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The Effects of Roasting and Microwave Processes at Different pH Values on Enrofloxacin, Oxytetracycline, and Sulfadiazine Residues in Chicken Meat

Mohammadtaghi Heydarian[1], Mohammadreza Khani[2]*, Ashkan Jebelli-Javan[3], Alireza Rahman[2]

[1]Ph.D. student, Department of Food Hygiene, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran

[2]Assistant Professor, Department of Food Science and Technology and Food Hygiene, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran

[3]Associate Professor, Department of Food Hygiene and Quality Control, Semnan University, Semnan, Iran

ABSTRACT

The present research aimed to evaluate the effects of roasting and microwave thermal processes along with pH change on the amount of residues of three commonly used antibiotics, enrofloxacin, oxytetracycline and sulfadiazine in chicken meat. For this purpose, first the three antibiotics were added to the chicken meat samples in amounts 4 times the remaining limit. Meat samples containing residues of each antibiotic were subjected to roasting (at 200 °C for 30 minutes) and microwave (at 100 °C for 3 minutes) treatments at pH 5.8 and 4.8. Then, the residual amount of each of the antibiotics was investigated by high-performance liquid chromatography (HPLC) along with colorimetry and evaluation of cooking loss. The results showed that both treatments were able to significantly reduce the amount of antibiotic residues, but both processes were more effective at pH 4.8 than 5.8 ($P<0.05$). The highest amount of reduction of antibiotic residue at pH 4.8 in both microwave and roasting treatments was observed in oxytetracycline residue with 76.2 and 72.4% respectively. Roasting and microwave treatments decreased L* index, but a* index decreased in roasting and increased in microwave, and b* index increased in microwave, but there was no significant change in roasting. Also, the amount of cooking loss in roasting in the range of 58 to 71% was more than that of microwave in the range of 48 to 62% ($P<0.05$). Finally, microwave treatment can be introduced along with reducing the pH of chicken meat to 4.8 as the best treatment to reduce antibiotic residues.

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*Corresponding Author E-Mail:
m.khani@qodsiau.ac.ir

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1- Introduction

In the poultry farming industry, various antibiotics are widely used to treat diseases and as growth promoters, and the residues of these antibiotics in meat in excessive amounts can cause problems for the health of consumers. One of the reasons for the isolation of antibiotic residues in chicken meat is the non-observance of the time of abstinence or prohibition of medicinal use[1] It is by poultry farmers. This causes their residual concentration in tissues such as muscle, liver and kidney to exceed the maximum allowed amount.[2] (MRL) to increase [1]. Continuous consumption of chicken meat with antibiotic residues can cause serious risks to human health, including antibiotic sensitivity, allergic reactions, mutation in cells, disturbing the balance of intestinal microflora, and bacterial resistance to antibiotics [2 and 3]. [.

Therefore, in order to prevent such health hazards and to reduce the amount of antibiotic residues in poultry meat, various solutions have been investigated and it has been determined that some processes such as cooking, washing, freezing and ultrasound are able to reduce antibiotic residues to some extent [4]. 5, 6, 7 and 8. It was also found in recent research that radiationUV It can also be effective on antibiotics [9].

Food is cooked in various ways such as boiling, frying, grilling and microwave, each of which has a different effect on antibiotics. In fact, the temperature and heating time during the cooking process have a very significant effect on reducing antibiotic residues. Reports have shown that the temperature of the center of the meat in the grilling and microwave methods is higher than the temperature created in the boiling method.[10 and 11]. Also, a recent research showed that in some cooking methods, reducing the pH can reduce the amount of antibiotic residue to an acceptable level. As a result, by reducing the pH of the cooking environment or the sample itself, cooking methods can be modified to create more effects]12[.

Studies investigating the effects of thermal processes on antibiotic residues usually present their findings in terms of percentage degradation of residues after treatment. From the available studies, it can be concluded that, in general, most thermal processes lead to the destruction of antibiotic residues and, as a result, reduce their concentration in food. However, the effects reported in different studies depend on the type of process used, the type of food, the temperature and time of heating, the amountpH etc. has been very different]13[. For example in the reviewThe effect of different cooking temperatures on the residue of oxytetracycline in chicken has been observed.]14[.

Shaltout et al. (2019) in the study of the effect of various cooking and freezing methods on the residues of ciprofloxacin and oxytetracycline in chicken meat found that quinolones are very stable compared to thermal treatments, but the freezing method for 6 months could reduce the residue of ciprofloxacin by 62%]11[. In another study byunderstandet al. (2019), investigated the effect of cooking methods including microwave and grilling on beef and reported that the residue of ciprofloxacin and oxytetracycline in the microwave method was 38.14 and 86.95%, respectively, and in the grilling method was 84%, respectively. 12.98 and 73.98% decreased]15[. alsoNagi et al. (2021) investigated the effect of cooking by boiling method on the residual amount of enrofloxacin in poultry meat and its edible contaminants, and the results showed that the effect of a specific method can also depend on different meat parts such as chicken breast and thigh and Meat organs such as liver and kidney are different]16[.

