

Theses of a doctoral (PhD) dissertation

**Impact of home frying and cooking methods on chemical
and microbiological safety and quality characteristics of
poultry meat**

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History

The International Agency for Research on Cancer (IARC) issued a resolution in 2015 classifying red (mammal) meat as a carcinogen in humans and meat products as proven to be carcinogenic, as they may contain genotoxic carcinogens during processing, home frying and cooking. The resolution links the potential carcinogenic effects of red meat and meat products to the formation of three groups of compounds, N-nitroso compounds, polycyclic aromatic hydrocarbons and heterocyclic amines (HCA). In addition to red meat, HCAs can also be formed in the meat of poultry and fish as a result of heat treatment, but the IARC did not address this.

The amount of carcinogenic compounds, such as HCAs, formed during the frying and grilling of meats can generally be reduced if the heat treatment is carried out at a lower temperature and for a shorter time. At the same time, it is a question of how all these affects the destruction of zoonotic bacteria in and on the surface of meat by heat treatment, so the microbiological safety of heat-treated foods, as well as the quality and enjoyment value of the prepared food.

Objectives

In the framework of my research work, the following main aspects were studied:

1. The extent of HCA formation in grilled skin-covered and skinless chicken breasts and chicken thighs as a function of temperature and time used
2. The extent of HCA formation on the surface and inside of meat in skin-covered and skinless chicken breasts under open and closed grilling conditions using different temperature-time combinations and the study of the relationship between HCA formation and meat colour change
3. Investigation of the effect of grilling with different temperature-time combinations on the thermal death of *Salmonella* Enteritidis

From the results of the experiments, statistical correlations were deduced between HCA formation and *Salmonella* elimination, and HCA formation and discolouration of meat slices.

Materials and Methods

Barbecue

The tests were performed at the Department of Food Hygiene of the University of Veterinary Medicine. For the experiments we used meat parts of Ross 308 meat hybrid chicken, which were obtained from the retail market (Budapest, Hungary). The breast and thigh fillets used for the experiments were cut into 40 g 1.6 cm thick slices, and 3 parallel samples were prepared for each grilling set. For the heat treatment, we used an Electrolux ETG340 electric single-grill grill in the first series of experiments, and a DeLonghi CGH 1012D electric contact grill in the second, which allowed the recording of several cooking parameters. The grilling conditions were three temperatures (Experiment 1: 150/180/210; Experiment 2: 150/190/230 °C) and three durations (Experiment 1 2.5/5/10; Experiment 2 5/10/15 minutes) included nine combinations, cooking time should be interpreted separately for each side of samples. Heat-treated samples for colour and microbiological examinations were treated as described in the second series of experiments.

Sample preparation for LC-MS / MS analysis

The samples were comminuted with a stick mixer (for the 2nd series of experiments, treating the inner and outer layers separately), then homogenized, followed by saponification with NaOH solution. The mixture was shaken on a water bath shaker, then 10 ml aliquots were taken into centrifuge tubes, centrifuged, and all supernatants were applied to silica gel SPE columns. The sample was eluted with ethyl acetate and evaporated to dryness under N₂. The remainder of the NaOH mixture previously run on the SPE column was applied to a C18 column, and then that was dissolved in acetonitrile into the same tube in which the HCA had previously been dissolved from the silica gel and evaporated again. The evaporated product was dissolved in 0.5 ml of acetonitrile containing internal standard caffeine.

LC-MS / MS analysis

Quantitative chemical analysis was performed on two pyrolytic (harman, norharman) and three thermic (MeIQx, 4,8-DiMeIQx, PhIP) HCAs and performed on a Shimadzu LCMS 8030 HPLC-MS/MS system. Chromatographic separation was performed on a C18 EVO column equipped with a protective column. Liquid chromatographic analysis was performed by gradient elution: eluent "A" was 50 mM ammonium acetate in water (pH 5 adjusted with acetic acid) and "B" was 0.1 V / V% formic acid in acetonitrile. A quadrupole tandem mass

spectrometer was used with an electrospray ionization (ESI) ion source in positive mode and multiple reaction monitoring (MRM).

