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Effects of various freezing and cooking processes on the residues of sulfamethazine in broiler tissues*

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Summary: This study was conducted to investigate the effects of frying, boiling and freezing processes on sulfamethazine (SMZ) residues in chick breast meat and liver. Chicks were fed with feed not containing sulfonamide residues for 30 days. After 30 days, the suspensions of SMZ (110 mg/kg) were given to 10 chicken by gavage to craw once daily for 5 days. After 35 day, all animals were slaughtered and their breast meat and liver were taken. The part of the tissues were stored in -20 °C freezer for 30 and 45 days. The levels of SMZ in raw, grilled and boiled and freezed tissues were determined by HPLC-DAD detector. As a result of the study, it has been determined that, boiling and grilling processes reduced the residues of SMZ at different rates in broiler tissues; storing in the deep freezer did not cause significant changes on drug residues.

Key words: Breast meat, broiler, cooking, freezing, liver, sulfamethazine

Sülfametazinin etçi piliç dokularındaki kalıntıları üzerine çeşitli pişirme ve dondurma işlemlerinin etkileri

Özet: Bu araştırmayla kızartma, haşlama ve dondurma işlemlerinin etçi piliç göğüs eti ve karaciğer dokularındaki sülfametazin (SMZ) kalıntılarına yönelik etkilerinin ortaya konulması amaçlandı. Civcivler 30 gün boyunca sülfonamid içermeyen yemle beslendi. Otuzuncu günün sonunda deneme grubuna SMZ (110 mg/kg) günde 1 kez ve 5 gün boyunca sonda ile kursağa verildi. 35. günün sonunda tüm hayvanlar kesilerek sağ göğüs eti ve karaciğerleri alındı. Dokuların bir kısmı 30 ve 45 gün süresince -20 °C'lik derin dondurucuda saklandı. Çiğ dokular, kızartma ve haşlama yapılan dokular ile derin dondurucuda muhafaza edilen dokulardaki SMZ düzeyleri HPLC-DAD dedektörle belirlendi. Çalışma sonucunda broyler göğüs eti ve karaciğer dokularındaki SMZ düzeylerinin ızgara ve haşlama işlemleri ile değişik oranlarda azaldığı; dokularda SMZ kalıntıları üzerinde derin dondurucuda muhafaza işleminin önemli bir değişime yol açmadığı belirlendi.

Anahtar sözcükler: Dondurma, etçi piliç, göğüs eti, karaciğer, pişirme, sülfametazin

Introduction

Sulfonamides are the first drugs used systemically for the treatment and prevention of bacterial diseases. It is widely used with trimethoprim in domestic animals for the treatment of bacterial and parasitic respiratory, gastrointestinal and urinary tract diseases (3, 12, 17, 20). Sulfamethazine (SMZ) is a sulfonamide derivative drug that is used on a large scale in veterinary medicine especially for cattle, horses, pigs, small ruminants, rabbits and birds. The drug provides 50 µg/mL of plasma level for at least 24 hours after oral administration of 100-110 mg/kg in all animal species (3, 6, 14, 16).

Drug residues in food cause undesirable effects such as allergy, cancer and teratogenecity on organism, also it is seen development of resistance on bacteria and decrease in antibacterial activity and failure of treatment of bacterial infections. According to previous researches, aminoglycosides, macrolides and tetracyclines remain in animal tissues without any disrupting for a long time. However, frying, roasting and canning processes destroy residues of several sulfonamide, chloramphenicol, carbadoks, ampicillin, levamisole and clenbuterol in meats (1, 2, 6).

The purpose of this study was to investigate the effects some cooking and freezing processes on SMZ residues in broiler meat and liver.

Material and Method

In this study, fifteen one-day-old Ross-PM3 chicken were used. Chickens were fed with commercial chicken

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diet. It was taken an Ethical Board Approval from Erciyes University Local Ethical Committee of Animal (ERU-HADYEK).

The chickens were fed with SMZ free ration for 30 days. At the end of the thirtieth day, SMZ (110 mg/kg) was given to 10 chicken by gavage to craw, once a day for 5 days (6). Followed by 24 hours the last administration, all animals were slaughtered and their right breast meat and liver were taken out. Five SMZ untreated chicken were cut and their right breast meat and liver were taken. 10 gram of samples were taken from each breast and liver, the remaining tissues were stored at -20 °C freezer for 30 and 45 days.