Therefore, according to the variable effects of thermal processes on different antibiotic residues, the present study aims to compare

[1]- Withdrawal period

[2]- Maximum residue limit

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the effects of two thermal processes, grilling and microwave, along with changingpH Meat was investigated on the reduction of drug residues of several commonly used antibiotics in chicken meat along with the evaluation of cooking loss and colorimetry.

2- Materials and methods

2-1- Raw materials

Whole chicken carcasses were prepared from the samples sent to the central laboratory of the General Veterinary Department of Semnan Province for the purpose of antibiotic residue monitoring and were used to separate the chicken breast. 100% raw materials of each of the antibiotics enrofloxacin, oxytetracycline and sulfadiazine were purchased from Royan Daru Company (Iran). Lemon juice was prepared by squeezing fresh limes. To measure antibiotic residues, standards of oxytetracycline, enrofloxacin and sulfadiazine from Sigma (USA), sodium hydroxide and alumina powder from Sigma (USA), formic acid, succinic acid and oxalic acid from Merck (Germany), methanol and acetonitrile were purchased and prepared from Majalli Company (Iran) and deionized distilled water from Kimia Company (Iran).

2-2- Method of preparation and preparation of chicken meat samples

For this purpose, the chicken carcasses that were free of any antibiotics in the antibiotic monitoring quality plan of the General Veterinary Department of Semnan province and were negative for antibiotic residues using diagnostic kits were selected and transferred to the laboratory by maintaining the cold chain. Then, 20 chicken breasts were separated from the whole carcasses (total weight of 5 kg) and ground together and kept in the freezer at -20 degrees Celsius for 24 hours until the preparation of the treatments. Before applying the desired processes, different doses (about four times more than the permissible limit of residual in meat) of the three studied antibiotics including oxytetracycline at a dose of 800 µg/kg (permissible limit of 200 µg/kg)]17[. Sulfadiazine with a dose of 400 µg/kg (limit 100 µg/kg)]17[. and enrofloxacin with a dose of 400 µg/kg (limit 100 µg/kg)]18[. it was prepared. For this purpose, two antibiotics, enrofloxacin and sulfadiazine, were dissolved in the amount of 0.02 g in 40 ml of water and oxytetracycline in the amount of 0.04 g in 40 ml of water, and then 10 ml of each was taken and dissolved in 1000 ml of water separately. and by digital stirrer (MST- yellow line, made in Italy) was thoroughly mixed. Next, 100 ml of each prepared antibiotic solution was added to 500 grams of minced chicken meat separately and completely mixed with a mixer.SFP1040- SUNNY, made in Türkiye) for 3 minutes were mixed[10 and 19].In the control sample, 100 ml of distilled water without antibiotics was used and added. Then, in order to equalize the conditions for different treatment methods, a certain mass of chicken meat (weighing 10 grams) was molded in the form of balls with a diameter of 5 cm.[12 and 20].The pellets were kept in a freezer at -20°C until treatment (about 48 hours). Before applying the procedures, the pellets were thawed at room temperature (25°C) and They were placed in a nylon zip cap for grilling and microwave treatments.

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2-3- Grilling method

In this process, minced chicken meat impregnated with each of the studied antibiotics and molded were divided into two groups. A group was roasted in the same way at the natural pH of chicken meat (pH = 5.8) in an electric oven (Memert, made in Germany) at a temperature of 200 degrees Celsius for 30 minutes. The other group was impregnated with a suitable amount of lemon juice with a pH of 2.6. For this purpose, 46 ml of lemon juice was added to 54 ml of distilled water and 100 ml of it was added to the chicken meat so that the pH reached 4.8. Then, like the above method, the grilling process was applied. Then each sample was cooled to ambient temperature after applying heat treatment and before extracting and measuring the antibiotic residue.]12[.

2-4- Microwave treatment method

In this process, minced chicken meat impregnated with each of the studied antibiotics and molded were divided into two groups. A group was heated in the same way at the natural pH of chicken meat (pH = 5.8) inside a microwave (Samsung, Korea) with a power of 900 W at a temperature of 100 degrees Celsius for 3 minutes. The other group was impregnated with a suitable amount of lemon juice with a pH of 2.6. For this purpose, 46 ml of lemon juice was added to 54 ml of distilled water and 100 ml of it was added to the chicken meat so that the pH reached 4.8. Then the microwave process was applied as above. Each sample was cooled to ambient temperature after heat treatment and before extracting and measuring the antibiotic residue.[7 and 12].