Sensory analysis

The sensory analysis was performed by university staff who did not have specialized training, so they could represent the average consumer. One study included ten subjects (6 women, 4 men; 24–62 years of age) who had to examine 3 samples at one time in a laboratory room. The sample to be tested consisted of a standard slice in one piece (for colour analysis) and a slice of diced meat for taste and stock analysis. The testers had to evaluate the colour of the meat according to lightness and darkness on a scale of 1 to 5, the combination of which consisted of the Light-Darkness combined parameter of 1 to 9.

In the case of skin meat samples, the skin-covered surface was examined. The responses were then analyzed, summarized, and compared.

Colorimetric examination

The Konica Minolta CHROMA METER CHR-400 tristimulus colorimetric system was used for objective colour analysis. The device analyzed the surface colour with CIELAB values:

L* - brightness, where 100 is perfect white and 0 is completely black

a* - redness, where the more positive the value, the redder the sample (negative - green)

b* - yellowness, where the more positive the value, the yellower the sample (negative - blue)

Ten individual measurements were made in each combination of grilling parameters under the same external light conditions each time. In the case of skin meat samples, the colour of the skin-covered surface was determined.

Salmonella testing

In eight cases, 10 grams of raw, non-contaminated meat were taken to detect the level of the original *Salmonella* contamination, which was performed by redox potential measurement in the Food Microbiology Laboratory of the department. During the redox potential measurement, the number of living *Salmonella* in the initial sample was determined in a Microtester redox potential meter.

Real-time PCR analysis of raw, non-contaminated samples was also performed to detect serotypes of *S. Enteritidis*, *S. Typhimurium*, and *S. Infantis*. The kits used were suitable for the detection of all *Salmonella enterica* serotypes and the three differentiated serotypes.

To investigate the heat elimination of *S. Enteritidis*, artificially contaminated samples of known contamination level were prepared by soaking in contaminated medium for 4 or 16 hours. Detection of *Salmonella* in this case was also performed by redox potential measurement, similar to raw, uncontaminated samples. The resulting slices were grilled, during which the core temperature of the samples was monitored with a core thermometer. After grilling, 10 grams of these were taken for redox potential measurement as described above.

Results

Examination of HCA content in skin-covered and skinless chicken breast and thigh samples (Experimental series 1)

When examining different chicken parts, the HCA content of skinless breast and thigh samples was compared for grilling at 150, 180 and 210 °C.

In chicken breast, at low temperature (150 °C) HCA production was not observed at all, and at medium temperature it did not induce significant HCA formation in a short time (180 °C for 2.5 minutes). During grilling at 210 °C, all tested HCAs were detectable after all treatment times, however, their concentrations depended significantly on the grilling time used. In the case of thigh samples, the occurrence of harman was more significant, it was already present in measurable amounts at 150 °C after 5 minutes of heat treatment. The absolute values of the other HCAs were generally lower compared to breast meat treated at the same temperature and duration.

The presence of skin on chicken breast slices demonstrably affected the incidence of the HCAs tested. Already at 150 °C almost all compounds' quantity was above the quantification limit (except for MeIQx). In contrast, even at 210 °C, the amount of some amines remained below this value, and the total HCA concentration was also lower than in the case of skinless chicken breast fillets. In the case of other temperature-time combinations, we were able to detect more HCA from the skin-covered sample (or showed no significant difference) compared to the skinless one.

Investigation of the effect of skin and grilling procedure on HCA content in the surface and deeper layers of chicken breast samples (Experimental series 2)

The quantitative and qualitative distributions of the HCAs formed differed slightly depending on the temperature used. While at 150 °C the thermic compounds, especially the harman, were the most frequently and most abundantly detected HCA components, at higher temperatures the PhIP dominated: grilling at 230 °C for 15 minutes it took more than 50 % of the total HCA ratio. In the case of contact (closed) grilling, the amount of HCAs was usually higher compared to open samples grilled in the same way. Internal samples grilled at 150 °C and 190 °C for 5 and 10 min contained less HCA in the case of skin-covered samples. At higher temperatures and / or for longer grilling, this trend has changed. The skin always contained more HCA than the skinless meat surface treated in the same way.

