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RESEARCH ARTICLE

Effects of Various Cooking and Freezing Processes on the Residues of Sulfachlorpyridazine-Trimethoprim Combination in Broiler Tissues [1] [2]

Murat KANBUR [1] Bilal Cem LİMAN [1] Gökhan ERASLAN [1]
Emine BAYDAN [2] Erdal DİNÇ [3] Mürsel KARABACAK [4]

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[1] Erciyes Üniversitesi, Veteriner Fakültesi, Farmakoloji ve Toksikoloji Anabilim Dalı, TR-38039 Kayseri - TÜRKİYE
[2] Ankara Üniversitesi, Veteriner Fakültesi, Farmakoloji ve Toksikoloji Anabilim Dalı, TR-06110 Dışkapı, Ankara - TÜRKİYE
[3] Ankara Üniversitesi, Eczacılık Fakültesi, Analitik Kimya Anabilim Dalı, TR-06100 Ankara - TÜRKİYE
[4] Erciyes Üniversitesi, Safiye Çıkrıkçıoğlu Meslek Yüksekokulu, TR-38039 Kayseri - TÜRKİYE

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Summary

This study was conducted to determine the effects frying, boiling and freezing processes on the levels of sulfachlorpyridazine (SCP) and trimethoprim (TMP) in broiler chest meat and liver. Male broiler chicks were assigned to two groups as control and treatment groups. Animals were fed on commercial diet not containing SCP and TMP for 35 days. At 30th day, experimental group received suspensions of SCP-TMP mixture (30 mg/kg SCP and 6 mg/kg TMP) via craw by gavage once daily for 5 days. At the end of 35th day, the chickens were sacrified and right chest tissue and the liver were taken out. A portion of the tissues were stored in -20°C freezer for 30 and 45 days. After sacrifiying, the raw tissues were exposed to frying and boiling processes. SCP and TMP analysis of tissues were performed by HPLC-DAD detector and reverse phase column. In conclusion, it has been determined that boiled and grilled processes caused a reduction of SCP and TMP residues at different rates in broiler tissues; while storing in the deep freezer did not cause a significant change on SCP and TMP residues.

Keywords: *Sulfachlorpyridazine, Trimethoprim, Broiler meat, Liver, Cooking, Freezing, HPLC*

Sülfaklorpridazin-Trimetoprim Karışımının Broyler Dokularındaki Kalıntıları Üzerine Çeşitli Pişirme ve Dondurma İşlemlerinin Etkileri

Özet

Bu araştırmayla kızartma, haşlama ve dondurma işlemlerinin piliç göğüs eti ve karaciğer dokularındaki sülfaklorpridazin (SCP) ve trimetoprim (TMP) kalıntılarına yönelik etkilerinin ortaya konulması amaçlandı. Erkek broyler civcivlerden kontrol ve deneme olmak üzere 2 çalışma grubu oluşturuldu. Hayvanlar 35 gün boyunca SCP ve TMP içermeyen yemle beslendi. Deneme grubundaki civcivlere otuzuncu günden itibaren SCP-TMP karışımı (30 mg/kg SCP, 6 mg/kg TMP) 5 gün boyunca günde 1 kez sonda ile kursağa verildi. 35. günün sonunda hayvanlar kesilerek sağ göğüs dokusu ve karaciğerleri alındı. Dokuların bir kısmı 30 ve 45 gün boyunca -20°C'lik derin dondurucuda saklandı. Kesim sonrası alınan çiğ dokulara kızartma ve haşlama işlemleri uygulandı. Dokuların SCP ve TMP analizleri DAD dedektör ve ters faz kolon ile HPLC'de gerçekleştirildi. Sonuç olarak, haşlama ve ızgara işlemlerinin broyler dokularında SCP ve TMP kalıntılarında değişik oranlarda azalmaya neden olduğu; derin dondurucuda bekletmenin ise SCP ve TMP kalıntıları üzerinde önemli bir değişime yol açmadığı belirlendi.

Anahtar sözcükler: *Sülfaklorpridazin, Trimetoprim, Etlik piliç eti, Karaciğer, Pişirme, Dondurma, HPLC*

INTRODUCTION

Sulfonamides block folic acid synthesis in bacteria and coccidia by competing with PABA. SCP is effective against many Gram-positive and Gram-negative microorganisms and coccidia *(E. necatrix, E. maxima, E. tenella, E. brunetti)*.

