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Effect of Microwave and Roast Treatment on the Degradation of Sulfamethazine Residue in Tilapia Meat

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ABSTRACT

Tilapia (*Tilapia mossambica*) was force-fed sulfamethazine (SMA) and residue of this antibiotic remained in the meat. We measured the change of residual SMA after microwave and roast treatment on Tilapia by different levels.

The content of SMA in Tilapia increased slowly 6 hrs after feeding, had a sharp climb in 6~12 hrs and decreased gradually in 12~24 hrs. Taking the Tilapia after feeding in 1, 6, 12 and 24 hrs, we measured SMA amount in fish meat is 0.3, 0.45, 1.60 and 1.2 ppm respectively. Microwaving the fish meat after feeding in 6, 12, 24 hrs, we found SMA levels in fish degraded rapidly. SMA in fish had the fastest degradation rate in the first two mins and declined moderately in 2~5 mins. It made no difference in residual SMA among testing groups with different feeding times. Fish was soaked in 5% salt water for 30 mins and SMA in which was also decreased by microwave treatment. However, the degradation of SMA in the salt soaked sample was lower than that of the unsoaked one. The lower SMA content in fish soaked in salt, the less percentage of SMA residual remains.

By roast treatment at 120˚C or 200˚C, SMA in the fish meat degraded with time in both cases no matter how much SMA the Tilapia first contained. The residual level of SMA was less in 200˚C case. Furthermore, the lower SMA content in fish at first, the less residual level was left after heating.

Key words: roast treatment, microwave treatment, sulfamethazine

INTRODUCTION

In recent years residual medicine has been frequently report to remain in the meat of livestock. In 1988, pork exported from Taiwan to Japan was found to contain high SMA residue that cost the proprietors much because of merchandise returning. About 5% of the pork in the United States has also been found to exceed the criteria of the Federal government[1-2]. Therefore, SMA residual in dead animal bodies has long term been controlled strictly by inspection agencies of all countries. Aquatic products also face the same problem. In Taiwan, the aquatic products industry has limited breeding areas and usually employs intensive farming. Because of high-density cultivation, improper feeding and processing, fish is easily invaded by bacterial disease[3,4] that must be prevented or treated by medicine. SMA is an often used antibiotic for aquatic products[4]. SMA is a broad-spectrum, strong antibiotic with limited bacterial resistance, making it popular with aquaculture industry[5,6]. Because proprietors abuse this drug and harvest the aquatic products regardless of the stop-dosing code, exported or domestic fish are sometimes examined and found to contain SMA residue. Consequently, fish products with drug residue that are not inspected by public health departments may enter the market and harm consumers. Antibiotics of common meat, fish and crustacean are examined in the living animals. The ROC government has also started to take fresh meat as a major investigated item. Although the evaluation of SMA residual in processed meat of livestock and its influence on human health after intake have been reported before[7], the influence of SMA residual in meat after heat treatment is much less studied. There is 93% antibiotic residues remaining in heating beef by biologically active analysis[6,8]. In processed pork, there is about 60%[6,8]or 50%[7,9] residual remained, 41%~46% for processed sausage, 83%~86% for canned meat, and 57%~67% for meatballs[10]. However, the SMA residual in processed aquatic products has never been studied. Therefore, we discuss the SMA variations in fish by different heating processes here to understand SMA degradation and residual level for reference.

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MATERIAL AND METHODS

I. *Material*

The *Tilapia mossambica* in this study were purchased from a Pingtung County aquatic farm. Each fish weighed about 600 g. The fish were cultivated in a 1.5 m long, 0.5 m wide, and 0.5 m deep fresh water tank. Groups of 25 fish were bred each time in the aerated tank without feeding them. After one-day cultivation, we force-fed them with SMA 20 mg/kg bw by soft tubes. Taking dorsal meat from the fish, we measured the content of SMA in it at 1, 6, 12, and 24 hrs after feeding; 5 fish were handled in each batch. The sampled fish meat was then heat-processed by the following conditions.

II. *Heat Treatment Condition*

(I) *Microwave Treatment*

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We sampled the dorsal meat of Tilapia at different times as described above. Taken 100 g from each group, the fish meat was put into a microwave (TECO, YM1012CB) and microwaved under 2650 MHz for 0, 1, 2 and 5 minutes. Another group was soaked in 5% salt water for 4 hrs and microwaved for the same time intervals as described above. SMA content in the fish meat was subsequently measured after this heat treatment.

(II) *Roast Treatment*

By the same sampling condition as for the microwave treatment, we placed 100 g fish meat into a 120˚C and a 200˚C oven respectively for roasting at 0, 10, 20 and 30 minutes. The SMA residual was then measured after removing.

