Studies of HAA accumulation in meat products, depending on the raw materials type and the heat treatment duration

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Abstract. This article addresses the issue of heterocyclic aromatic amines (HAA) accumulation during the process of heat treatment of meat products. There are methodological conditions of HAAs determination using high performance liquid chromatography with mass-spectrometer detection. During the research the most important factors that affect HAAs formation in meat products were identified. The most important factors are heat treatment duration, product contact with the heating surface, and temperature of the heat treatment should be above 150 °C. During research work, the information about HAAs concentration in beef, chicken and pork was obtained. The highest amount of HAAs was formed in beef, in the same time the lowest concentration was detected in poultry. Also the relation between heat treatment duration and HAAs concentration was identified every 5 mins of heat treatment leads to an increase of about 50% of HAAs concentration. Studies have shown that HAA are unique products of post-translational modifications occurring in animal proteins, which can be formed as a result of a biochemical condensation reaction of carboxyl and amine compounds. Safe products manufacturing is impossible without studying the ways of metabolism of protein and the mechanisms of action of organic contaminants.

1. Introduction
During the last two decades more attention has been paid to foods safety by science society. Meat products are the most popular food to be researched because of the increasing number of scientific articles and reports of scientific organizations about potential harm of red meat [1-4]. A number of studies proved that during the heat treatment of meat products there are new chemical compounds with carcinogenic and mutagenic features. HAA belongs to that kind of compounds. HAA is a group of chemical compounds formed in foods with high protein content (such as meat, chicken and fish) during high temperature heat treatment (e.g. frying, barbeque, grill etc.). Supposedly they are formed from creatine/creatinine, carbohydrates and aminoacids as products of Maillard reaction [5-7].

Based on the results [8] the main factors contributing to the formation of the HAA in the product were determined. They are the temperature (above 150 °C), the duration of heat treatment, the contact of the product and the heating surface. Regarding this, the HAA will mainly be formed in products cooked at home, and also at public catering enterprises. The range of the HAA values content in food products varies from 1 µg / kg to 100 µg / kg. The quantitative content of the HAA in food products is directly proportional to the temperature of heat treatment. The profile of the substances related to the HAA depends on the temperature in the same way [1, 9-10].
2. The purpose of the study
The research works aimed to find the ways of decreasing the HAAs amount in meat products are necessary, because of HAAs carcinogenic and mutagenic features. To find the ways of HAAs concentration decrease, the first factors that have the most affect in reaction of HAAs formation in meat products should be identified. According to literature analysis the most important factors in reaction of HAAs formation are type of raw meat, heat treatment temperature its duration and type. The purpose of studies in this article was identification of effect of raw materials and heat treatment duration to the amount of HAAs formed in meat products.

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3. The object of the study
The meat products were the objects of the study:
- Fried boneless chicken (m. Pectoralis major).
- Fried boneless pork (m. Longissimus dorsi).
- Fried boneless beef (m. Longissimus dorsi).

From each type of meat we formed steaks with a mass of 150.0±1.0 g and 2.5 cm in thickness. After steaks formation they were fried using electric grill at a temperature of 230˚C. The first set of steaks were fried during 5 min on each side, second one – during 7.5 min, and the third set was fried during 10 min on each side.

4. Materials and methods

4.1 Method development
For the development of the method of HAAs determination in meat products as standards we used:
- 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) – manufactured by Toronto Research Chemicals (Canada) with purity of above 99.0%;
- 2-Amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) – manufactured by ChemCruz (USA) with purity of above 95.0%.

Determination of HAAs in meat products was made using HPLC system (Agilent 1200) with QQQ MC/MC detector (Agilent 6410B). Column C18 4.6x50 mm, 1.8 μm (Agilent, USA) were used for analytes separation. Acetonitrile (Panreac, France), formic acid (Merc, USA), deionized water obtained on the MilliQDirect 8 system (France) were used as reagents.

4.2 Sample preparation
After frying the samples were cooled at room temperature then the samples were ground. Ground samples were hydrolase in 1M NaOH solution in ethanol. After that the hydrolysis analytes were extracted by the diethyl ester. Then the diethyl ester was evaporated to dry residue, which was dissolved in 1 ml of acetonitrile transferred into chromatographic vial for analysis.