5-2- Determining antibiotic residue by HPLC

The residual amount of each of the three studied antibiotics in the chicken meat samples, before applying each of the processes, using a WATERS (2695) HPLC device with a C18 column (made in America) equipped with a WATERS (2475) and UV WATERS (2487) fluorescence detector. (Made in USA) Measured. Also, after applying each of the processes, antibiotic residues were measured in three replicates in each treatment.

2-5-1- Antibiotic extraction from chicken samples

The mobile phase and solvent used for extraction included phosphoric acid (75 ml), acetonitrile (25 ml) and sodium hydroxide buffer and the stationary phase of a C18 chromatography column. To make the buffer, 0.1 M phosphoric acid was prepared and its pH was adjusted to 3 with 0.1 M sodium hydroxide. To prepare the samples, 5 grams of each chicken sample was added to 15 ml solvent and vortexed for 3 minutes.YOU ModelMS3B (Made in USA) mixed until uniform. Then mix for 5 minutes with model centrifugeSigma 2.16 (Made in Germany) isolated. The supernatant was removed and its pH was adjusted to 7 using 0.1 M sodium hydroxide. 1 ml of the solution was taken and poured into the test tube and 5 ml of distilled water was added to it and mixed. Then 200 microliters of chloroform was added to the solution and vortexed for 2 minutes. The bottom layer was removed, poured into a vial and dried with nitrogen. 100 µl of the mobile phase was poured into the vial and mixed by vortex. Finally, samples with HPLC according to the method Jammoul et al. (2019)were analyzed [21].

2-5-2- Preparation of standard stock solution

10 mg of each antibiotic standard was dissolved in acetonitrile and made up to 100 ml with acetonitrile. The concentration of this standard was 100 mg/ml, from which concentrations of 20, 30, 40, 80, 100, 200 and 400 µg/ml were prepared. Then 20 µl of each was injected into the HPLC device at a flow rate of 1 ml/min. A standard curve was drawn based on different standard concentrations and the area under the peaks was calculated [21].

2-5-3- determination of antibiotic concentration

The concentration of antibiotics in the samples was calculated based on the equation 1 below:

$$\text{Antibiotic concentration})_{ppb} = \left(\frac{Concentration\ standard}{Sample\ weight \times 2} \times \right.$$

$$\frac{\textit{The curve under the sample area}}{\textit{The curve below the standard level}}$$

Relationship (1):

2-5-4- calculation method of antibiotic residue reduction

Antibiotic residues were measured before and after each treatment, and the reduction percentage was calculated according to equation 2 below:

$$\frac{\textit{The sample has not been treated in anti-residue biotic.The sample has been treated in anti-residue biotic}}{\textit{The sample has not been treated with anti-residual biotics}} \times$$

= (%) reduction of antibiotic residue Relationship (2): 100

6-2- Determining the cooking loss

The weight of all samples before and after treatments was measured using a digital scale (Sartorius model A120S, made in Germany) with an accuracy of 0.001 grams, and the percentage of weight loss was calculated using equation 3:

$$\text{weight loss (\%)} = \frac{\textit{Treatment before the sample weight} - \textit{treatment after the sample weight}}{\textit{Pretreatment sample weight}} \times 100$$

relationship (3)

2-7- Evaluation of color changes

Before and after the studied thermal treatments, the color indicators of $L^*a^*b^*$ In other words, the brightness, redness and yellowness of the meat samples were measured and recorded. The used system consisted of three components: an imaging box, a camera and a computer equipped with Image J 1.53e software. The interior of the box was white and its light was supplied by a halogen lamp. To capture images, a camera (Canon 12.1MP model SX-260, made in Japan) was installed perpendicular to the sample surface. The distance between the sample and the camera lens was 40 cm. Pictures of previous samples And after the treatments, it was taken to check the effect of each treatment. Then all the images were saved as JPEG and processed using the software. To extract the feature, the obtained image was transferred to different color channels and finally the desired features were extracted from the channels [22].

8-2- Statistical analysis method

All treatments were performed in three repetitions and the results were reported as mean ± standard deviation (Mean ± SD). To compare the effects of grilling and microwave, one-way analysis of variance (ANOVA) was used at a significance level of 5%, and Duncan's multiple range test was used to compare significant means. Data analysis was done using SAS 2.9 software and graphs were drawn using Excel 2016 software.

3. Results and Discussion

3-1- The effect of grilling and microwave processes along with pH change on the amount of antibiotic residuesand their reduction percentage

The results of grilling and microwave treatments at two different pH levels on the amount of antibiotic residues in chicken meat samples are presented in Table 1 and the percentage reduction of antibiotic residues in each of these treatments is presented in Chart 1. As can be seen, the effect of both processes and pH change on the amount of residues of all three studied antibiotics is significant ($p<0.05$). Although the thermal treatments of grilling and microwave in both investigated pH caused a decrease in the amount of antibiotic residues, but the effect of both treatments at pH=4.8 was significantly higher than pH=5.8 ($p<0.05$).

