SHORT COMMUNICATION

Degradation kinetics of six sulfonamides in hen eggs under simulated cooking temperatures

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Abstract: Six sulfonamides, *i.e*., sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole and sulfamonomethoxine, were applied to spike whole hen eggs at 0.1 mg kg^{-1} eggs. The spiked hen eggs were heated at 80 and 100 °C to investigate the degradation kinetics of the sulfonamides under simulated cooking conditions. The sulfonamides added were extracted twice from the spiked eggs with dichloromethane by an ultrasonic-assisted extraction, and analyzed by a HPLC method after purification. The first-order rate constants and half-life times of the sulfonamides were calculated, and the corresponding apparent activation energy of their degradation was also obtained by application of the Arrhenius equation. The results indicated that all six sulfonamides degraded faster at the higher heating temperature, with first-order rate constants ranging from 0.0056 to 0.0108 min^{-1} at 80 °C and from 0.0147 to 0.0394 min^{-1} at 100 °C. The apparent activation energies for the degradation of the sulfonamides were estimated to be in the range 30.9 to 77.5 kJ mol^{-1}. Sulfadiazine and sulfadimethoxine had the shortest and longest half-life time, respectively, and were the most instable and stable.

Keywords: sulfonamide; hen eggs; thermal degradation; kinetics; HPLC.

INTRODUCTION

Sulfonamides are synthetic antibiotics with a wide spectrum against most gram-positive and many gram-negative organisms, and are used in human medicine for therapeutic and prophylactic purposes. Sulfonamides are also sometimes used as additives in animal feed for the prevention and treatment of bacterial diseases in livestock, and might be accumulated in meat, eggs, milk and

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fish. In a recent study, Wang *et al.* found that 24 of 56 egg samples had sulfadiazine contents larger than 100 μg kg^{-1}.[1] Sulfonamide residues remaining in foods are considered to be a harmful risk for consumers. A legal maximum residue limit for sulfonamides was set at 100 μg kg^{-1} in the United States and the European Union.[2] Various LC-based analysis methods with UV,[3] diode array or photo-diode array detector,[4,5] fluorescence detector,[6] and MS or MS/MS detection [7–9] have been developed to determine sulfonamide residues in various food matrices. Other analysis techniques based on immunoassay[10,11] and capillary zone electrophoresis[12] were also applied in recent studies.

The residue levels of sulfonamides in foods are affected by food processing, especially heat treatment. It is of interest to determine if sulfonamide residues can be decreased by the usual cooking methods of foods. Furusawa and Hanabusa used three different cooking procedures to study the net change of four sulfonamide residues in chicken muscles.[13] Rose *et al.* studied the heat stability of sulfamethazine in animal tissues subjected to a range of cooking processes, including boiling, roasting, grilling, frying, pressure cooking and microwaving.[14] Fischer *et al.* reported cooking-related changes in sulfamethazine residues and its metabolites in pork products.[15] Xu *et al.* also investigated the effect of cooking on sulfadimethoxine residues in the muscle of channel catfish.[16] All these mentioned results showed that heat treatment decreased the residue levels of sulfonamides to different extents. Unfortunately, the degradation kinetics of the sulfonamides were neither measured nor studied.

In the present work, six sulfonamides, *i.e.*, sulfadiazine (SDZ), sulfadimethoxine (SDM), sulfamerazine (SMR), sulfamethazine (SMZ), sulfamethoxazole (SMX) and sulfamonomethoxine (SMM), were selected to study their degradation kinetics in hen eggs under simulated cooking conditions. The spiked whole hen eggs were heated at two temperatures for different periods. The sulfonamide residues left in the cooked eggs were separated by ultrasonic-assisted extraction and analyzed by a HPLC method. The degradation kinetics of the sulfonamides were calculated and compared, whereby the degradations of the sulfonamides were treated as first-order reactions.

