大韓獸醫學會誌(1994) 第34卷 第1號
*Korean J Vet Res* (1994) 34(1):141-146

# 육가공시 설폰아미드의 분해에 관한 연구

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(1993년 11월 18일 접수)

# A study on decomposition of sulfonamide during meat processing

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(Received Nov 18, 1993)

**초록** : 돈육의 가열과 냉동저장시 잔류된 설폰아미드가 증가 또는 감소되는 정도를 조사하였다. 설파메라진, 설파메타진, 설파모노메톡신, 설파디메톡신, 설파퀴녹사린을 돈육에 100ng/g씩 주사하고 30분간 60℃로 가열하였을 때 설파메타진은 40% 증가하였으나, 60-120℃로 가열한 다른 설폰아미드는 12-22% 감소하였다. 4주간 -20℃이하로 냉동하였을 때, 설파메라진과 설파퀴녹사린은 각각 12%, 19% 감소하였으나 다른 설폰아미드는 변화가 없었다. 설폰아미드의 검출한계는 고상분산을 이용한 정제시 2.6-27ng/g이었으며, 액상분배를 이용한 정제시 25-36ng/g이었다.

본 실험의 결과 돈육중의 설폰아미드는 가열과 냉동저장에 따라 안정성이 변화될 수 있으며, 잔류물 정제방법으로는 고상분산과 액상분배방법을 이용할 수 있을 것으로 생각된다.

**Key words** : pork, sulfonamide, sulfamethazine, cooking, freezing, residues

## Introduction

Drug residue in food from animal origin is a continuing problem. The development of bacterial populations resistant to antibiotics and antimicrobials was such potential health hazards[1]. Residue in meat could be converted to its metabolites by cooking or cooled storage. Drug-related antibacterial activity was decreased 7% by cooking of beef[2]. Loss of sulfamethazine was 40% in a cured pork product and 13.9% in pork after 15 days storage at -20℃[3,4]. Fisher et al[5]. found no loss of sulfamethazine in pork loin, liver, and cured ham as a result of cooking. Regulatory safe levels of sulfonamide are directed toward concentrations of drugs in uncooked meat. Sulfonamide metabolites could be converted to its parent drug, and one drug could be converted to its metabolites during extraction, cooking or cooled storage of meat samples. In the literature, these compounds were sulfamethazine, benzylpenicillin, and chloramphenicol[3,5,6]. The present study was conducted to examine the effect of cooking and freezing on residues of 5 sulfonamides in pork tissues.

The change of concentration of sulfonamide during cooking or cooled storage might be explained by sample weight loss or analytical error. Analytical methods for drug residue in food have been developed to improve accuracy and reproducibility. High pressure liquid chromatographic methods have

* This paper was supported in part by 'non-directed research fund', Korea Research Foundation, 1992.

been improved to simple, reliable and sensitive methods[7]. Weber and Smedly[8] reported determination of sulfamethazine residues in milk at 10ng/g by liquid partision clean-up techniques. Solid phase dispersion techniques simplified toilsome clean-up step[9]. The present study compared solid phase dispersion techniques with liquid phase partition techniques.

Depending new analytical method development for drug residue in food, trace residue less than ng/kg level could be measured. However, the observance of withdrawal time of drug is essential to decrease the violation rate. The violation rate of sulfamethazine residue in pork could be decreased by screening[10,11]. The present study surveyed sulfonamide residue in pork in the Cheju area.

## Materials and Methods

Pork samples were purchased in meat shops. Meat samples were sliced chops into 10cm thick(ca 10 g). All meat was sealed and frozen in polypropylene bags until the experiment. Five sulfonamides were sulfamerazine, sulfamethazine, sulfamonomethoxine, sulfadimethoxine, and sulfaquinoxaline(Sigma Chemical Co). Pork tissues were spiked by injection with 5 sulfonamides at concentration of 100ng/g. Unspiked samples served as controls. Sample weight was measured before cooking or freezing step to permit a direct comparision of concentrations in cooked, frozen, and uncooked meat. The equipments for cooking and freezing experiment were a convection oven and a deep freezer, respectively. The oven temperature was set at either 60℃ or 120℃, and meat samples were put on alumimum plates for 30 minutes in the temperature controled oven. The freezer temperature was set at either -20℃ or -70℃, and meat samples in polypropylene bags were stored in the freezer for 4 weeks. Duplicate samples of cooked, frozen, and uncooked meat were extracted using the solid phase dispersion[9] or the liquid phase partition method. The pork sample was extracted with acetone, and the acetone was evaporated. The residue was suspended in hexane, and partitioned with saline. The pH of saline with hexane in liquid phase partition method was adjusted to 7.0 with 0.1 N NaOH. Residues were extracted with chloroform from saline, and eluted through an alumina column. The LC condition was as follows[12]. The column was a 25cm × 4.6 mm id μBondapak(Waters Co). The mobile phase was water-acetic acidacetonitrile(88-1-12). The sample was eluted isocratically at a flow rate of 0.5 ml/min. Absorbance was measured using an UV detector at 270nm. Recoveries were determined by comparsion of the LC peak area with a standard curve prepared from varying concentrations of sulfonamide. Screening of sulfonamide in pork was done by above mentioned HPLC method.