III. *Assay of SMA*

Quantification by HPLC analysis[11].

(I) *Specimen Preparation*

Slicing the specimens and stirring to homogeneity in a juice blender, 25 g of the broth was put in the homogenizer, decanted to centrifugal tubes after adding 50 mL methanol, and centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and the precipitate was extracted again after adding 50 mL methanol as described above. The combined supernatant was subsequently dried and 10 mL 1N HCl was added. After adsorption in a Sep-Pak C_{18} column, the column was washed with 20 mL distilled water and finally eluted by 5 mL methanol. The collected eluent was called the “testing liquor”.

(II) *Quantification Analysis*

Taking 10 μL of each of the testing liquor and standard liquid, we injected them respectively into a HPLC column. The analysis column was a Licrospher 100RP-18 (3.9 mm × 30 cm). The mobile phase is 7% isopropanol in 0.001M Na-EDTA (Ethylenediaminetetraacetic acid disodium salt) and 0.1M DEA (Diethanol- amine) liquid solution, titrated to pH7.3 by phosphoric acid and with flow rate of 0.5 mL/min. The adsorption peak was detected under 254 nm by UV detector.

IV. *Calculation of SMA Residual Percentage (Residue %) in Fish Meat*

The SMA content of the fish meat was measured without heat treatment or with heat treatment by roast and microwave. The SMA content was further calculated and expressed by dry fish meat (water will reduce during process) and the water content was measured by the AOAC method[12]. The residual level was indicated by the percentage of SMA content after heat treatment divided by that before heat treatment.

V. *Statistical Analysis of the Results of Heat Treatment*

Sampling the fish meat at different times after feeding, we sliced two pieces of fish meat for subsequent roast and microwave treatments and analyzed the SMA content after the triple repeated extraction as described above. The data were processed by ANOVA (analysis of variance) analysis via SAS statistical software to examine the differences in testing groups. If there is a significant difference ($P<0.05$), we acquired the significantly different sample by Duncan's multiple range test and eliminated that during average calculation. Data were then analyzed again by the same method.

RESULTS AND DISCUSSION

I. *Standard and Recovered Curve of SMA*

(I) *Standard Curve Establishment*

10 μL SMA solutions of concentration 8 ppm, 16 ppm, and 32 ppm were injected into the HPLC column respectively. The standard curve was established by drawing integrated area at different concentrations. This experiment employed the average of three repeated tests.

(II) *Recovered Curve Establishment*

1 mL 8 ppm, 16 ppm and 32 ppm SMA solution added to fish meat samples that contained no SMA at first. The fish meat samples were then ground, homogenized and extracted following the procedure described above and 10 μL samples of each were injected into the HPLC column. The result is shown in Figure. 1.

(III) *The Difference of Standard and Recovered Curve*

The first regression function for the standard curve established from standard SMA solution was Y = 41537.7 × -12610.5 (r = 0.999) and that of the recovered curve was Y = 31239.9 × -28029.5 (r = 0.999). As shown on the plot, SMA in fish showed a reduction during the extraction process. A lower recovered rate around 70%~73% was obtained.

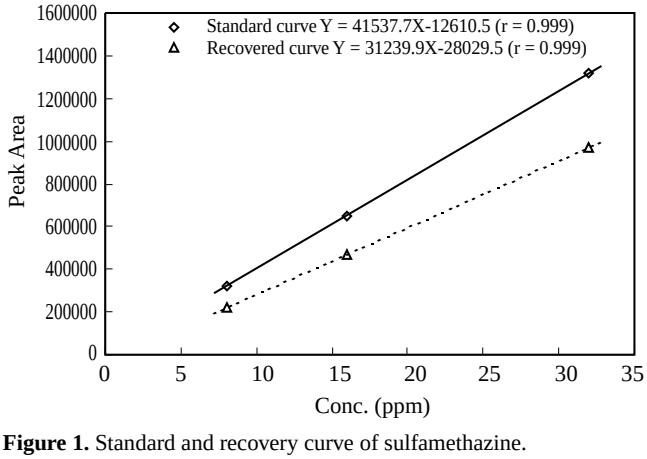

Figure 1. Standard and recovery curve of sulfamethazine.