EXPERIMENTAL

Reagents and materials

Six sulfonamide standards, SDZ, SDM, SMR, SMZ, SMX and SMM, were purchased from Sigma (St. Louis, MO, USA) with declared purity of more than 99.5 %, and stored at −18 °C. The first stock solutions (200 mg L^{-1}) of the individual sulfonamides were prepared by dissolving the sulfonamides in methanol separately. The second stock solutions of the individual sulfonamide were prepared daily by diluting the first stock solution with mobile phase to 10 mg L^{-1}. Working solution of the mixed sulfonamides was daily prepared by combining the second stock solution of the single sulfonamide in different volumes and diluting to a fixed volume with mobile phase. These were used to determine the standard curve or to spike hen eggs. The first stock solutions of the sulfonamides were stored at 4 °C for one week and

then discarded later. The methanol used was of chromatographic grade; other employed chemicals and solvents were analytical grade, and all were procured from Sigma-Aldrich (Schnelldorf, Germany). Water used was highly purified water prepared with Milli-Q PLUS (Millipore Corporation, New York, NY, USA). Hen eggs were purchased from a supermarket in Harbin, Heilongjiang Province and stored at 4 °C before use.

Preparation of spiked hen eggs

Some hen eggs were broken by hand and the contents of the eggs (whole hen eggs) were collected and mixed well with a hand blender. After this, working solution of the mixed sulfonamides was added to the eggs to insure the content of the sulfonamides was 100 μg kg^{-1} eggs. The spiked eggs were mixed well to distribute the sulfonamides. Portions of spiked eggs, about 50.0 g, were put in beakers of 100 mL capacity, stood at ambient temperature for 1 h, and then heated gently in a water bath to 80 or 100 °C with gentle stirring at the beginning. After the central temperature of the sample had reached the selected cooking temperature, zero reaction time was set and the eggs were heated continuously for different periods (4, 8, 12, 16 and 20 min). Then, the portions were rapidly cooled in ice–water to ambient temperature and stored at 4 °C until sulfonamide extraction and analysis. These samples were used for the kinetics study.

Whole hen eggs were heated at 100 °C for 20 min without sulfonamide addition. The heated eggs were cooled in ice–water both to ambient temperature and milled into fine particles. The cooked eggs were spiked and mixed well with the six sulfonamides at three levels, 0, 0.05 and 0.1 mg kg^{-1} egg, left at ambient temperature for 1 h, and then used as the controls for recovery evaluation. The cooked eggs were also spiked and mixed well with the six sulfonamides at 6, 8, 10, 15 and 20 μg kg^{-1} eggs, and used to determine detection limits of the sulfonamides.

Extraction and purification of sulfonamides from spiked hen eggs

The procedure for the extraction of the sulfonamides from the prepared samples was as per the method of Kishida[17] and our previous work[18] with some modifications. The spiked, cooked eggs, 3.0 g, were put in a centrifuge tube together with 15.0 mL of dichloromethane, and homogenized for 30 s with a handheld homogenizer. Then, the tube was put in an ultrasonic extractor and subjected to further extraction for 10 min at 10 °C. The mixture was centrifuged at 400×g for 5 min, and the liquid phase was collected. The residue left was re-extracted once with 15.0 mL of dichloromethane as above. The combined liquid phase was filtered through anhydrous sodium sulfate (2.0 g) into a 50 mL heart-shaped flask.

Glycol about 0.1 mL was added to the flask and the content in the flask was evaporated with nitrogen gas in an evaporation station to remove the dichloromethane. The residue was reconstituted to a volume of 1.50 mL with mobile phase, extracted with 3.0 mL *n*-hexane three times to remove the lipids, and filtered through a 0.45 μm microporous membrane before HPLC analysis.