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In the grilling treatment, the highest percentage reduction of antibiotic residues at pH = 4.8 was obtained in oxytetracycline with a value of 72.4%, which was reduced by enrofloxacin (with a value of 69.9%) and sulfadiazine (with a value of 66.6%). There was a statistically significant difference ($p>0.05$). Also, in microwave treatment, the highest percentage reduction of antibiotic residue at pH = 4.8 was observed in oxytetracycline with a value of 76.2%, with the reduction rate of enrofloxacin (with a value of 71.2%) and sulfadiazine (with a value of 54.5%).) had a significant difference ($p>0.05$). In the comparison between the two heat treatments, the microwave process was able to reduce the residues of the two antibiotics oxytetracycline and enrofloxacin at both pH levels, while the grilling process was more effective in reducing the amount of sulfadiazine residues than the microwave ($p<0.05$). Microwave electromagnetic radiation leads to the movement of ions and vibrations of polar molecules (such as water, proteins and some liquids), heat production and extensive collisions, which leads to the reduction of antibiotic residues.]23[.

In various thermal processes, the percentage of degradation of tetracyclines has been reported as varying from 2 to 100%. Studies have shown that doxytetracycline is the most thermally stable compound, while oxytetracycline is the least thermally stable. It has been reported that oxytetracycline is very sensitive to heat and completely decomposes in about half an hour of boiling in water, but during deep frying in oil at high temperature, less degradation was obtained than by boiling.]20[.In another research, it was reported that among the various methods studied, microwave was the most effective method. Also, during the thermal process, the most stable and unstable tetracyclines were identified, respectively doxycycline and oxytetracycline.[12]. These results are consistent with the results of the current research that microwave treatment has a greater effect on reducing the residual percentage of oxytetracycline, as well as the different effects of thermal processes on reducing antibiotic residues. In the present research The grilling treatment along with reducing the pH of chicken meat to 4.8 compared to the natural pH was able to reduce the amount of antibiotic residues studied to a greater extent by about 13-14%. Also, microwave treatment along with reducing the pH of chicken meat to 4.8 was able to reduce the amount of studied antibiotic residues to a greater extent than the normal pH by about 8-9%. In other words, decreasing the pH of meat in both grilling and microwave treatments significantly reduced the amount of residual antibiotics more than the normal pH, which is close to the studied pH to the isoelectric pH of chicken meat (pH=5). It is relevant that in these conditions water storage capacity[3] (WHC) has reached its lowest level and causes a part of the internal residues to come out along with the secretion of water from the meat and causes more loss of antibiotic residues in a more acidic pH [12].

In a similar study by Vivienne et al. (2018), the concentration of oxytetracycline in meat after microwave, grilling and boiling processes at pH = 6 was 49.1%, 53.6% and 69.6%, respectively, but at 2 7/pH decreased by 34.3%, 53.2% and 67.7%, respectively, which are not consistent with the results of the present research, which can be attributed to the different pH of the studied samples and the different amounts of the antibiotic residues under study. be related]12[.

Tetracycline has different epimeric forms - isomers that differ only in the arrangement of one carbon in the position of OH and H groups other than the last asymmetric carbon - which may be degraded under different conditions. For example, the pathways related to the degradation of different tetracycline epimers are mainly dependent on pH. Tetracycline can under certain conditions to epimers such as 4-epi-oxytetracycline[4], alpha-apooxytetracycline[5] and beta-apooxytetracycline[6] be decomposed 4-epi-oxytetracycline is biologically active and can be converted back to the parent compound. Therefore, the degradation products of antibiotics may be a potential threat to human health. In this regard, the type of thermal process and cooking method is one of the most important factors that affect the residue of tetracyclines. It has been reported that the antibiotic oxytetracycline in chicken meat is reduced during the grilling process, and grilling leads to the destruction of oxytetracycline and the production of two epimers, alpha and beta apooxytetracycline, which reduces the toxic concentration of this antibiotic.]24[.

Thermal degradation of beta-lactams, quinolones, sulfonamides, macrolides, tetracyclines, and aminoglycosides depends on temperature, and at a certain temperature, increasing the duration of heating helps to cause further degradation. In addition, the composition of the food (such as fat content) and its physicochemical characteristics (such as pH), cooking methods used and the presence of food additives are all factors.

[3]- Water holding capacity

[4]- 4epi-Oxytetracycline

[5]- α-apo-Oxytetracycline

[6]- β-apo-Oxytetracycline

which affect the degradation rate of antibiotics. Hydrolysis of oxytetracycline has been reported to occur at pHs of 3 to 10.5. Also, using a solution with a neutral pH and then lowering the pH are more effective in destroying antibiotics]25[.