Correlations between colour and HCA content of grilled chicken breast

Among the parameters of the colorimetric measurement (L^* , brightness; a^* , redness; b^* , yellowness), the relationship between L^* and a^* was observed with both temperature and time: L^* showed a decreasing trend, a^* an increasing trend with both grilling parameters (temperature and time). In the sensory examination, the value of 5 was the code of the desired colour (golden brown) to be achieved during grilling. As expected, both temperature and duration of heat treatment affected the colour of the grilled meat. The results of the sensory test confirm that the open grilling method was indeed considered milder and the results obtained were generally lower than for meats prepared with the same parameters but closed. The presence of skin had a slowing effect on burning: scoring values for skinned meats were generally lower compared to the same results without skin.

In the present experiments, three different data sets were compared: chemical HCA analysis, sensory colour analysis, and instrumental colorimetric analysis for similarly heat-treated meats. Comparing the results of sensory and objective colour analysis, it was found that the L^* (brightness) and a^* (redness) indices show a very strong correlation for both closed and open skinless combinations, skin-covered openly grilled samples did not show this strong correlation, nor is the b^* (yellowness) index at any time.

When examining the relationship between objective instrumental colorimetric results and the HCA content of meat, L^* and a^* proved to be the most relevant, especially for skin-covered closed and skinless open grilling, for both internal and cortical / skin results for all HCAs. For methods without skin-cover and closedly grilled only a weak correlation could be detected, and no correlation was detected at all with the skinful-open combination. The relationship between HCA content and b^* was only detectable in some cases and there was never a strong correlation. In most cases, we found a strong correlation between subjective human colour judgment and the amount of instrumentally measured HCA for each HCAs, as well as for the thermic, pyrolytic, and sum-HCA groups.

Effect of grilling on heat destruction of Salmonella Enteritidis

Our results showed that all the eight not artificially contaminated retail samples contained *Salmonella*, and one sample showed *S. Typhimurium* serotype. When comparing the two contamination soaking periods (4 or 16 hours), no significant difference was detected in the final concentrations of *Salmonella*.

In the case of open skin-covered cooking, pathogens stayed alive even at 150 and 190 °C even after 15 minutes of bilateral grilling, but at 230 °C they were completely destroyed in 10 minutes. In contrast, open skinless grilling at all three temperatures resulted in complete *Salmonella*-free samples as early as 10 min, and significant destruction was detected already after 5 minutes. Closed grilling resulted in a much more effective elimination effect. During closed skin-covered frying at 150 °C, there was a measurable amount of live microbes in the meat even after 15 minutes (although the core temperature has reached 100 °C here so far). However, at 190 and 230 °C grilling, 15 minutes was enough for complete destruction. Closed skinless grilling at all temperatures caused complete destruction of *Salmonella* as early as 5 minutes. Core temperatures were higher compared to the skin-covered samples for shorter grilling times, but this difference gradually decreased for longer grilling times.

Correlation between HCA production and Salmonella death

We compared our chemical and microbiological results by examining the correlation between the resulting HCA amounts and the number of surviving *Salmonellae*. The results of closed, skinless grilling were unsuitable for this type of observation because complete *Salmonella* destruction was observed for each temperature-time combinations. For the other grilling combinations, however, the correlation could be examined. Open, skinless grilling showed negative but weak correlation values, while the results of skin-covered samples showed medium correlation for closed and strong correlation for open grilling. That is, especially when grilling skinned chicken breast, the combination of temperature and time required for significant *Salmonella* elimination also induces significant HCA formation.

Discussion

Examination of HCA content in skin covered and skinless chicken breast and thigh samples (Experimental series 1)

Comparing the results obtained by grilling chicken breast and chicken thigh fillet samples using the same temperature and time combinations, we found that higher temperature resulted in higher total HCA production in the breast than in the thigh. All but one of the HCAs were significantly higher in the chicken breast than in the chicken leg. The difference in total HCA levels between the two meat types is presumably also due to the higher protein content of the breast. Differences in the proportions of each HCAs can be explained by the different amino acid profiles of the breast and thigh.