HPLC analysis of sulfonamides

The analysis of six sulfonamides was performed in a liquid chromatograph 2695 Series (Waters Corporation, Milford, MA, US) with a diode array detector (DAD), a C$_{18}$ reversed phase column (Hypersil ODS 250 mm×4.6 mm i.d. 5 μm, Elite Technologies, Dalian, Liaoning, China) at ambient temperature. The mobile phase consisted of methanol and 0.08 % acetic acid in a volume ratio of 38:62. The flow rate was 0.7 mL min^{-1}. Detection was realized at a wavelength of 275 nm and the injection volume of the sample was 20 μL. Qualitative identification of each sulfonamide in the samples was performed by comparing its UV spec-

trum and elution time with those of the corresponding standard. Quantification of six sulfonamides was performed by comparing the peak areas of the sulfonamides to a calibration curve of the standards, and used for the calculation of their degradation kinetics. Multitude-point calibration was used in this work.

Statistical analysis

All data are expressed as means from at least five independent trials. The rate constants of degradation of the sulfonamides were calculated by linear regression analysis using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Analysis of sulfonamides by HPLC

Chemical structures of six sulfonamides are given in Fig. 1. With the procedure employed in this work, the six sulfonamides were extracted efficiently from the cooked hen eggs and measured accurately by HPLC. Typical HPLC profiles of the six sulfonamides obtained from the standard solutions and the samples are given in Fig. 2, which shows that the six sulfonamides were well separated by the column with elution times ranging from about 6 to 17 min. SMM was identified in the raw hen eggs by its UV spectrum obtained using the DAD and its retention time. The analysis result revealed that the linear range for the determination of the six sulfonamides was from 0.01 to 1.0 mg kg^{-1} eggs ($R^2 \geq$ ≥ 0.999). The data listed in Table I demonstrates that the detection limits for the six sulfonamides ranged from 8 to 10 μg kg^{-1} eggs (peak area three times larger than the noise), and the addition recovery of the six sulfonamides at two addition levels ranged from 78.8 (SMR, at 0.1 mg kg^{-1} eggs) to 103 % (SMM, at 0.1 mg kg^{-1} eggs). The lower recovery of SMR might be due to its lower stability, *i.e.*, easier degradation during analysis sample preparation. The relative standard deviation of the recovery of the six sulfonamides was less than 8.5 %. This means that the applied extraction and purification procedure appeared to be efficient, and the HPLC analysis procedure was applicable to detect sulfonamide residues in the prepared egg samples.
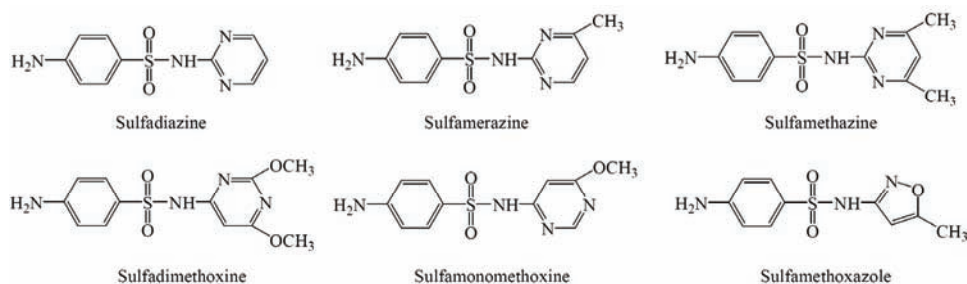