Although the compounds in the same group of antibiotics may have similarities in terms of chemical structure and bioactivity, their thermal stability may be very different. Also, according to previous studies, antibiotics cannot be ranked based on their group in terms of thermal stability, because the heating environment, i.e. matrix and pH, also have a great effect on the degradation of antibiotics. However, under certain conditions, antibiotics of the same family have similar thermal properties[13]. But More studies in this field are needed to systematically understand the effect of these parameters during thermal processes on different antibiotic residues..

Table 1. Effects of roasting and microwave treatments at different pH values on the amount of antibiotic residues in chicken meat samples (Mean ± SD)

Antibiotic	**Antibiotic residue before heat treatment (ppm)**	**Antibiotic residue after heat treatment (ppm)**			
		Roasting		Microwave	
		pH=5.8	**pH=4.8**	**pH=5.8**	pH=4.8
Enrofloxacin	385.3±0.50	165.6±0.57 ^{D}	116±0.76 ^{B}	139.6±0.81 ^{C}	110.8±0.61 ^{A}
Oxytetracycline	767.3±0.57	316±0.88 ^{D}	212±0.75 ^{B}	239.2±0.71 ^{C}	182.4±0.68 ^{A}
Sulfadiazine	356.5±0.52	170.8±0.63 ^{B}	119.2±0.70 ^{D}	193.6±0.43 ^{C}	162.4±0.50 ^{A}

A-D: Different superscripts in each row indicate significant differences among different heat treatment ($p<0.05$).

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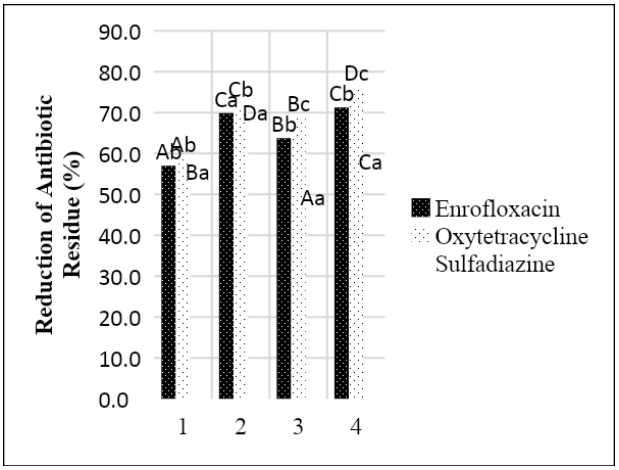

Fig 1. Reduction percentages of antibiotic residues by heat treatments at different pH values in chicken meat samples (Mean ± SD)

A-C: Different capital letters indicate significant differences between the different heat treatments ($p<0.05$). a-c: Different small letters indicate significant differences between the different antibiotics at each treatment ($p<0.05$).

3-2- Effect of grilling and microwave processes along with pH change on cooking loss percentage

The results of grilling and microwave treatments on the percentage of weight loss of chicken meat samples are shown in graph 2. As can be seen, the application of these thermal processes at different pH caused the weight loss of chicken meat samples and their effect on the amount of weight loss of the samples containing the residues of all three studied antibiotics is significant ($p<0.05$). Grilling treatment at pH = 5.8 in different chicken meat samples caused a decrease of 55-58% compared to the control sample and at pH = 4.8 it caused a decrease of 66-71% compared to the control sample. While microwave treatment at pH = 5.8 in various chicken meat samples caused a decrease of 48-58% compared to the control sample and at pH = 4.8 it caused a decrease of 55-62% compared to the control sample. In other words, the percentage of weight loss in both treatments was mainly at pH=4.8 than pH=5.8 ($p<0.05$). In general, the highest percentage of weight loss was obtained in the grilling treatment at pH=4.8 with a value of 71% and the lowest in the microwave treatment at pH=5.8 with a value of 48%, which had statistically significant differences with other treatments. ($p<0.05$).

In the first place, the reason for weight loss after thermal processes is related to the loss of moisture and the exit of water from the meat due to the rapid thermal contraction of the muscle and the structural changes caused by the denaturation and coagulation of proteins during heating. In addition, by reducing the pH of meat and changing its chemical structure, the release of interstitial water and the reduction of water absorption occurs.[26 and 27.]In addition, the distance between the proteins decreases with increasing cooking speed and more water is removed from the meat]28[.

In the present research, grilling treatment at pH 4.8 compared to microwave treatment at pH 4.8 caused a greater percentage of weight loss in all the same samples examined ($p<0.05$). The reason for these results can be the use of more temperature and duration of heating in the grilling treatment (200°C for 30 minutes) compared to the microwave treatment (100°C for 3 minutes), which caused more weight loss. These results are in agreement with the results of Li et al. (2019) and Muthulakshmi et al. (2022), who stated that higher cooking temperature and long cooking time cause more moisture loss through evaporation and the release of excess water inside the meat samples and lead to more weight loss. Correspond]29[.

