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Effect of Deep-Frying at Different Temperature and Time on Sulfonamide Residues in Chicken Meat-Balls

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ABSTRACT

A study on the effect of deep-frying at different times and temperatures on sulfonamide (SA) residues in chicken meat-balls has been carried out. The SAs used were sulfadiazine (SDZ), sulfamethazine (SMZ), sulfamethoxazole (SMX), and sulfaquinoxaline (SQX). Time factor (3, 6, and 9 min) showed greater effect on the reduction of SAs compared to the temperature factor (170, 180, and 190°C). Through the Pearson Correlation analysis, reduction of SA concentration was positively related to the increase of the internal temperature during deep-frying process. The weight loss of the chicken meat-balls after deep-frying also is another factor in the reduction of SA residues. The longer the duration of deep-frying and the higher the temperature, the lower SA concentrations were observed. Deep-frying process could help in reducing the SA residues in chicken meat-balls where the maximum reductions obtained were 37.5, 27.5, 40.7, and 27.6% for SDZ, SMZ, SMX, and SQX, respectively at the maximum frying time and temperature. However, controlling the time and temperature of cooking at its best is still needed to retain the taste and appearance of the food. In this study, chicken meat balls fried at 180°C for 6 min resulted in the best condition for outward appearance and consumption as well as reduction in SA residues.

Key words: sulfonamide, deep-frying, chicken meat-balls

INTRODUCTION

Chicken meat and chicken meat products are among the most common food consumed worldwide. Numerous chicken products have been developed for convenience and variety, including chicken meat-ball. Despite the frequent intake of chicken meat, consumers are exposed to the adverse effect caused by veterinary residues, including sulfonamides (SAs). The residues are mainly found in chicken meat due to the over dosage in the farm and slaughtering the chicken before withdrawal period[(1)]. For consumers' safety, the EU has restricted the SA residues to 100 μg/kg of food products[(2)]. However, possibility for the residues to exceed the limit is still high. Furthermore, the residues could be carried over from raw chicken meat to chicken meat products.

Few studies have addressed the effect of cooking or heat treatments on the SA residues in different samples and sulfonamides. Dehai *et al.*[(3)] had used sulfadimethoxine as antibiotic in muscle of channel catfish and had an average of 46.1% reduction from raw fillet of fish after baking, smoking and frying (in canola oil and vegetable oils). Meanwhile, Lan *et al.*[(4)] applied sulfamethazine in the research on tilapia meat for microwave and roast treatments and resulted in 90% and 85% reductions, respectively. A study by Furusawa and Hanabusa[(5)] was carried out on various heat treatments against sulfadiazine, sulfamethoxazole, sulfamonomethoxine and sulfaquinoxaline residues in chicken thigh muscle. The results were 45-61% reduction with boiling, 38-40% reduction with roasting except for sulfadiazine, and 35-41% reduction with microwaving in all four sulfonamides.

Chicken meat-balls were chosen as the samples because it has common ingredients with other chicken meat products. Deep-frying is one of the most popular thermal processes of chicken meat-balls. In addition, different frying times and temperatures were used as study parameters. Based on these factors, sulfonamide residues in deep-fried chicken meat-balls could result in

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various outcomes compared to previous studies. Therefore, the objectives of this study were to observe the effect of frying temperature and time on selected sulfonamide residues in chicken meat-balls and to determine the relationship of deep-frying time and temperature, internal temperature and weight loss of the chicken meat-balls as the factors of the SAs residues reduction.

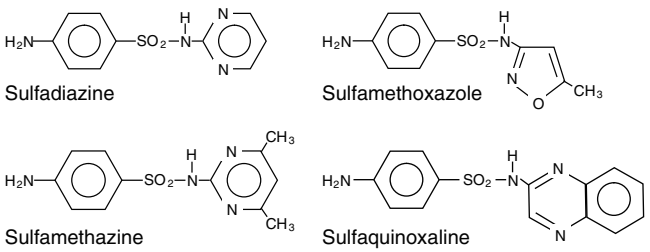

Figure 1. Chemical structures of sulfonamides.

MATERIALS AND METHODS

I. *Materials*

Free antibiotic chicken (Nutriplus), corn starch (Nona), ground black pepper (McCormick), garlic powder (McCormick), fine granulated sugar (CSR) and pure dry vacuum salt were purchased from supermarket whereas sodium tripolyhosphate was from Meilun Food Chemical Sdn. Bhd. (Selangor, Malaysia).