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II. *Variation of SMA Content in Fish after Feeding*

Tilapia was fed on 20 mg/kg bw SMA and the residual level was tested at different times (Figure 2). Within 6 hrs after feeding, fish meat still had a low SMA content, around 0.3~0.45 ppm. In the 6th ~12th hr, SMA in fish had a sharp climb, achieved the maximum content of 1.6 ppm at the 12th hr and degraded gradually after that. In the 24th hr, the SMA declined to 1.2 ppm, almost the 3/4 of the maximum. The result showed the SMA transferred to fish meat slowly after Tilapia was feeding but metabolized out gradually after getting into fish meat. The SMA content in fish meat went down gradually after the 12th hr.

III. *Effect of Heat Treatment on SMA in Fish*

(I) *Microwave Treatment*

Tilapia was fed SMA first. The fish meat with SMA inside had a residual level of 55.0%~62.5% after one minute microwave treatment, of 24.0%~36.5% after 2 minutes treatment and of 10.0%~12.5% after 5 minutes treatment (Figure 3). Experiment data showed SMA in fish meat decreased with an extended time in the microwave and the three groups had similar decayed rates. The SMA residual level of fish meat went down after microwave treatment for 5 minutes and was only around 10%. This meant almost 90% loss in this case. We can assume from this result that microwave treatment caused the degradation of SMA or the combination of fish tissue. Therefore, insufficient extraction amount was collected or the extract cannot be dissolved in solvent that made a lower analyzed consequence on HPLC chart.

(II) *Microwave Treatment of the Salt-soaked Fish Meat*

The SMA in fish meat had a residual level of 92.0%~94.5% after fish was first soaked in the salt water, of 47.0%~79.5% after microwaved for one minute, of 30.5%~59.0% after microwaved for two minutes, and of 19.0%~26.0% after microwaved for five minutes (Figure 4). Compared with the unsoaked fish (Figure 3), there was a higher SMA residual level in the soaked case. This implied higher ionic strength may alleviate SMA degradation and combination of fish meat.

The residual level of SMA in fish meat after soaked in salt declined with time in the microwave. The residual level of fish meat soaked in salt and microwaved at the 6th hr after feeding had a lower residual level than that at the 12th and 24th hr after feeding. From Figure 2, we know that fish meat at the 6th hr after feeding had the lowest SMA content. Therefore, the lower SMA content in fish first soaked in salt, the smaller the residual level was left after microwave treatment. Groups of fish meat first soaked in salt had a higher residual level and a lower degradation rate than that unsoaked. It showed higher ionic strength promoting SMA extraction from the fish meat and decreasing the chance of combination of meat and SMA.

(III) *Roast Treatment at 120˚C*

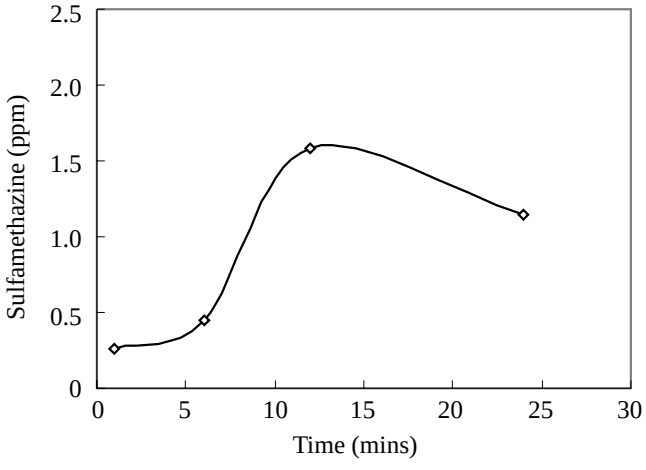

Figure 2. The concentration of sulfamethazine in dorsal meat of Tilapia force-fed 20 mg/kg bw sulfamethazine.

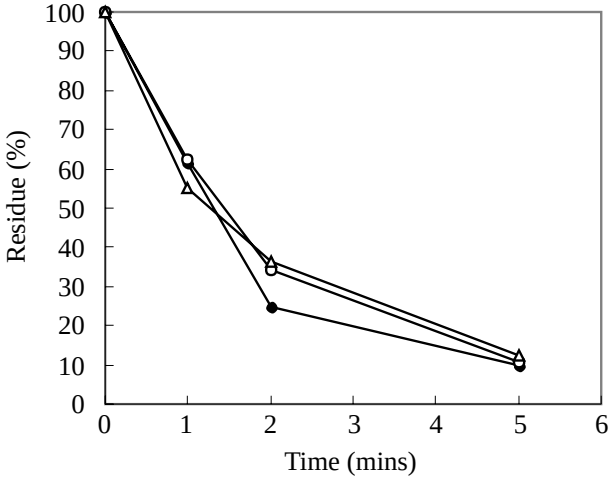

Figure 3. Effect of microwave treatment on degradation of sulfamethazine in dorsal meat of Tilapia force-fed with 20 mg/kg bw of sulfamethazine. (-●-: fed 6 hrs, content SMA 0.45 ppm. -○-: fed 12 hrs, content SMA 0.45 ppm. -△-: fed 24 hrs, content SMA 0.45 ppm).