İletişim (Correspondence)
+90 352 2076666/29972
kanburm@hotmail.com

SCP is used orally and well tolerated by the animals. It is used for the treatment of coli-bacteriosis, cholera and coccidiosis in drinking water for 3-6 days, at the dose of 30-50 mg/kg. If it is necessary, daily dose can be given up to 400 mg/kg [1-4]. SCP is also used in aquaculture and as a feed additive especially for pigs and cattle [5,6]. The forms of sulfonamides combined with TMP have very broad spectrum; these combinations are used diarrhea, coli-septicemia, CRD, salpingitis, coryza, cholera, staphylococcal infections and coccidiosis of poultry [1,2].

SCP are rapidly absorbed and moderate exctrected. This drug are bound to plasma proteins, especially albumin; it is distributed widely to the body and readily enter body fluids. Levels of SCP are highest in the liver, kidney and lung. Metabolism is by acetylation and glucuronidation in the liver. Like SCP, TMP are widely distributed in tissue and interstitial fluid. Concentrations of TMP are higher in tissues than serum. TMP is metabolized by the liver to oxide and hydroxylated metabolites [4]. After the sulfonamide administration of animals, sulfonamide residues can be found in meat for a long time and they migh cause allergic reactions for human and animals. Also, sulfonamide residues of meat can increase occurence of bacterial resistance [7,8]. According to international codex [7] and Turkish Food Codex [9] maximum residue limit of sulfonamides and trimethoprim are 100 µg/kg and 50 µg/kg for animal-originated food, respectively.

Today, the use of drugs for the treatment and prevention of diseases in animals has become an indispensable application. However, the medications can be harmful for human health through the residues of animal products such as allergy, carcinogenic and teratogenic effects, the development of resistancy in bacteria and decreasing the activity of antibiotics [3,10-12].

This study was conducted to determine the effects of some cooking and freezing processes on SCP and TMP residues in broiler meat and liver tissues.

MATERIAL and METHODS

In this study, 15 Ross-PM3 male chicken were used. Chicken were fed with commercial chicken diet containing all nutrients accepted by National Research Council (NRC) [13]. The diet and water were given as *ad libitum*. For this research it was taken an ethical approval by Ethical Committee of Erciyes University Veterinary Faculty (2005/056/071).

Two groups were assigned as control and SCP-TMP given group. For SCP-TMP group 10 male broiler chicks were used. Five broilers were kept as control group to study validation of SCP and TMP analysis. The chicks were fed with SCP and TMP free feed for 35 days. At the end of the 30th day, SCP-TMP combination was given by gavage to craw at the dose of 30 mg/kg SCP and 6 mg/kg TMP [3] in water, once a day for 5 days to the SCP-TMP group. 24 h after the last administration, the chicks were sacrified and right chest and livers were taken out. Broilers in control group were cut without any drug administration; right chest and liver were taken to use for validation studies. Ten grams of samples were taken from each chest tissue and livers. The remaining tissues were stored at -20°C freezer during 30 and 45 days to determine the effect of cold storage on SCP and TMP residues.

Grilling process was performed in teflon pan for 5 min, boiling process was performed on a tray with heater set to 100°C with the addition of 50 mL salin (50 mg/50 ml NaCl) on the tissues at 10 min. After boiling process, tissues and boiled water were seperated [10]. Extraction and analysis of SCP and TMP in raw, grilled, freezed tissues at 30 and 45th days, boiled tissue and meat stock were performed according to Papapanagiotou et al.[14] with several modification.

For extraction of tissue, 30 ml of dichloromethane was added to 3 g of tissue. Sulfamethazine (80 µg) was added on it as internal standard. The tube was homogenized by homogenizer for 10 min in cold water medium at 20.000 rpm. The mixture was collected by filtration through Whatman 40 filter paper. 1 mL of 3 N HCl was added on 10 ml of the filtrate and centrifuged at 3.000 rpm for 5 min. 250 µl of fluid was from the upper side and 250 µl of 3.8 M sodium acetate solution was added on it and vortexed for 15 sec. 20 µl of this solution was applied to HPLC.

Validation of SCP and TMP analysis were evaluated with the parameters of recovery, corelation coefficient, limit of detection and limit of quantitation by using chemometric techniques [15]. For preparation of calibration curve 1-80 µg/ml or g range standard solutions as different 7 concentrations were prepared. The recovery rates were calculated in drug-free tissue samples at the same range of standard solutions for each samples.