Grilling and boiling processes of raw tissue samples were performed to Baydan et al. (2). After boiling processes, tissue and water were separated. The exctraction and analysis of SMZ residues of raw, grilled, freezed (30 and 45 days) and boiled tissues and boiled water were performed according to the method by Papapanagiotou et al. (10).

For calculation of calibration equation, stock solution (1.0 g/100 mL SMZ in dichlormethan) and standard solutions (range of 1-80.0 mg/mL) were prepared. SMZ solutions were added to tubes as 1.0, 5.0, 10.0, 20.0, 40.0, 60.0 and 80.0 µg respectively and their volumes were completed to 1 mL of 3 N HCI. They were centrifuged at 3000 rpm for 5 minutes. 250 µL of the upper phase was transfered to glass tube and 250 µL of sodium acetate solution (3.8 M) was added and vortexed for 15 seconds. 20 µL of this solution was applied to HPLC (10).

For recovery studies of meat and liver, 3 gram of chest meat or liver tissue collected by SMZ untreated chickens was placed to 7 tubes. 1.0, 5.0, 10.0, 20.0, 40.0, 60.0 and 80.0 µg amounts of SMZ solutions were added to the tubes. The samples were homogenized and analysed by Papapanagiotou et al. (10).

Fort the exctraction of the samples, 3 g of chest meat and liver or whole boiled water were taken into a plastic centrifuge tube and sulfachlorpyridazine (80 µg) were added as internal standard. The samples were homogenized with mechanical homogenizator for 1 minute by adding 30 ml of dichloromethane. The mixture was collected by filtration through Whatman 40 filter paper. 10 mL of the filtrate was taken to 15 mm centrifuge tube and 1 mL of 3 N HCl was added and centrifuged at 3000 rpm for 5 minutes. 250 µL fluid from the upper phase was transfered to 15 mm centrifuge tube and 250 µL of sodium acetate solution (3.8 M) was added and vortexed for 15 seconds. 20 µL of this solution was applied to HPLC.

SMZ analysis were carried out by HPLC equipped with DAD detector and C18 reverse phase column (ACE-121-2546, 250x4.6 mm) by using methanol: water carrier system (60:40) with pH 3 (added 10% orthophosphoric acid) as 1.8 mL/min flow rate (10). For calculation of Limit of Detection (LOD) and Limit of Quantification (LOQ), signal/noise ratio was selected as 3/10.

The analysis of data were performed using SPSS 15.0 software package. Data were evaluated with one-way analysis of variance (ANOVA) ($p<0.05$). Differences between groups were determined with the Duncan's test.

Results

The peak retention time of SMZ and SCP were found as 8.3 and 17.7 minutes, respectively. The standard curve showed linearity in range of 1-80 µg/ml; the recovery values of SMZ, r^2 values, LOD and LOQ were seen in Table 1.

Table 1. Validation data of SMZ
Tablo 1. SMZ'ye ait validasyon sonuçları

	Recovery	r^2	LOD (µg/g)	LOQ (µg/g)
SMZ (Chest)	90.13±4.85	0.99234	0.161996	0.539988
SMZ (Liver)	96.60±3.82	0.99889	0.120655	0.402183
SMZ (Boiled tissue)	98.91±5.12	0.99874	0.152171	0.362941

SMZ: Sulfamethazine, r^2: Correlation coefficient, LOD: Limit of Detection, LOQ: Limit of Quantification
SMZ: Sülfametazin, r^2: Korelasyon katsayısı, LOD: Tespit sınırı, LOQ: Tayin sınırı

SMZ levels of breast and liver tissue were shown in Table 2. SMZ levels of boiled tissue were decreased when compared with other various freezing and cooking processes of liver tissues ($p<0.05$).

Table 2. SMZ levels of tissues (µg/g).
Tablo 2. Dokulardaki SMZ düzeyleri (µg/g).