Muscle pH also plays an important role in the rate of weight loss because it directly affects the amount of negative charges on protein molecules that bond with water molecules. At a pH close to the isoelectric point, due to the equal positive and negative charges of proteins, less water can be attached to them. The reason for this is the absence of electrostatic repulsion in muscle fibers, which causes the space between them to decrease and the amount of water trapped in the space between them to be minimized. Therefore, at a pH higher than the isoelectric pH of meat (2.5), the binding ability of proteins and water increases, and as a result, weight loss decreases.]30[. On the other hand, with an increase in the concentration of organic acids and a further decrease in pH, the muscle membrane begins to tear, and the collagen that begins to dissolve in acidic conditions is slowly removed from the muscle and the amount of muscle connective tissue decreases. Also, lysosomal enzymes are released in acidic conditions, which lead to the destruction of myofibrillar proteins, and as the pH of meat decreases, the rate of moisture loss during cooking increases.[26 and 31].

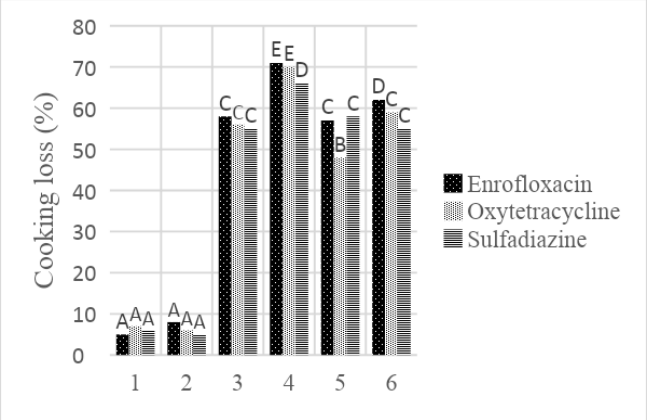

Fig 2. Percentage of cooking loss in chicken meat samples treated with roasting and microwave at different pH values (Mean ± SD)

A-E: Different capital letters indicate significant differences between the applied treatments ($p<0.05$).

3-3- The effect of grilling and microwave processes along with pH change on the color indicators of L* a*b*

The results of grilling and microwave treatments on the color changes of chicken meat samples containing antibiotic residues are presented in Table 2. Color is one of the most important sensory characteristics of any food, including chicken meat. L*a*b color model is one of the most complete and common color space that has been introduced to display colors visible to the human eye. The investigated parameters include L* or brightness and transparency, which varies from zero to 100, that is, from black to white. a* and b* both vary from -120 to +120 and a* varies from green (negative) to red (positive) and b* varies from blue (negative) to yellow (positive)]32[.

As can be seen, the effects of both grilling and microwave treatments and pH change on the investigated color indicators are significant ($p<0.05$). However, both treatments produced more color changes at pH=5.8 compared to the control sample at pH=4.8. Grilling and microwave treatments decrease the L index* compared to the control sample, and at pH 8.5, the amount of this index shows a significant decrease compared to pH 4.8. In other words, the samples in both treatments and in both pH were less transparent compared to the control sample. However, at pH = 4.8, the amount of index L* increased significantly ($p>0.05$).

Based on the obtained results, more transparency in meat samples can be related to its higher moisture, which causes less Maillard reaction and more brightness. Muscle structure and meat color are strongly related to each other are. It has been reported that there is a negative and significant correlation between brightness and pH of chicken breast meat, and at higher pH values, the color of the meat becomes darker.]33[. This issue is consistent with the results of the present research, which reduced the transparency of chicken meat samples in each of the heating methods at higher pH. The addition of citric acid increases the L* values of the meat and leads to a lighter overall color in the meat. The reason for the increase in transparency can be attributed to the denaturation of sarcoplasmic and myofibrillar proteins in the acid solution, which changes the water retention and binding capacity of the muscle as a result of their denaturation, and finally, the amount of water dispersed among the muscle fibers affects the ability of meat to reflect light. Lays]34[.

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Allen and colleagues (1997) reported that the amount of L* of chicken fillet has a positive relationship with the loss caused by cooking, and the reason for the increase in brightness can be related to the high loss during cooking.]35[. Wattanachant et al. (2004) in the investigation of pH and L* values of thigh and breast of broiler chickens found that the thigh had a pH of 6.62 and L* value of 32.53, while the breast with a pH of 5.93 had a L* value of 38.79, which It indicates the darkening of meat color in higher pH values]36[.