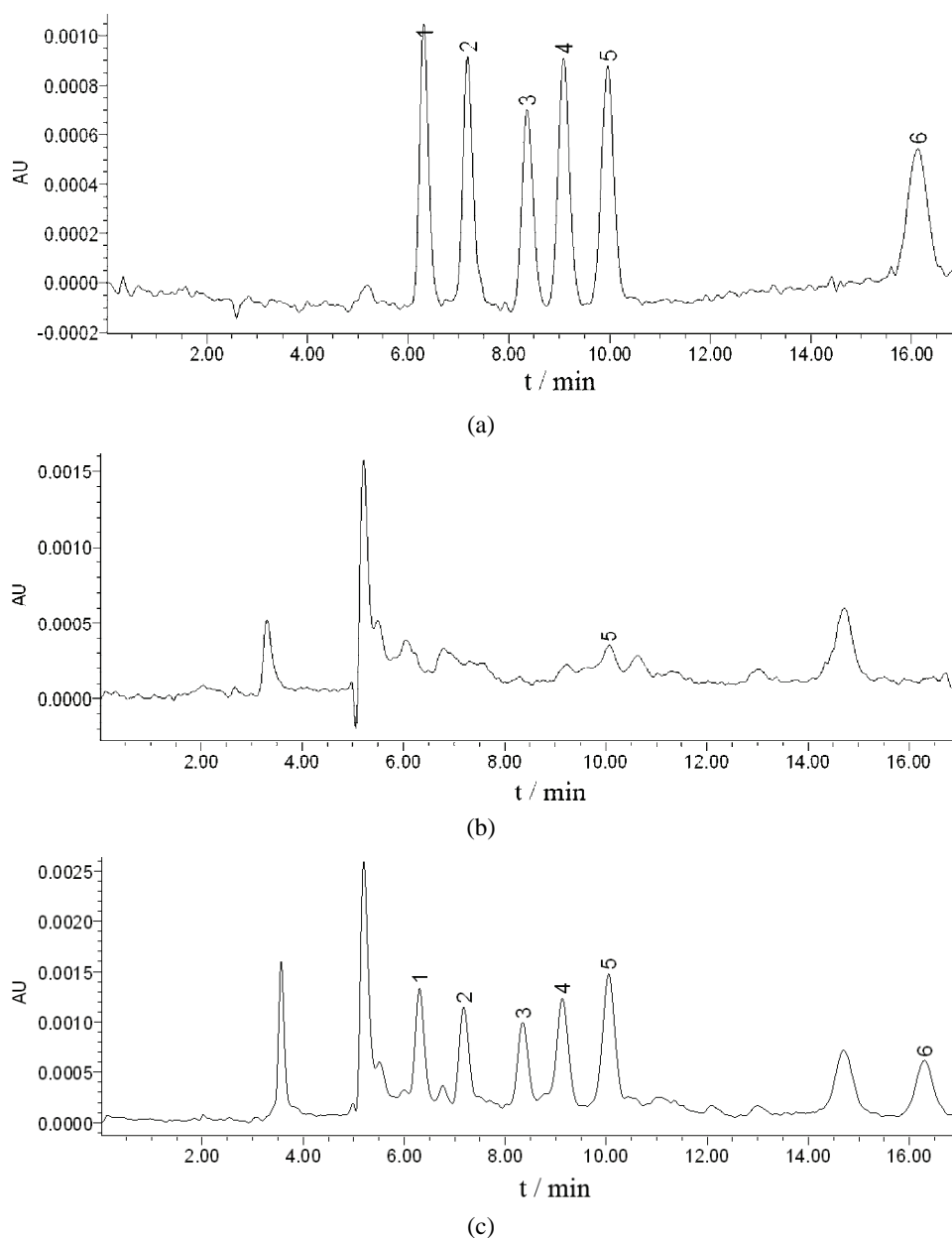Fig. 1. Chemical structures of the six studied sulfonamides.

Fig. 2. Typical HPLC profiles of the six sulfonamides obtained from standard solution of 0.05 mg kg^{-1} (a), control (b) and extract of 0.1 mg kg^{-1} spiked whole hen eggs heated at 80 °C for 4 min (c). Peaks 1 to 6 in the profiles represent SDZ, SMR, SMZ, SMX, SMM and SDM, respectively. As SMM was identified in the raw hen eggs, its content served as the background and it was discarded from the experimental data of the degradation kinetics.