II. *Apparatus and Reagents*

(I) *Apparatus*

Bowl cutter/mixer (ADE SL-18, Hamburg, Germany), digital water bath (EYELA, SB-1000, Japan) and deep-fryer (Philips HD 6155 Cucina, Philips Malaysia Sdn. Bhd., Malaysia) were used to process and cook chicken meat-balls. Digital thermometer (Thermocouple, Cole-Parmer, Chicago, IL, USA) and electronic balance (PB503-S/FACT Precision Balance, Mettler Toledo, USA) were used for internal temperature and weight changes measurements, respectively. Homogenizer Ultra Turrax basic (IKA Labortechnik, Germany), Clements 2000 benchtop centrifuge (Sydney, Australia), sonicator Ultrasonik 104X (Neytech, USA), a rotary evaporator (EYELA, 1L Rotary Evaporator, N-1001S-W, equipped with an aspirator, A-1000S and a digital water bath, SB-1000, Japan), a nitrogen-evaporating unit (Pierce, ReactiTherm Heating Module, Rockford, IL, USA), auto-vortex mixer (Stuart, USA), and eppendorf microfuge (EBA 12, Hettich Zentrifugen, Germany) were used for sample preparation.

(II) *Solvents and Other Reagents*

Acetonitrile, acetone, methanol, dichloromethane and *n*-hexane (all HPLC grade) were purchased from Merck (Darmstadt, Germany).

(III) *Standards*

Sulfadiazine (SDZ), Sulfamethazine (SMZ), Sulfmethoxazole (SMX) and Sulfaquinoxaline (SQX) were produced by Sigma (St. Louis, MO, USA), which were provided by Department of Veterinary Services, Salak Tinggi, Malaysia. Stock standard solution was prepared by dissolving 0.01 g of each SA standard with 10 mL of 90% acetonitrile (*n*-hexane saturated), separately. Mix standard solution was prepared by combining 1.25 mL of each stock standard solution and was made up to 50 mL with 50% methanol in 0.01 M ammonium acetate (pH 4.6). Working mix standard solution at a concentration of 0.5 μg/mL was prepared by diluting the mix standard solution with 50% methanol in 0.01 M ammonium acetate (pH 4.6) prior to the analysis.

III. *Preparation of Chicken Meat-Balls*

(I) *Production of Chicken Meat-Balls and Fortification of the Sulfonamides*

Chicken meat-ball production was carried out according to formulation developed in the Faculty of Food Science and Technology, UPM. The chicken breast meat (1 kg) was weighed and cut into small pieces before being minced in the bowl cutter / mixer until the chicken meat was completely ground. While mincing, the chicken meat was fortified with SA mix standard solution at 0.5 μg/g level of chicken meat. The corn starch, (100 g), sodium tripolyphosphate (25 g), sugar (5 g), salt (5 g), black pepper (2.5 g) and garlic (2.5 g) were added portion by portion into the bowl cutter / mixer while mixing. Ice cubes were added to the mixture to control the temperature. All chicken breast meat, sulfonamides and ingredients were well homogenized during the mixing. The chicken dough was made into portions of 10 g and stored at -20°C prior to cooking process.

(II) *Deep-Frying of Chicken Meat-Balls*

One liter of distilled water was preheated to 100 ± 1°C in a water bath before 10 portions of chicken ball dough weighed 10 g each were boiled for 3 min. The process was repeated with other batches with new distilled water for every boiling process to prevent contamination of excess residues. The boiled chicken meat-balls were stored over night below -4°C prior to deep-frying. One liter of Seri Murni pure vegetable cooking oil (palm oil) was preheated in deep-fryer to 170 ± 1°C before the boiled chicken meat-balls were deep fried for 3, 6, and 9 min. The processes were repeated at 180 ± 1 and 190 ± 1°C for 3, 6, and 9 min.

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(III) *Internal Temperature Measurement*

The internal temperature was measured by striking the sensor probe of digital thermometer into the center of the chicken meat-ball before deep-frying process. Then it was immersed into the oil and reading was taken.

(IV) *Weight Changes Measurement*

The weights of chicken meat-balls were measured using an electronic balance before and after every deep-frying process to calculate any changes in weight.