When both the temperature rises and as the exposure time increases, the temperature in most of the meat samples may rise above a critical level for HCA formation. This is 150 °C for thermic HCAs according to previous literature, 300 °C for pyrolytics, but recent results and our own study also call these limit temperatures into question. In our experiments, the temperature in even the most heated point of the meat could not exceed 230 °C, yet in many cases we measured harman and norharman concentrations above the LOQ.

The role of skin proved to be twofold based on the results of our experiments. For skin samples grilled with or without skin, skinless results were generally lower than the skin-covered ones. This phenomenon may be due to the higher fat content of the skin, which may increase HCA production by increasing heat transfer. Due to the different protein composition of the skin, the concentration was significantly higher for MeIQx and harman (3.5-fold and 2.3-fold, respectively) compared to skinless samples. However, the effect of skin presence on increased HCA production was not seen in all combinations. In some cases, especially for shorter treatment times, it has been observed that until the fat in the skin melts, it has an insulating effect and thus the heat required for HCA production reaches less protein-rich tissues. During shorter grilling times, lower core temperatures were usually measured for skin-covered samples, which may explain the lower rate of HCA formation.

Investigation of the effect of skin and grilling procedure on HCA content in the surface and deeper layers of chicken breast samples (Experimental series 2)

In addition to temperature and time, the presence or absence of skin also had a measurable effect on the amount of HCAs produced. The hypothesis of the dual role of skin could be confirmed in these experiments as well. In the skin itself, higher total HCA levels were measured in 2/3

of the cases compared to skinless surface values. However, after grilling at lower temperatures and for shorter periods of time, this trend is reversed, the thermal insulating effect of the skin acting to a certain level may explain the reduced HCA formation.

Comparing the two grilling modes (closed and open), we found that in most cases the HCA level was higher during closed grilling compared to open grilling, probably due to the reduced heat loss from both directions and the closedness of the system at the same time.

In line with our expectations, PhIP was the most characteristic HCA for chicken meat, especially at higher treatment temperatures, among the compounds we tested. This is consistent with literature data. This thermic HCA is in category 2B (potential carcinogen) on the IARC list, consequently, its high concentration may pose a risk to consumers. Its proportion compared to other HCAs was particularly high in the skin. In addition to PhIP, the levels of co-carcinogenic pyrolytic amines, especially harman, were high.

Chemical risk assessment and evaluation

The degree of chemical risk was characterized by evaluating the toxicological significance of PhIP.

For the toxicological characterization of the measured HCA concentrations, the poultry meat consumption data, the HCA amounts we measured and the benchmark dose with a 10% probability of carcinogenic effect (BMDL10) observed in animal experiments were taken into account. The results of our risk assessment suggest that, based on the principle of maximum safety, the amount of PhIP generated during grilling at 230 °C for 15 minutes may pose a risk to the consumer for prostate and breast tumors in all three model countries studied (Israel, USA, Hungary). When grilling at 230 °C for 10 and 5 minutes, respectively, the MOE (Margin of Exposure) value indicates still a risk to the prostate and the 10-minute frying process for the breast in the two “high-consumption” countries, Israel and the United States.

Correlations between colour and HCA content of grilled chicken breast

For our studies, we used the specific sensory colour determination and combined with the instrumental measurement that made the subjective-objective colour measurement comparable with each other and with the HCA content of the sample. The evaluation of colour measurement thus took place in three forms: sensory-instrumental, sensory-toxicological, instrumental-toxicological.

The parameters L^* and a^* of the instrumental measurement mostly showed a very strong relationship with sensory scoring. In the case of L^* we could find a positive correlation, while the a^* values were negatively correlated with the sensory scale system. From these results, we can conclude that the colour change of the meat during frying changes mainly on the brightness and redness scale and these changes are similarly perceived by the human eye.