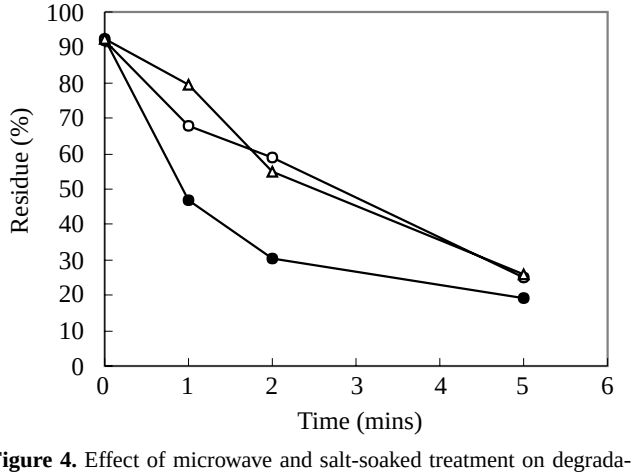

Figure 4. Effect of microwave and salt-soaked treatment on degradation of sulfamethazine in dorsal meat of Tilapia force-fed with 20 mg/kg bw of sulfamethazine. (-●-: fed 6 hrs, content SMA 0.45 ppm. -○-: fed 12 hrs, content SMA 1.6 ppm. -△-: fed 24 hrs, content SMA 1.2 ppm).

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Fish meat with SMA contained was roasted at 120˚C for 10 minutes and had a residual level of SMA of 84.5%~98.0%, of 72.0%~93.0% by roast treatment at 120˚C for 20 minutes and of 31.5%~86.0% by roast treatment at 120˚C for 30 minutes (Figure 5). It showed the residual level of SMA in fish meat declined with time after the roast treatment at 120˚C. The fish meat roasted in the 12th hr after feeding had the highest residual level. The fish meat at the 6th hr after feeding had the least SMA before heat treatment. With heating time prolonged to 20 minutes, the degradation rate declined rapidly and greatly. This result agreed with the salt-soaked case which was subsequently heated by microwave. Both cases showed that lower concentrations of SMA in fish caused a lower residual level after heating. However, fish meat after roast treatment had a more obvious variation.

(IV) *Roast Treatment at 200˚C*

Fish meat containing SMA was roasted at 200˚C for 10 minutes and had a residual level of SMA of 32.5%~79.0%, of 27.0%~38.5% by roast treatment at 200˚C for 20 minutes and of 15%~23.5% by roast treatment at 200˚C for 30 minutes (Figure 6). It showed the residual level of SMA in fish meat declined with time after the roast treatment at 200˚C. The same result was obtained when case was heated at 120˚C; the residual level of fish meat roasted at the 6th hr after feeding had a rapid and great loss. The fish meat at different SMA concentration roasted at 200˚C for 10 minutes had a similar variation as that roasted at 120˚C for 30 minutes. SMA content had a moderate degradation rate at 20~30 minutes under 200˚C roasting and the difference of residual level between each groups get smaller with an extended heating time. The most significant reasons for reduction of residual level and increase of loss were temperature and time effects. We can therefore conclude from the experiment results that extending the heating time at 120˚C may cause a reduction of residual level of all the groups and the difference of residual level between each other will get much closer as shown in the 200˚C case.

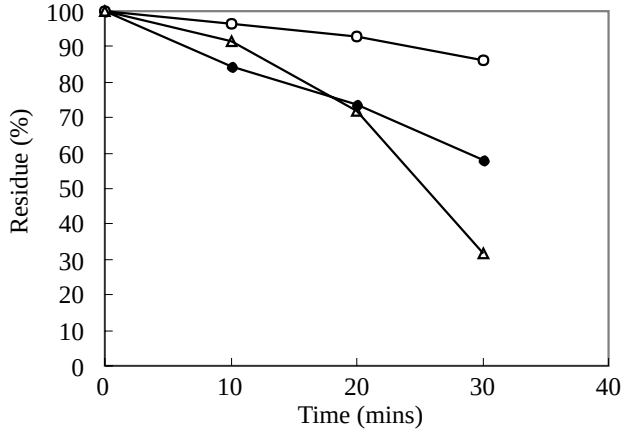

Figure 5. Effect of roast treatment at 120˚C on degradation of sulfamethazine in dorsal meat of Tilapia force-fed with 20 mg/kg bw of sulfamethazine. (-●-: fed 6 hrs, content SMA 0.45 ppm. -○-: fed 12 hrs, content SMA 1.60 ppm. -△-: fed 24 hrs, content SMA 1.20 ppm).