SCP and TMP analyzes were performed in HPLC equipped with DAD detector and C18 reverse phase column (ACE-121-2546, 250x4.6 mm) by using methanol:water (60:40) carrier system with pH 3 set by 10% orthophosphoric acid with 1.8 ml/min flow rate [14].

The analysis of data was 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 Duncan's test.

RESULTS

Validation Results

In analyzes of samples in HPLC, retention time of

TMP, SMZ and SCP were found as 6.857 min, 8.168 min and 17.736 min, respectively (*Fig. 1* and *Fig. 2*). The standard curve showed linearity in range of 1-80 µg/ml for SCP and TMP mixtures. For SCP, recovery (%), r^2, the limit of detection (LOD; µg/g) and limit of quantitation (LOQ: µg/g) were found 93.25±4.46, 0.9992, 0.857 and 2.859 of chest; 87.95±4.5, 0.9992, 0.739, 2.464 of liver; 98.12±6.51, 0.9993, 0.512, 0.812 of boiled tissue, respectively *(Table 1)*. For TMP, recovery, r^2, the limit of detection (LOD) and limit of quantitation (LOQ) were found 97.55±3.58, 0.9923, 0.255, 0.851 of chest; 95.38±6.15, 0.9983, 0.390, 1.303 of liver; 98.87±7.49, 0.9994, 0.432, 0.502 of boiled tissue, respectively *(Table 1)*.

Experimental Results

SCP levels of chest meats in raw tissue, raw tissue frozen for 30 days, raw tissue frozen for 45 days, grilled tissue, boiled tissue were found as 49.10 µg/g, 44.93 µg/g, 48.14 µg/g, 25.33 µg/g, 21.46 µg/g, respectively; SCP levels of boiled water could not be determined *(Table 2)*.

TMP levels in chest meats in raw tissue, raw tissue frozen for 30 days, raw tissue frozen for 45 days, grilled tissue, boiled tissue and boiled water were found as 1.91 µg/g, 1.8 µg/g, 1.84 µg/g, 1.48 µg/g, 1.15 µg/g and 0.68 µg/ml, respectively *(Table 2)*.

SCP levels of liver in raw tissue, frozen tissue for 30 days, frozen tissue for 45 days, grilled tissue, boiled tissue were found as 60.31 µg/g, 61.87 µg/g, 60.91 µg/g, 20.97 µg/g and 27.33 µg/g, respectively; SCP residues could not detected in boiled water. One of the most important finding of the study is that the levels of SCP were statistically decreased in grilled and boiled tissue and boiled water as compared to raw liver tissue ($P<0.05$) *(Table 2)*.

TMP levels for liver tissue in raw tissue, frozen tissue for 30 days, frozen tissue for 45 days, grilled tissue, boiled tissue and boiled water were found as 1.56 µg/g, 1.52 µg/g, 1.59 µg/g, 1.34 µg/g, 0.97 µg/g and 0.57 µg/ml. TMP levels of liver in boiled water were reduced significantly as compared to raw tissue ($P<0.05$) *(Table 2)*.

DISCUSSION

There are several HPLC techniques for determining sulfachlorpyridazine residues in meat [7,17,18]. According to the method developed by Kowalski et al.[18] which conducted for analysis of SCP, residues in meat samples were

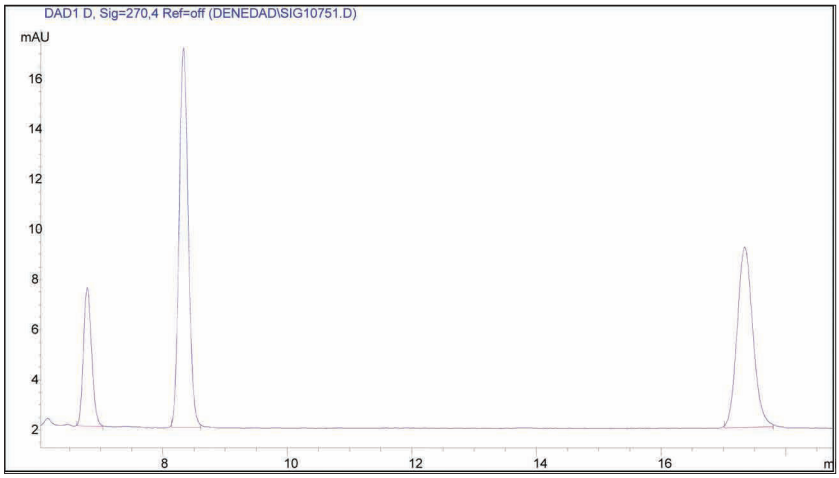

Fig 1. Chromatograms of TMP, SMZ ve SCP in chest samples at 270 nm

Şekil 1. Göğüs eti örneklerinde 270 nm'deki TMP, SMZ ve SCP'ye ait kromatogramlar

