments on the degradation of antibiotic residues in food. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 3760–3770. [[CrossRef](#)]
39. Oliver, C.; Dallinger, D. Microwave Processing Techniques. In *Practical Microwave Synthesis for Organic Chemists*; John Wiley & Sons: Hoboken, NJ, USA, 2008; pp. 87–160.
40. Chen, Z.; Dou, X.; Zhang, Y.; Yang, M.; Wei, D. Rapid thermal-acid hydrolysis of spiramycin by silicotungstic acid under microwave irradiation. *Environ. Pollut.* **2019**, *249*, 36–44. [[CrossRef](#)]
41. Thompson, T.S.; van den Heever, J.P. Degradation of erythromycin in honey and selection of suitable marker residues for food safety analysis. *Food Chem.* **2012**, *133*, 1510–1520. [[CrossRef](#)]
42. Okocha, R.C.; Olatoye, I.O.; Adedeji, O.B. Food safety impacts of antimicrobial use and their residues in aquaculture. *Public Health Rev.* **2018**, *39*, 21. [[CrossRef](#)] [[PubMed](#)]
43. Johnson, A.R.; Carlson, E.E. Structure Elucidation of Macrolide Antibiotics Using MS(n) Analysis and Deuterium Labelling. *J. Am. Soc. Mass Spectrom.* **2019**, *30*, 1464–1480. [[CrossRef](#)] [[PubMed](#)]
44. Grover, R.K.; Joshi, B.S.; Batra, S.; Roy, R.; Bhaduri, A.P. Translactonization of erythromycin A during oximation: Mixture analysis and reaction monitoring by NMR. *Magn. Reson. Chem.* **2001**, *39*, 355–360. [[CrossRef](#)]
45. Amsden, G.W. Erythromycin, clarithromycin, and azithromycin: Are the differences real? *Clin. Ther.* **1996**, *18*, 56–72. [[CrossRef](#)] [[PubMed](#)]

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