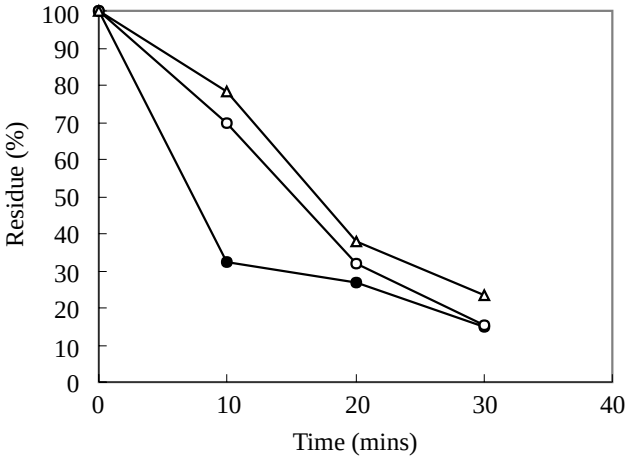

Figure 6. Effect of roast treatment at 200˚C on degradation of sulfamethazine in dorsal meat of Tilapia force-fed with 20 mg/kg bw of sulfamethazine. (-●-: fed 6 hrs, content SMA 0.45 ppm. -○-: fed 12 hrs, content SMA 1.60 ppm. -△-: fed 24 hrs, content SMA 1.20 ppm).

In this study, SMA was directly added into *Tilapia mossambica.* After fish meat was ground to homogeneity and extracted by methanol, the residual level was obtained with a percentage of 70.0%~73.0% (Figure 1). Fish meat after roast and microwave treatment had even lower residual levels and the residual level straightly declined with extended heating time. After being roasted at 200˚C for 30 minutes or microwaved for 5 minutes, SMA in fish meat decreased to about 20.0%. Fish meat roasted at 120˚C had higher residual levels of SMA and a moderate degradation rate, but levels did reduce with extended roasting time. Results showed that fish meat might reduce the extraction effect of SMA after protein denaturing. Judging from the reduction of residual levels with extended heating time, we propose the possibility of SMA's degradation by heat effect and combination of fish meat and protein tissue. This was further verified by the evidence that less SMA content in fish had smaller residual levels after heating. Different residual levels also occurred in the processed meat of livestock, with levels from 41.0% to 86.0%[6-10]. The residual level of SMA was also lowered down by reducing the dosing amount and raising the temperature[9-10]. This result also proved that this occurred in all the meat of livestock and the degradation of SMA in fish meat after heat treatment was much more obvious and faster.

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微波及燒烤處理對吳郭魚肉中磺胺二甲嘧啶殘留之影響

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摘 要

以強迫方式餵食吳郭魚抗菌劑磺胺二甲嘧啶，使其魚肉中殘留此抗菌劑，將魚肉以不同程度的微波及燒烤處理，再測定其加熱後魚肉中磺胺二甲嘧啶含量的變化。

吳郭魚經餵食磺胺二甲嘧啶6小時，魚肉中的磺胺二甲嘧啶含量並未快速上升，從餵食後6小時至12小時急速上升，餵食後12小時至24小時呈緩慢下降趨勢。取上述經餵食後不同時間1、6、12及24小時之吳郭魚，其魚肉中所含之磺胺二甲嘧啶量分別為0.3、0.45、1.60及1.2ppm，取餵食後6、12、24小時之魚肉經微波處理後磺胺二甲嘧啶殘存率迅速下降，微波處理2分鐘內之殘存率較為快速，2至5分鐘則趨緩和，各組之殘存率差異並不大。以5%鹽醃30分鐘之魚肉，經微波處理後亦使殘存率下降，但魚肉經鹽醃處理後殘存率較未鹽醃者大，且鹽醃魚肉之磺胺二甲嘧啶含量愈低，則殘存率有較小之現象。

以120˚C或200˚C燒烤處理，無論魚肉中含磺胺二甲嘧啶之多寡，均隨加熱時間之延長殘存率均有下降之現象。但以200˚C加熱者殘存率較小，且魚肉中含磺胺二甲嘧啶濃度越低，加熱後之殘存率亦愈小。

關鍵詞：燒烤處理，微波處理，磺胺二甲嘧啶