## Results

The changes in the concentrations of sulfonamide in pork by cooking or cold storage were found to differ with the type of sulfonamides tested(Table 1). Cooked pork at 60℃ contained the highest concentration of sulfamethazine. The level of sulfamerazine was the lowest concentration of the pork samples investigated. Cooled pork showed no changes in mean values for residues of sulfamethazine, sulfamonomethoxine, and sulfadimethoxine. Sulfamerazine and sulfaquinoxaline decreased 12% and 19%, respectively, by freezing. These cooking and freezing related data indicated the unstability of sulfonamide by heat treatment.

Detection limit and recovery of solid phase dispersion techniques and of liquid phase partition techniques were calculated(Table 2). The average recovery from samples fortified at 100 ng/g was 78.4% for solid phase dispersion techniques and 84.2% for liquid phase partition techniques. The range of detection limit was 3.1-27ng/g for solid phase dispersion techniques and 25-36ng/g for liquid phase partition techniques. From these data, both methods could be used to determine sulfamethazine residues in pork at 100ng/g.

Sulfonamide residues in pork were surveyed in the Cheju area(Table 3). One case out of 79 specimens violated the safe residue level of sulfamethazine in

Table 1. Changes in concentrations of sulfonamide in pork by heating or freezing

| | Sulfonamide(ng/g)* | | | | |
|---|---|---|---|---|---|
| | SME | SMZ | SMM | SDM | SQX |
| Uncooked | 100 | 100 | 100 | 100 | 100 |
| Heating | | | | | |
| 60℃ | 77 | 140 | 98 | 101 | 96 |
| 120℃ | 78 | 100 | 72 | 75 | 72 |
| mean | 78 | 121 | 85 | 88 | 85 |
| Heating change, % | -22 | +21 | -15 | -12 | -15 |
| Freezing | | | | | |
| -20℃ | 69 | 100 | 92 | 95 | 85 |
| -70℃ | 92 | 101 | 100 | 102 | 90 |
| mean | 81 | 101 | 96 | 98 | 88 |
| Freezing change,% | -19 | +1 | -4 | -2 | -12 |

*SME : sulfamerazine, SMZ : sulfamethazine, SMM : sulfamonomethoxine, SDM : sulfamonomethoxine, SQX : sulfaquinoxaline*

Table 2. Comparision of clean-up methods for sulfonamide in pork

| Sulfonamide | Solide phase dispersion | | Liquid phase partition | |
|---|---|---|---|---|
| | detection limit(ng/g) | recovery % | detection limit(ng/g) | recovery % |
| Sulfamerazine | 4.8 | 90 | 25 | 68 |
| Sulfamethazine | 3.1 | 82 | 26 | 89 |
| Sulfamonomethoxine | 2.6 | 85 | 32 | 88 |
| Sulfadimethoxine | 27 | 75 | 34 | 87 |
| Sulfaquinoxaline | 13 | 60 | 36 | 89 |

Table 3. Sulfonamide residues in pork tissue in the Cheju

| Method | Positive | Doubtful | Negative | Total |
|---|---|---|---|---|
| HPLC* | | | | |
| -No. of sample | 1 | 0 | 78 | 79 |
| -Percentage | 1.3 | 0 | 98.7 | 100 |

* Sulfamethazine was measured

pork, and the violation rate was 1.3% by HPLC method. The insufficient amount of data obtained from the HPLC method precluded a direct comparision with published data. These results indicated low violation rate of sulfamethazine residue in pork in the Cheju area.

## Discussion

Meat is sold either fresh or in a frozen storage condition. The meat samples which are to be used for residue analysis should be stored in a cooled state prior to a regulatory assay. The regulatory residue level in uncooked meat was not concern neither the storage temperature or the storage period of meat samples. In the literature, the storage temperature and period of meat samples has been reported to affect the chemical characteristics of the residues in meat samples, which might result in false positive or false negative reports of the residue level in meat samples. O'Brien et al[2]. showed the stability of ampicillin at -20℃ in tissues. Boison et al[13]. reported the effect of cold-temperature storage on stability of benzylpenicillin in tissues. Fisher et al[5]. measured the change of sulfamethazine glucoside into sulfamethazine in pork ham by various cooking methods, which indicated a 26% increase by cooking.