TABLE I. Mean recovery and limits of detection (*LOD* / %) of six sulfonamides from the spiked hen egg using HPLC analysis (the number of replicate measurements was five)

| Sulfonamide | Spiking level of the sulfonamides | | | | *LOD* / μg kg^{-1} |
| | 0.05 mg kg^{-1} | | 0.1 mg kg^{-1} | | |
	Recovery, %	*RSD* / %	Recovery, %	*RSD* / %	
SDM	83.5	7.7	91.8	5.8	10
SDZ	83.0	7.6	79.4	8.4	8
SMM	98.6	6.3	103.0	8.2	8
SMR	89.1	5.2	78.8	7.6	8
SMX	85.2	5.7	83.5	3.3	8
SMZ	86.8	4.3	89.7	4.1	10

Degradation parameters of the six sulfonamides in hen eggs during cooking

The sulfonamide residues remaining in the cooked hen eggs subjected to different heating times were extracted and analyzed. Significant loss of the six sulfonamides was found before zero reaction time. The final results are given in Table II. The amount of sulfonamide residues showed a decreasing trend as the heating time increased, indicating the degradation of the six sulfonamides.

TABLE II. Contents of six sulfonamides remaining in the spiked hen eggs after heating at different temperatures for various times (the number of replicate measurements was five)

| Cooking temperature, °C | Heat time, min | Content of the sulfonamides, μg kg^{-1} | | | | | |
		SDM	SDZ	SMM	SMR	SMX	SMZ
80	4	78.4	41.0	69.1	65.8	67.1	76.7
	8	76.9	40.0	68.0	64.0	65.7	75.0
	12	74.4	38.9	64.4	63.0	64.0	73.0
	16	73.3	36.1	62.7	60.8	61.8	69.1
	20	71.7	34.8	61.0	57.8	61.0	65.5
100	4	68.0	29.3	53.5	47.5	59.1	61.9
	8	64.0	25.2	51.5	45.4	54.0	60.5
	12	60.0	24.5	44.2	40	47.2	54.9
	16	56.4	17.5	37.6	32.1	46.4	49.2
	20	54.0	16.0	36.0	29.7	42.6	48.5

Furusawa and Hanabusa found that when chicken muscles were cooked by boiling, roasting or microwaving, the amount of SDM, SMX, SMM and sulfaquinoxaline (SQ) remaining could be well-described by exponential equations.[13] Their result meant that the degradation of four sulfonamides might be first-order reaction, and the content variation of each sulfonamide in chicken muscles at different cooking times would behave as Eq. (1).

$$-\frac{\mathrm{d}c}{\mathrm{d}t} = kt \tag{1}$$

where c is the content of the sulfonamide, t is cooking time and k is the first--order rate constant of degradation of the sulfonamides.

The first-order rate constant of degradation and the half-life times of the six sulfonamides in hen eggs at two cooking temperatures were thus calculated from the data listed in Table II using Eqs. (2) and (3):

$$\ln c = -kt + A \tag{2}$$

$$t_{1/2} = \frac{0.693}{k} \tag{3}$$

where A is a constant and $t_{1/2}$ is the half-life time of the sulfonamide. The obtained values are given in Table III.

TABLE III. Kinetic parameters of degradation of six sulfonamides in hen eggs at two cooking temperatures (k / min^{-1}, first-order rate constant of degradation; R, coefficient of regression; $t_{1/2}$ / min, half-life time)

Sulfonamide	Kinetic parameter	80 °C	100 °C	R^2
SDM	k	0.00560	0.0147	0.990–0.996
	$t_{1/2}$	124	47.1	
SDZ	k	0.0108	0.0394	0.933–0.960
	$t_{1/2}$	64.2	17.6	
SMM	k	0.00830	0.0277	0.957–0.976
	$t_{1/2}$	83.5	25.0	
SMR	k	0.00780	0.0321	0.956–0.960
	$t_{1/2}$	88.8	21.6	
SMX	k	0.00630	0.0202	0.957–0.985
	$t_{1/2}$	110	34.3	
SMZ	k	0.00990	0.0174	0.943–0.962
	$t_{1/2}$	70.0	39.8	