In the present research, the grilling treatment caused an increase in the a index* Compared to the control sample, the highest amount of this index was measured at pH 8.5, which did not have a statistically significant difference with pH 4.8 ($p<0.05$). But by applying microwave treatment, the amount of index a* It decreased in the samples compared to the control sample, and these results were not statistically significantly different from each other at different pH levels ($p<0.05$). In other words, the amount of redness index in the grilling treatment significantly increased compared to the control sample, but in the microwave treatment, the amount of redness decreased significantly ($p<0.05$).

The reason for the reduction of redness in the heat treatment can be related to the oxidation of myoglobin and its conversion to met-myoglobin due to the low oxygen pressure in the meat immersed in the lemon juice solution. In fact, simultaneously with the formation of methemoglobin, the amount of myoglobin and oxymyoglobin decreases and the gradual color change of the surface of the meat occurs from red to brown. Also, under the influence of heat and as a result of the denaturation of myoglobin, two pigments, hemochrome and hemichrome, are likely to be created in meat, which have dark red and brown colors, respectively, and can increase the redness of the meat. The obtained results are consistent with the results of Sabzi et al. (2016) that the parameters of meat color under the influence of boiling water, the amount of parameter a* decreased with increasing pH.]31[.

As can be seen, microwave treatment at both pH (especially at pH 4.8) causes an increase in b index.* It was compared to the control sample, but with the application of grilling treatment, the amount of index b* There was no significant change in the samples compared to the control sample ($p<0.05$). In addition, decreasing the pH from 5.8 to 4.8 in both grilling and microwave treatments significantly increased the b index.* ($p>0.05$)

Latif (2011) showed that in marinite samples[7] (flavored) with orange juice, phosphate, salt and spices along with grilling treatment (temperature 85 degrees Celsius for 20 minutes) and microwave (temperature 78 degrees for 3 minutes) increased b* value of chicken breast samples and L* value decreased]37[. Muthulakshmi et al. (2022) also found that by heating chicken breast at temperatures of 75 to 94 degrees Celsius for 5 minutes in the oven, the b* index increased and the L* index decreased, mainly They are consistent with the results of the present study]29[.

[7]- Marination

Table 2. Effects of heat treatments at different pH on L*a*b* values in chicken meat samples (Mean ± SD)

Antibiotics	Color values	Control		Color changes after Roasting		Color changes after Microwave	
		5.8= pH	4.8= pH	5.8= pH	4.8= pH	5.8= pH	4.8= pH
Enrofloxacin	L*	49.5±0.74^{Not}	55.6±0.71^{That}	44.1±0.76^{Aa}	49.0±0.69^{Not}	42.1±0.81^{Aa}	49.9± 0.80 ^{Not}
Oxytetracycline		51.4±0.71^{Not}	53.4±0.50^{That}	45.9±0.40^{Aa}	50.1±0.70^{Not}	43.9± 0.89 ^{Aa}	46.8± 0.76 ^{Aa}
Sulfadiazine		51.4±0.75^{Not}	53.3±0.59^{That}	44.5±0.45^{Aa}	50.5±0.38^{Not}	44.8± 0.90 ^{Aa}	45.4± 0.22 ^{Aa}
Enrofloxacin	a*	6.0±0.69^{Not}	5.2±0.59^{Not}	9.1±0.75^{That}	8.7±0.77^{That}	4.8±0.17^{Not}	3.3±0.15^{Aa}
Oxytetracycline		6.3±0.66^{Not}	6.1±0.27^{Not}	8.6±0.3^{Not}	7.0±0.40^{Not}	4.1±0.28^{Aa}	3.7±0.40^{Aa}
Sulfadiazine		6.6±0.84^{Not}	5.8±0.21^{Not}	7.8±0.08^{Not}	6.6±0.18^{Not}	4.5±0.37^{Aa}	4.1±0.28^{Aa}
Enrofloxacin	b*	18.5±0.82^{Aa}	19.9±0.77^{Not}	18.4±0.50^{Aa}	19.7±0.61^{Not}	20.4±0.68^{Not}	21.6±0.69^{That}
Oxytetracycline		18.8±0.57^{Aa}	20.5±0.63^{Not}	18.8±0.73^{Aa}	19.2±0.70^{Aa}	20.2±0.64^{Not}	21.5±0.22 ^{That}
Sulfadiazine		17.8±0.57^{Aa}	19.5±0.33^{Not}	18.7±0.55^{Aa}	18.9±0.30^{Aa}	20.9±0.24^{Not}	22.5±0.67^{That}

A-D: Different capital letters in each row indicate significant differences between the applied treatments ($p<0.05$).

a-c: Different small letters in each column corresponding to each color value, indicate significant differences between different antibiotics ($p<0.05$).