The present study showed changes in mean values for cooked pork samples. The level of

sulfamethanzine increased by 21% after heating, but those of other sulfonamides decreased by 12-22%. In frozen pork samples, the mean values decreased by 12-19%, while the values of sulfamethazine, sulfamonomethoxine, and sulfadimethoxine did not change. The changes could be explained by conversion of sulfonamide into its metabolites. The extent of cooking-related change in the concentrations of sulfamethazine was tissue dependent. The increase in sulfamethazine caused by cooking was high for liver(67%) and ham(26%), and low for loin(-8%). The conversion of sulfamethazine glucoside to sulfamethazine was rapid at higher temperatures and in acidic environments. The change in the concentration of sulfamethazine was more rapid at 60℃ than at 120℃. This was not consitstent with a previous report[5]. The fractions of sulfonamide metabolites in pork samples were not measured in this study. Howerver, the data presented here make it apparent that sulfonamide was unstable during cooking or frozen storage, and metabolites of sulfonamides should be considered in safe residue level. In Germany and Switzerland, sulfonamide and its metabolites(such as glucoside or acetate) were included within the safe residue level(100ng/g)[14].

Sulfamethazine glucoside could be formed in liver under cold storage conditions[14,15]. The level of sulfamethanzine in the pork liver stored at -20℃ decreased from 0.57μg/g to 0.04μg/g over a 12 month period. No decrease in the level of sulfonamide except sulfaquinoxaline was observed in the frozen pork during the experimental period(4 weeks). Sulfaquinoxaline decreased 12% in frozen pork. Autolysis and hydrolysis might be inhibited in frozen tissues, and the concentration of sulfonamide in frozen pork did not change significantly. Pork seemed to be consumed before significant change of sulfonamide in the pork tissue.

For the determination of sulfonamide or its metabolites in food of animal origin, a general approach involves extraction, sample clean-up and analysis steps. The HPLC methodology is used conventionaly in this field, and reviewed recently by Agarwal[7]. Clena-up is a tedious and time consuming step for HPLC, which may restrict the daily analysis capability in a laboratory. A matrix solid-phase dispersion technique, which is based upon the use of C18 polymer phase bound to a silica support, disrupt the cell membrane of biological matrixes. The detection limit of these methods is 31.2ng/g for tissue[9]. In this experiment, the detection limit and the recovery of sulfamethazine was 3.1ng/g and 82% by solid phase dispersion technique, and 26ng/g and 89% by the liquid phase partition technique. The solid phase dispersion technique could be used as one of clean-up steps. However, it is necessary to adjust the pH of the extract a low pH of 1-2 before loading on to the solid phase dispersion column[16]. With a milk sample, more solution must be eluted when using the solid phase technique. Liquid phase partition techniques also need a pH adjustment for good recovery. In this experiment, the pH of saline with hexane was 3, and it required adjustment to 7. This value is the same as that reported by Ackermans et al[17], who separated sixteen sulfonamides in pork meat extracts by capillary zone electrophoresis.

The potential of the development of bacteria resistant to antibiotics and antimicrobials residues levels have been reported. The use of a combination of three kinds of drugs increased the incidence of *Staphylococcus aureus* 4.6 times over the incidence found with a single drug. The incidence found with a single drug was 6.0 times the control incidence[1]. To reduce the possible hazards from drug residues in food of animal origin, the required drug withdrawal time should be strictly observed. Surveys of the residue level in food has been found to decrease the violation rate. Screening of sulfamethazine in pork liver decreased the violation rate from 13% in 1977 to 3.8% in 1987[10]. This experiment showed 1.3% of violation rate. This rate was low compared with the reported rate of the 20.8% in 1989 by our laboratory[18]. The detection of sulfonamide in the screening method was accomplished by bioassays or TLC. To reduce non-specific growth inhibition by lysozyme in bioassay, raw milk samples were heated for 10 minutes at 80℃. Inhibition of growth could then be overcome by the addition of para-amino benzoic acid. In addition the initial pH of the test medium was adjusted to 8.0 rather than 7.0[19]. Unruh et al[20]. reported a sensitive TLC method for sulfamethazine in

pork tissue, 0.25ng/g, using solid phase extraction, derivatization with fluorescamine, and scanning densitometer.

## Conclusion

The stability of sulfonamides in pork tissues can be changed by cooking or cold storage, depending upon the particular sulfonamide being studied. Sulfamethazine, sulfamonomethoxine, and sulfadimethoxine were stable when stored at -20℃ for 4 weeks. The levels of sulfamerazine, sulfamonomethoxine, sulfadimethoxine, and sulfaquinoxaline decreased by cooking at 60-120℃ for 30 minutes. Two kinds of clean-up methods, solid phase dispersion and liquid phase partition, appeared to be reliable to measure the residue in pork at 100 ng/g.

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