Fig 2. Chromatograms of TMP, SMZ ve SCP in liver samples at 270 nm

Şekil 2. Karaciğer örneklerinde 270 nm'deki TMP, SMZ ve SCP'ye ait kromatogramlar

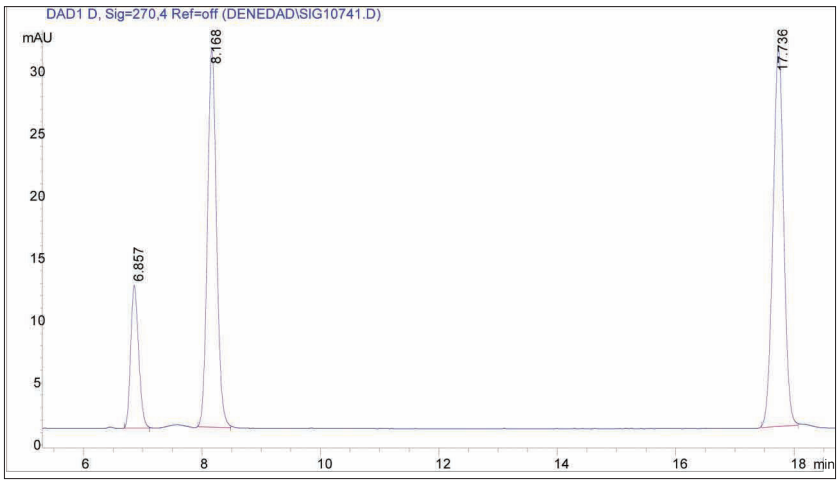

Table 1. *Validation data of SCP and TMP*
Tablo1. *SCP ve TMP analizlerine ait validasyon verileri*

Samples	Recovery (%)	r^2	LOD (µg/g)	LOQ (µg/g)
SCP (Chest)	93.25±4.46	0.9992	0.857	2.859
SCP (Liver)	87.95±4.5	0.9992	0.739	2.464
TMP (Chest)	97.55±3.58	0.9923	0.255	0.851
TMP(Liver)	95.38±6.15	0.9983	0.390	1.303
SCP (Boiled tissue)	98.12±6.51	0.9993	0.512	0.812
TMP (Boiled tissue)	98.87±7.49	0.9994	0.432	0.502

Table 2. *SCP and TMP levels of samples*
Tablo 2. *Örneklerde SCP ve TMP düzeyleri*

Samples	Chest		Liver	
	SCP	TMP	SCP	TMP
Raw tissue (µg/g)	49.10±7.26^{c}	1.91±0.19^{d}	60.31±10.47^{c}	1.56±0.67^{b}
Freezed tissue (30 day) (µg/g)	44.93±10.63^{c}	1.8±0.21^{cd}	61.87±7.24^{c}	1.52±0.33^{b}
Freezed tissue (45 day) (µg/g)	48.14±10.03^{c}	1.84±0.24^{cd}	60.91±11.62^{c}	1.59±0.63^{b}
Griled tissue (µg/g)	25.33±3.66^{b}	1.48±0.24^{bc}	20.97±8.53^{b}	1.34±0.41^{b}
Boiled tissue (µg/g)	21.46±3.68^{b}	1.15±0.57^{b}	27.33±5.84^{b}	0.97±0.07^{ab}
Boiled water (µg/ml)	00.00±00.00^{a}	0.68±0.04^{a}	00.00±00.00^{a}	0.57±0.05^{a}

deproteinized with acetonitrile, followed by treated with acetonitrile-hexane. They have obtained clean samples with dichloromethane-phosphate buffer exctraction and evaporation under nitrogen gas. In our study, tissues were extracted with dichloromethane, followed by the filtering with filter paper, treated with 3 N HCl and 3.8 M sodium acetate solution. As a result, purity of the samples is sufficient for applying to HPLC.