4- Conclusion

The results of this research indicated that the use of each of the grilling and microwave heat treatments along with reducing the pH value of meat from 5.8 to 4.8 had a significant effect on the reduction of antibiotic residues and the greatest reduction of residues in each The two processes of grilling and microwave at pH 4.8 were assigned to the antibiotic oxytetracycline with the amount of 73.5 and 77.2%, respectively. Also, by applying thermal treatments at pH 4.8, more weight loss up to about 14% in grilling treatment and less weight loss up to about 11% in microwave treatment was observed in most of the samples. Therefore, although the decrease in pH reduces the percentage of antibiotic residues during thermal processes, it can also cause more weight loss in chicken meat samples. On the other hand, in terms of color changes of chicken meat samples,

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grilling and microwave treatments cause a decrease in L index* compared to the control sample, and at pH 5.8, the amount of this index significantly decreased compared to pH 4.8. In addition, the grilling treatment caused an increase in the a index* But microwave treatment causes a decrease in index a* compared to the witness sample. But these results in different pH mostly did not have statistically significant differences with each other. Also, microwave treatment (especially at pH 8.4) increases the b index* compared to the control sample, but with the application of grilling treatment, the amount of index b* There was no significant change in the samples compared to the control sample.Finally, according to the results of this research, it is possible to use both grilling processes and especially microwaves along with reducing the pH of the meat in order to reduce the studied antibiotic residues in chicken meat, in addition to monitoring the observance of the antibiotic prohibition period during Poultry breeding and control of antibiotic residue limit by relevant organizations recommended.

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اثرات فرآیندهای کباب کردن و مایکروویو در pH های مختلف بر میزان بقایای انروفلوکساسین، اکسی-تتراسایکلین و سولفادیازین در گوشت مرغ

محمدتقی حیدریان[۱]، محمدرضا خانی[۲*]، اشکان جبلی جوان[۳]، علیرضا رحمن[۲]

۱-دانشجوی دکتری تخصصی گروه بهداشت مواد غذایی، واحد شهر قدس، دانشگاه آزاد اسلامی، تهران، ایران

۲- استادیار گروه علوم و صنایع غذایی و بهداشت مواد غذایی، واحد شهر قدس، دانشگاه آزاد اسلامی، تهران، ایران

۳- دانشیار گروه بهداشت و کنترل کیفی مواد غذایی، دانشگاه سمنان، سمنان، ایران

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* مسئول مکاتبات:

m.khani@qodsiau.ac.ir

چکیده

تحقیق حاضر با هدف ارزیابی اثرات فرآیندهای حرارتی کباب کردن و مایکروویو همراه با تغییر pH بر میزان بقایای سه آنتی‌بیوتیک پرمصرف انروفلوکساسین، اکسی تتراسایکلین و سولفادیازین در گوشت مرغ مورد بررسی قرار گرفت. برای این منظور، ابتدا سه آنتی‌بیوتیک مورد نظر در مقادیر ۴ برابر حد مجاز باقیمانده به نمونه‌های گوشت سینه مرغ افزوده شد. نمونه‌های گوشت حاوی بقایای هر آنتی‌بیوتیک به طور جداگانه در دو pH ۵/۸ و ۴/۸ تحت تیمارهای کباب کردن (در دمای ۲۰۰ درجه سانتیگراد به مدت ۳۰ دقیقه) و مایکروویو (در دمای ۱۰۰ درجه سانتیگراد به مدت ۳ دقیقه) قرار گرفت. سپس میزان باقیمانده هر یک از آنتی‌بیوتیک‌ها با روش کروماتوگرافی مایع با عملکرد بالا (HPLC) همراه با رنگ سنجی و ارزیابی میزان افت پخت مورد بررسی قرار گرفت. نتایج نشان داد که هر دو تیمار توانستند میزان بقایای آنتی بیوتیکی را به طور معنی داری کاهش دهند اما هر دو فرآیند در pH ۴/۸ نسبت به ۵/۸ موثرتر بودند. بیشترین میزان کاهش باقیمانده آنتی‌بیوتیک در pH ۴/۸ در هر دو تیمار مایکروویو و کباب کردن در باقیمانده اکسی تتراسایکلین به ترتیب با ۷۶/۲ و ۷۲/۴ درصد کاهش مشاهده گردید. تیمارهای کباب کردن و مایکروویو سبب کاهش شاخص L^* شدند اما شاخص a^* در کباب کردن کاهش و در مایکروویو افزایش یافت و شاخص b^* در مایکروویو افزایش داشته اما در کباب کردن تغییر معنی داری نداشت. همچنین میزان افت پخت در کباب کردن در محدوده ۵۸ تا ۷۱ درصد بیشتر از مایکروویو در محدوده ۴۸ تا ۶۲ درصد بدست آمد. در نهایت می توان تیمار مایکروویو را توام با کاهش pH گوشت مرغ به ۴/۸ به عنوان تیمار برتر جهت کاهش بقایای آنتی بیوتیکی معرفی نمود.