The data listed in Table III indicate that first-order rate constant of degradation was the highest for SDZ and the lowest for SDM, meaning they were the most instable and stable, respectively, at the two studied cooking temperatures. When the cooking temperature was elevated from 80 to 100 °C, all six sulfonamides degraded faster with the increases ranging from about 1.8 (SMZ) to 4.1 (SMR) folds. Based on the relationship between the first-order rates of degradation and temperature, the apparent activation energy for degradation could be estimated from the Arrhenius equation; the calculated activation energies for the six studied sulfonamides ranged from 30.9 (SMZ) to 77.5 kJ mol^{-1} (SMR).

It was reported that the loss of SMZ from animal tissue cooked in oil at 260 °C was obvious, with a half-life time of about 5 min.[14] Fischer *et al.* found that cooking causeed on average a 46.1 % reduction of SDM in raw fillet of fish fried in vegetable oil at 190 °C.[15] In addition, SDM residues in fish muscles cooked at 190 °C for 45 min were decreased by 54 %.[16] The present result and the previously published results all demonstrate that the procedure of cooking could

decrease the residue levels of sulfonamides in food of animal-origin. The results from Furusawa and Hanabusa showed that the reductions of SDZ, SMX, SMM and SQ were 45–61 % after 12-min boiling and 38–54 % after 12-min roasting at 170 °C.[13] In their paper, it was also stated that when chicken muscle was cooked by boiling or other methods, SDZ appeared to be the most unstable and SMM the most stable, which is in agreement with the present finding. The study of Furusawa and Hanabusa also showed that the half-lives of SDZ, SMX, SMM and SQ in chicken muscle cooked by boiling and roasting decreased in the range from 7.3 to 20.3 min, which might support the results obtained in the present study at 100 °C.

CONCLUSIONS

The degradation kinetics of six sulfonamides, i.e., sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole and sulfamonomethoxine, in hen eggs at two simulated cooking temperatures were studied in the present work by measuring the sulfonamide residues in the cooked eggs by HPLC. The degradation of the sulfonamides was enhanced at higher temperature. The degradation rate constants of the sulfonamides were obtained assuming first-order reactions, and were found to range from 0.00560 to 0.0108 min^{-1} at 80 °C, or from 0.0147 to 0.0394 min^{-1} at 100 °C. The apparent activation energy for the degradation of the six sulfonamides was estimated to be 30.9 to 77.5 kJ mol^{-1}. Sulfadiazine appeared to be the least stable and sulfadimethoxine the most stable.

И З В О Д

КИНЕТИКА РАЗЛАГАЊА ШЕСТ СУЛФОНАМИДА У КОКОШИЈЕМ ЈАЈЕТУ У СИМУЛИРАНИМ УСЛОВИМА КУВАЊА

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Шест сулфонамида је унето у кокошије јаје (0,1 mg kg^{-1} јаја): сулфадиазин, сулфадиметоксин, сулфамеразин, сулфаметазин, сулфаметоксазол и сулфамонометоксин. Јаја су загревана на 80 и 100 °C да би се испитала кинетика разлагања ових сулфонамида у симулираним условима кувања. Суфонамиди су екстраховани из јаја дихлорметаном у ултразвучном купатилу и анализирани методом HPLC. Израчунате су константе реакције првог реда, полуживот сулфонамида и енергије активације применом Аренијусове једначине. Резултати су показали да се свих шест сулфонамида брже распада на вишим температурама, а константе брзине су биле 0,0056–0,0108 min^{-1} на 80 °C и 0,0147–0,0394 min^{-1} на 100 °C. Израчуната енергија активације за разлагање сулфонамида је била 30,9–77,5 kJ mol^{-1}. Сулфадиазин је имао најкраћи полуживот и био је најмање стабилан, док је најдужи полуживот и највећу стабилност имао сулфадиметоксин.

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