To previous HPLC methods conducted to determine sulfonamide residues in tissue, the wavelength was selected as 270 nm [16,19-21]. Although, the peak intensity of trimethoprim was reported to be better at 229 and 240 nm wavelengths, the wavelength of 270 nm was preferred in the analysis of TMP combined with sulfonamide [14,21,22]. In this study, the SCP, SMZ and TMP analysis were performed at the wavelength as 270 nm *(Fig. 1, Fig. 2)*. In previous studies, several solutions such as 20% acetonitrile, dichloromethane-phosphate buffer and columns such as C18, C4, C8 were used for determining sulfonamides and TMP [19-22]. In this study, the peaks for TMP, SMZ and SCP were taken as 6.857 min, 8.168 mine and 17.736 min respectively, by using methanol: water carrier system (60:40) with pH 3 (set by 10% orthophosphoric acid). Although, the retention time of SCP is longer than the other studies, obtaining the peaks of SMZ, SCP and TMP at different times and sufficient peak intensity may be considered as advantage of this method.

Kowalski et al.[18] reported that the r^2 value and recovery rate of SCP were found as 0.9997 and 72.8, respectively. Papapanagiotou et al.[14] found the recovery rate of sulphadiazine and TMP as 77.8-87.4% and 66.7-83.1%, respectively. In this study, high recovery values were obtained for both SCP and SMZ. According to the recovery and correleation coeeficient parameters of this study, Papapanagiotou's method [14] can be used for the analysis of SCP and TMP in chicken muscle and liver.

Unlike previous studies about sulfonamide analysis of tissues, it has found high recovery and, low LOD and LOQ values. This differencies can be related to modification of homogenisation procedures, high peak intensity and resolution with DAD at 270 nm, also chemometric calculation methods *(Fig. 1, Fig. 2)*.

Kostadinovic et al.[17] reported that, SCP residues in muscle tissue were eliminated faster than skin, liver, and kidney (7, 12 and 18 days respectively) in turkeys exposed to single administration of SCP; the highest tissue SCP level in liver was observed as 32.3 µg/kg. In our study, the highest SCP levels were also obtained from liver samples. The study results reveal that, the accumulation of SCP in liver was high like other studies [4].

There are several studies about the effects of cooking and storing on sulfonamides. According the studies, cooking processes have no significant effect on SMZ residues in pork meat [23,24]; ormethoprim and sulfadimethoxine residues decrease of fish meat [25]; sulfadiazine, sulfamethoxazole, sulfamonometoxin reduce of chicken meat, sulfadimethoxine, sulfaquinoxaline and sulfadoxine reduce

at different cooking and storing processes in broiler tissues [3,10,11,26]. In this study, a significant reduction was seen in the level of SCP and TMP in grilled and boiled chest tissue and boiled water as compared to raw chest tissue (P<0.05). The study results showed that, grilling and boiling processes have a reducing effect on both SCP residues in chest and TMP residues of grilling process. The SCP residues could not be identified in boiled water. This result can related to the weak passing of this drug to the water, disintegration of the drug with the effect of water and heat or transforming of the drug into different metabolites. These results compatible of several studies [10,11,26].

In the study SCP residue levels were decreased in grilled and boiled liver samples compared to raw liver samples (P<0.05), The SCP residues in boiled water were not within measurable levels. The results of the study reveal that, grilling and boiling processes have reducing effects on liver SCP residues.

In terms of TMP residue levels in liver, a significant reduction was seen only in boiled water compared to raw tissue (P<0.05), No difference was recorded between total TMP level of boiled tissue and water and raw liver TMP levels (P>0.05). These results suggested that, the TMP residue levels for liver were not affected from the storage in freezer, grilling and boiling processes.

There are many factors affecting drug residue levels in edible tissues like species, race, age and sex of animals; stability, solubility, pharmacokinetic and pharmaceutical differencies of drugs and administration route and time of drugs [10,11,23,27]. In this study, it is possible that, the reducing effects of cooking process on the residues of SCP and TMP in tissues are likely to be related to the above factors.

Although, many studies focused on the tolerance limits of drug residues in foodstuffs for raw tissues or organs, animal products such as meat, milk and eggs usually expose many process like cooking or canning before consuming. Aminoglycoside, macrolide and tetracyclines often remained in the tissues of slaughtered animals without disrupting for a long time, but some of the cooking and storing procedures could cause weak changes on drug residues [3,10,11,27]. During these processes many alterations may have seen in tissues like protein degradation, water and fat loss and change in pH. Therefore, it has been expected that, baking, roasting, frying, or cold storage processes may breakdown or convert of drugs to ineffective metabolites. So, cooking processes may be reduced drug residues taken in meat.

In conclusion, boiling and grilling processes caused a reduction in SCP and TMP residues at different proportions in broiler tissues; storing in freezer did not cause a significant change on residues of these drugs.

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