Assuming a relationship between the HCA content and the result of the colour analysis, the sensory test showed the highest score and the strongest correlation with the measured HCA content, followed by L^* and a^* values, the b^* correlation proved to be weak. Based on the results of the present studies, the colour of the surface of chicken breast may be a useful indicator of the HCA content formed during grilling at different temperatures and times.

Effect of grilling on heat destruction of Salmonella Enteritidis

According to the results of our research, grilling is an effective heat treatment method to kill *S. Enteritidis*. However, it is important to choose the right temperature-time combination because, as expected, higher temperatures and longer duration of heat treatment cause more pronounced bacterial death.

Closed contact grilling, presumably due to better heat retention, caused faster death of the bacteria. At the same time, the skin can form a heat-insulating layer that keeps the temperature lower for a longer period of time, thus helping *Salmonella* to survive in the meat. However, in determining the rate of elimination, the difference between the slopes of the curves did not fully reflect the expected decay time based on the literature, which may also be due to the fact that the temperature of the grilling surface does not correspond to the core temperature of the sample.

Joint assessment of toxicological and microbiological food safety and organoleptic aspects in the case of grilled poultry meat

From a microbiological food safety point of view, heat treatment with a higher temperature-time combination is preferred. At the same time, heat treatment at higher temperatures and for longer periods of time increases the amount of carcinogenic HCAs formed. In addition, we must pay attention to the organoleptic properties of grilled meat. In our previous studies, the colour of meat can be related to HCA content on the basis of both mechanical and organoleptic tests, so that the intake of these carcinogens among the population can be reduced with appropriate information about overcooked meals.

Based on the results of our studies, the food hygiene risk modeled by HCA and *S. Enteritidis* in the case of electric grilling can be reduced by considering the following aspects:

- Skinless frying: the skin can provide protection against pathogens in the inner layers of the meat, and at higher temperatures, melting fats can increase the heat transfer of the outer (but not only surface) layers, as well as the formation of HCA.
- Lower grilling temperature, longer grilling time: at 150 °C, even during long grilling times, high HCA levels were not observed in the cortical layer, and at 190 °C the MOE values of the averaged samples remained below 10,000 even after 15 minutes indicating low risk. However, with open but especially skin grilling, it is difficult to achieve the safe core temperature required for *Salmonella* elimination. At the same time, during grilling at low temperatures, the organoleptic characteristics of the meat do not meet consumer expectations.
- Contact grilling: HCAs are mainly formed on the contact surface of meat and the grilling pan, which is similar for open and closed (contact) grilling. At the same time, the destruction of *Salmonella* is much more a function of core temperature, which in turn can be increased more rapidly in a closed system with less ambient heat loss. The contact grill is thus able to produce a microbiologically safer product in a shorter time. In parallel, we found no significant difference between closed-open pairings grilled under the same conditions in terms of the amount of HCA formed in them. The reason for this can presumably be due to two factors, on the one hand the temperature of the grilling tray is not significantly reduced by open grilling, and the samples spent twice as much time on the grilling tray
- Colour change: The results of the sensory colour test showed a strong correlation with the HCA content of our samples. Therefore, consumers can deduce from the colour of the grilled meat the amount of carcinogenic amines in it. It is therefore important to draw the public's attention to the dangers of overcooking and to the sensory recognition of this danger.

Comparing the results of our chemical HCA measurements with the microbiological, *Salmonella* mortality studies, we found a negative correlation between the number of carcinogens produced and the number of surviving bacteria.

New Scientific Results

1. We were the first in Hungary to describe the basic factors influencing the formation of carcinogenic HCAs in poultry meat.
2. That was the first simultaneous study about the effect of the combination of grilling temperature and time on the formation of HCAs, the organoleptic properties of poultry meat and the rate of *Salmonella* elimination.
3. We were the first to investigate the correlation between sensory tests, instrumental colour analysis, and the HCA content of grilled poultry meat samples.
4. It was the first time to be investigated the correlation between HCA formation and *Salmonella* mortality during grilling under different conditions in poultry meat.

Publications

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