IV. *Sulfonamide Residues Analysis*

(I) *Extraction / Clean-Up*

The sulfonamide extraction was carried out using the modified method of Furusawa and Hanabusa[5], Stoev and Michailova[6], Kao *et al.*[7] and Hela *et al.*[8]. Ten grams of sample was weighed in glass centrifuge tube, after adding 30 mL of 90% acetonitrile (*n*-hexane saturated); the sample was homogenized for 1 min using Ultra Turrax homogenizer and centrifuged at 3500 rpm for 10 min. The supernatant was transferred into a pear-shaped flask. Twenty mililiter of acetone was added to the sediment before the mixture was sonicated for 10 min. The solution was centrifuged once again and the supernatant was added into the same pear-shaped flask. The mixed solution was evaporated at 50°C until near to dryness. Afterwards, 5 mL of dichloromethane was added, mixed using vortex and transferred into test tube. The step after the addition of dichloromethane was repeated three times and the combined dichloromethane was dried under nitrogen at 50°C. The residue was reconstituted with 1 mL of 50% methanol in 0.01 M ammonium acetate (pH 4.6) and mix properly by using vortex. Two mililiter of *n*-hexane was added into the test tube and mixed properly by using vortex before it was removed. The remaining solution was ready for injection into HPLC system.

(II) *HPLC Analysis*

The HPLC system consisted of a Photo diode array detector (Waters model 996), a HPLC auto sampler (Waters model 717 Plus), and two pumps (Waters model 510 and 590). The HPLC column used was Symmetry C18, 5 μm 3.9 × 150 mm (Waters). The HPLC analyses were performed by using gradient mobile phase as described by Hela *et al.*[8] with several modifications. The mobile phases were 0.01 M ammonium acetate pH 4.6 (A) and 100% acetonitrile (B). The flow rate was 1 mL/min. The detection wavelength was set at 266 nm.

V. *Statistical Analysis*

The statistical analysis has been performed by SAS version 9.1[9]. Two-way ANOVA was used to determine which factor gave greater effect on SA residues and Pearson Correlation was applied to determine the correlation between time, temperature, internal temperature and weight changes against the reduction of SAs.

Table 1. Gradient of mobile phases used in HPLC determination of SA residues in chicken meat and chicken meat balls

Time	% 0.01M ammonium acetate pH 4.6	% Acetonitrile
0	95	5
18	63	37
23	63	37
25	95	5
30	95	5
35	95	5

RESULTS AND DISCUSSION

Deep-frying processes at different times and temperatures on chicken meat balls showed various reduction effects on SA residues. Observation on the temperature factor indicated significant reductions ($p < 0.05$) of all SAs in deep-fried chicken meat-balls at 170 and 180°C compared to the control as shown in Table 2. However, only SDZ showed significant reduction ($p < 0.05$) between deep-frying of chicken meat balls at 170 and 180°C. There were no significant reductions ($p > 0.05$) for SDZ, SMX and SQX in samples, which were deep fried between 190°C and 180°C. The results might be influenced by the internal temperatures where there were increments of the internal temperatures as the outer temperatures increased. Table 3 shows that the internal temperatures continued rising from treatment of 170 to 190°C at 3, 6 and 9 min. It demonstrated that frying of the chicken meat balls especially the internal part was still not completed and reached the maximum temperature after frying at 190°C.

In terms of time factor, deep frying at 3 min showed all SAs were significantly reduced against the control ($p < 0.05$) except for SQX, which needed 6 min of deep frying to show significant reduction ($p < 0.05$). Significant reductions were also observed for 6 min against 3 min in all type of SAs with the exception of SMZ. Significant reductions were observed in all SAs after 6 and 9 min of deep frying. The results were fitted with the internal temperatures, which decreased at 9 min at all temperatures (Table 3). It showed that the internal part of the chicken meat-ball has completely fried.