	Chest (n=10)	Liver (n=10)
Raw tissue (Control)	$49.21±6.20^{c}$	$47.75±8.44^{c}$
Freezed tissue (30 day)	$48.93±7.02^{c}$	$44.33±13.11^{c}$
Freezed tissue (45 day)	$48.64±4.78^{bc}$	$41.97±7.18^{c}$
Grilled tissue	$38.11±9.55^{b}$	$33.23±3.30^{bc}$
Boiled tissue	$24.84±4.26^{a}$	$26.23±4.31^{ab}$
Water of boiled tissue	$15.71±1.65^{a}$	$14.11±3.89^{a}$
Boiled tissue+water	$40.55±6.53^{bc}$	$40.34±8.20^{c}$

a,b,c: Different characters indicate statistically significant differences in the same column ($p<0.05$).
a,b,c: Aynı sütünda farklı harflerle gösterilen gruplar arasındaki fark istatistiksel olarak önemlidir ($p<0.05$).

Discussion and Conclusion

In this study validation data of chest meat, liver and boiled tissue have found as 90.13, 96.60, 98.91 for recovery; 0.99234, 0.99889, 0.99874 for r^2; 0.161996, 0.120655, 0.152171 for LOD; 0.539988, 0.402183, 0.362941 for LOQ, respectively. The recovery value and

correlation coefficient are within acceptable ranges for analytical studies (11, 13, 19). According to validation data, methods of Papapanagiotou et al. (9, 10) are suitable for SMZ analysis in chicken meat and liver.

The changes of SMZ levels in frozen breast for 30 and 45 days were not significant when with compared to raw tissue ($p>0.05$). The reduction of SMZ levels in grilled, boiled tissue and boiled water were significant ($p<0.05$). These results reveal that, storing in frezer has no effect on breast SMZ residues; but thermal processing (grilling and boiling) has effective on SMZ residues in breast tissue. Absence of the detectable SMZ residue in boiled water may be associated with weak passage of the drug to water, disintegration of the drug because of heating (18). Although SMZ levels decreased boiled liver tissue and boiled water ($p<0.05$), SMZ levels didn't change significantly of boiled tissue+water as compared with raw tissue. This situation reveals that, the boiling process does not have significant effect on SMZ residues in liver tissue like storage in freezer and grilling process (18).

Studies previously shown that cooking processes have no significant effect on the SMZ residues in porks (7, 15), but ormethoprim and sulfadimethoxine residues decrease in fish meat due to various cooking processes (21). Furusava and Hanabusa (4) has observed a reduction of sulfadiazine, sulfamethoxazole, sulfamonometoxin levels in boiled chicken meat. Various cooking and storing in the freezer process for different durations reduce sulphadiazine residues in tissues (1, 21). Also, different cooking and storing in the freezer processes cause a significant reduction on the residues of sulfadimethoxine, sulfaquinoxaline and sulfadoxine in broiler tissues; the storage in freezer at different times caused a statistically significant reduction of sulfadoxine and sulfaquinoxaline residues (2). Our results show that, boiling and grilling processes cause a decrease at different rates on SMZ residues in breast and liver. The decrease of SMZ in boiled tissue shows that SMZ can transfer boiled water. These findings are compatible of previous studies about other sulfonamides (1, 2, 4). But the storage of freezer does not lead to a significant alteration on SMZ residues unlike previous studies on other sulfonamides (1, 2). This result may be related with species, race, age and sex of animals; stability, solubility, pharmacokinetic and pharmaceutical differencies of drugs and administration route and time of drugs (1, 2, 7).

As a result, boiling and grilling processes causes a reduction of SMZ residues in chest meat and liver tissue, also storing of freezer doesn't cause significant changes on SMZ residues. Boiling and grilling processes can reduce other antibiotic residues in meat.

The studies on the effects of various cooking and storage processes on antibiotic residues in tissues show that, cooking and storage of foods can not completely breakdown antibiotic residues. Although, the antibiotic residues in foods reduce to safe limits for human health in particular with long-term cooking at a high temperature, what kind of metabolites occur after cooking and their effects on human health are unknown (5, 7, 8). Drug residues in tissues may differ with animals age, race, sex, disease state, drug solubility, stability and pharmacokinetics, pharmaceutical formulations, route and time of administration, different cooking and storage methods and durations (1, 2, 7, 8). It needs detailed studies about effects of various cooking and storing processes on drug residue in foods.

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