In general, the temperature factor showed significant reduction ($p < 0.05$) of SA concentrations against the control but not among the treatments. As for the

Table 2. Effect of various temperature and time of deep frying on sulfonamide residues in chicken meat-balls

Temperature (°C)	Time (min)	Sulfonamides (μg/g) (n = 3) ± S.D.			
		Sulfadiazine	Sulfamethazine	Sulfamethoxazole	Sulfaquinoxaline
Control	Control	0.224 ± 0.009 ^{Aa}	0.323 ± 0.008 ^{Aa}	0.240 ± 0.009 ^{Aa}	0.226 ± 0.004 ^{Aa}
170	3	0.210 ± 0.011 ^{Bb}	0.285 ± 0.013 ^{Bb}	0.215 ± 0.006 ^{Bb}	0.225 ± 0.016 ^{Ba}
170	6	0.174 ± 0.002 ^{Bc}	0.282 ± 0.005 ^{Bb}	0.194 ± 0.018 ^{Bc}	0.197 ± 0.009 ^{Bb}
170	9	0.149 ± 0.009 ^{Bd}	0.268 ± 0.006 ^{Bc}	0.181 ± 0.025 ^{Bd}	0.181 ± 0.003 ^{Bc}
180	3	0.199 ± 0.002 ^{Cb}	0.285 ± 0.008 ^{Bb}	0.213 ± 0.017 ^{BCb}	0.217 ± 0.013 ^{Ba}
180	6	0.157 ± 0.003 ^{Cc}	0.282 ± 0.002 ^{Bb}	0.188 ± 0.005 ^{BCc}	0.195 ± 0.010 ^{Bb}
180	9	0.143 ± 0.007 ^{Cd}	0.258 ± 0.007 ^{Bc}	0.159 ± 0.013 ^{BCd}	0.179 ± 0.015 ^{Bc}
190	3	0.186 ± 0.003 ^{Cb}	0.286 ± 0.006 ^{Cb}	0.199 ± 0.002 ^{Cb}	0.206 ± 0.002 ^{Ba}
190	6	0.155 ± 0.006 ^{Cc}	0.272 ± 0.004 ^{Cb}	0.182 ± 0.017 ^{Cc}	0.190 ± 0.025 ^{Bb}
190	9	0.140 ± 0.013 ^{Cd}	0.234 ± 0.015 ^{Cc}	0.142 ± 0.002 ^{Cd}	0.164 ± 0.028 ^{Bc}

$^{A-C}$ Means with different capital letters in the same column are significantly reduced ($p < 0.05$) against temperature.
$^{a-d}$ Means with different small letters in the same column are significantly reduced ($p < 0.05$) against time.

time factor, there were significant reductions ($p < 0.05$) of SA concentrations against the control and among all the treatments. Therefore, it can be concluded that the time has greater effect than temperature in reducing SA concentrations in the deep-frying process.

No previous studies on the stability of the sulfonamide standard against direct heating up to 180°C and above were found. However, Dehai *et al.*[3] observed reduction of sulfadimethoxine (SDM) when carried out frying on breaded channel catfish using canola oil. This was supported by the study of Rose *et al.*[10] in which reductions occurred on SMZ with frying at 180 and 260°C after the weight losses were measured. In this study, it was found that the higher temperature and longer time of deep-frying, the greater loss on the weight of chicken meat balls (Table 4). At some point during the deep-frying process, the moisture in the chicken meat balls leached out and was replaced by the frying oil[11]. The exchange of moisture and oil could remove SAs out from the chicken meat balls. This process produced a crust outside the chicken meat balls, which could become a barrier and prevent the leached out SAs from re-entering the chicken meat balls.

A Pearson correlation coefficient analysis was carried out to observe the relationship after deep-frying process for SAs concentration against the temperature, time, internal temperature and weight changes (Table 5). The results showed that the time factor gives greater effect in reducing SAs concentration with Pearson correlation coefficient from -0.802 to -0.933, suggesting that longer deep-frying time resulted in lower SAs concentration. Meanwhile, the temperature factor resulted in Pearson correlation coefficient near to zero with probability values more than 0.05, suggesting lack of correlation between SA concentration and temperature factor.

Table 3. The internal temperature of chicken meat balls during deep frying

Time (min)	Temperature (°C)		
	170	180	190
0	-2.3 ± 0.2	-2.3 ± 0.3	-2.3 ± 0.4
3	94.9 ± 1.6	100.0 ± 3.3	100.4 ± 4.8
6	103.9 ± 0.7	104.5 ± 0.8	107.3 ± 2.4
9	102.5 ± 0.8	103.4 ± 0.6	106.6 ± 3.1

Table 4. Percentage of weight change after deep-frying

Time (min)	Weight change (%)		
	170°C	180°C	190°C
3	-16.38	-16.98	-20.23
6	-24.38	-25.44	-25.57
9	-26.27	-28.72	-29.02

The weight changes showed high percentage of loss (Table 4). The Pearson correlation coefficients between the SA concentration and weight loss were close to one (0.751 to 0.984). This indicates that the more weight loss, the lower the SA concentration. The correlation between SA concentration and internal temperature ranged from -0.564 to -0.843, which indicated the inverse relationship between internal temperature and SA concentration. Overall, the reduction in SA concentration was mainly because of the increase in internal temperature and weight loss during the deep-frying process. This result is

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Table 5. Pearson correlation coefficient matrix between dependent and independent variables of chicken meat balls during deep-frying (probabilities in parentheses)

	Temperature	Time	Internal temperature	Weight change
Temperature	1.000	0.000	0.495	-0.238
		(1.000)	(0.175)	(0.537)
Time	0.000	1.000	0.655	-0.931
	(1.000)		(0.056)	(0.000)
Internal temperature	0.495	0.655	1.000	-0.836
	(0.175)	(0.056)		(0.005)
Weight change	-0.238	-0.931	-0.836	1.000
	(0.537)	(0.000)	(0.005)	
SDZ concentration	-0.296	-0.928	-0.843	0.984
	(0.439)	(0.000)	(0.004)	(0.000)
SMZ concentration	-0.359	-0.802	-0.564	0.751
	(0.342)	(0.009)	(0.114)	(0.020)
SMX concentration	-0.407	-0.881	-0.727	0.915
	0.277	(0.002)	(0.026)	(0.001)
SQX concentration	-0.323	-0.933	-0.805	0.960
	0.396	(0.000)	(0.009)	(0.000)

supported by study of Papapagiotou *et al.*[12] where reduction of sulfamethazine was recorded after weight reduction was measured. The decrease in weight might be due to reduced water-holding capacity after the increase of heat temperature. Murphy and Marks[13] also supported this finding by suggesting that muscle and connective tissue changes during heating may influence texture and cook loss from processed poultry meat.

All the deep-frying treatments resulted in reductions of all SA concentrations. The longer the cooking time, the more reduction occurred. However, the rates were different between different temperatures even with the same frying time. Figure 2 shows that the reduction of SAs against deep-frying at 190°C for 9 min, the percentage were 37.5, 27.5, 40.7, and 27.6% for SDZ, SMZ, SMX, and SQX, respectively. In ascending order, the percent of reductions were 170°C (3 min) < 180°C (3 min) < 190°C (3 min) < 170°C (6 min) < 180°C (6 min) < 190°C (6 min) < 170°C (9 min) < 180°C (9 min) < 190°C (9 min).

Quality of the outer appearance and the internal texture of the chicken meat-balls deep fried at the times and temperatures used in this study were both acceptable for consumption. This study also found that deep-frying longer than 9 min at higher than 190°C resulted in unacceptable quality for consumption. Deep-frying the chicken meat-balls at 180°C for 6 min resulted in the best

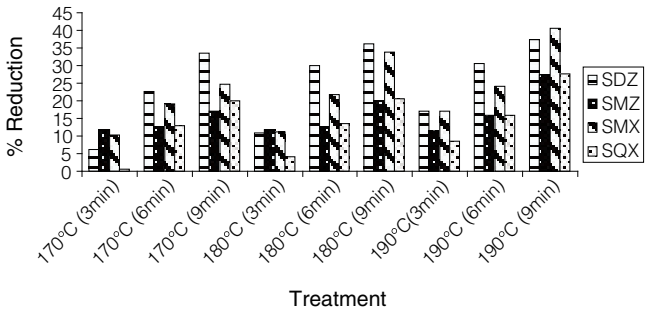

Figure 2. Percentage of SAs reduction against deep-frying processes.

acceptable quality in terms of outward appearance and consumption as well as reduction in SA residues.

CONCLUSIONS

Deep-frying despite of cooking can be a device to ensure the safety of food consumes from SA residues. Time has greater effect than temperature does in reducing SAs residues. The longer the duration of deep-frying and higher the temperature applied, the more reduction of SA residues was observed. However, over cooking could damage the food properties and taste. Therefore, appearance and taste of the food still need to be retained by controlling the time and temperature of cooking at its best, while the risk from SA residues could be reduced at the same time. From this study, it is suggested that deep-frying chicken meat balls at 180°C for 6 min is the best treatment for outward appearance, consumption and reduction in SA residues.

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