5. Discussion of the results
Analysis of Russian technology of manufacturing of meat products showed that the critical technology in terms of HAAs formation is the manufacturing of culinary products. According to the results of a causal analysis, raw materials should affect the amount of HAAs formation. In accordance with this statement, studies were immersed to determine the accumulation of HAA depending on the type of raw material. The most widely used types of raw materials in industry were selected as objects of the study - poultry meat (chicken), pork and beef. Studies have shown that the type of raw material does not affect the amount of HAA so significantly as temperature and heat treatment duration do. The results of the study are shown in table 1 and in figure 1.
Table 1. Results of studies on the formation of HAA in meat products obtained from different types of raw meat

| Steaks made of | Heat treatment duration on each side, min | Amount of determined HAAs, µg/kg | MelQx | PhIP |
|---------------|------------------------------------------|----------------------------------|-------|------|
| Poultry       | 5.0                                      | 3.84±1.34                       | 18.61±6.51 |
| Pork          | 5.0                                      | 12.02±4.21                      | 35.07±12.27 |
| Beef          | 5.0                                      | 6.20±2.17                       | 36.36±12.72 |
| Poultry       | 7.5                                      | 5.99±2.10                       | 29.77±10.42 |
| Pork          | 7.5                                      | 18.63±6.52                      | 55.05±19.27 |
| Beef          | 7.5                                      | 9.92±3.47                       | 51.99±18.20 |
| Poultry       | 10.0                                     | 9.11±3.19                       | 45.25±15.84 |
| Pork          | 10.0                                     | 29.26±10.24                     | 83.13±29.10 |
| Beef          | 10.0                                     | 15.27±5.35                      | 76.94±26.93 |

The studies did not show a large difference in the total concentration of HAA formed during frying of pork and beef, but they showed that the amount of HAA in frying chicken is 1.5-2 times less than that in pork or beef. This may be caused by the lowest fat content in poultry meat relative to other types of meat. At the same time, an increase in the duration of heat treatment by 2.5 minutes on each side increases the amount of HAA formed in the product almost 1.5 times.

Figure 1. The amount of HAA formed during frying of steaks made from various types of raw materials, µg/kg, 1, 2, 3 - chicken, pork, beef fried for 5 minutes on each side, 4, 5, 6 - chicken, pork, beef fried for 7.5 minutes on each side, 7, 8, 9 - chicken, pork, beef fried for 10 minutes on each side, the ordinate - concentration µg/kg
Also cinder formed during the frying of meat was studied by HAAs concentration. Three steaks made of different types of raw meat were sequentially grilled for 10 minutes each at a temperature of 230 °C without cleaning the grill between the samples. About 1.0 g of cinder was collected from the heating surface after frying. The results of the study are shown in table 2.

Table 2. The results of studies of the HAAs concentration in cinder

| Sample                      | MeIQx, µg/kg       | PhIP, µg/kg       |
|-----------------------------|--------------------|------------------|
| Cinder formed during poultry frying (chicken) | 436.63±152.82  | 3179.95±1112.98  |
| Cinder formed during pork frying | 480.30±168.10  | 3402.55±1190.89  |
| Cinder formed during beef frying | 414.80±145.18  | 3275.35±1146.37  |

Studies shows that the difference between the samples obtained by frying various types of meat is insignificant, but the amounts exceed the values obtained in meat more than 30 times. This suggests that the heating surface must be cleaned, and eating this cinder is unacceptable.

6. Conclusion

The conducted studies allow one to establish a direct proportional relationship between the increase in the duration of the heat treatment and the concentration of HAA formed in meat products. Studies show that the longer the meat is treated at high temperatures, the greater the risk it poses to human health.

Determination of the main factors affecting the formation of HAA in meat products will allow the most efficient selection and study of measures to manage the risk of HAAs formation, which in the long run will reduce the carcinogenic and mutagenic load on the human body and make food manufacturing safer.

References

[1] Utyanov D A., Kulikovskii A V, Vostrikova N L and Kuznetsova O A 2019 Products of chemical reactions that occur during high-temperature heat treatment of the meat products Theory and practice of meat processing 4 (4) 17-22 https://doi.org/10.21323/2414-438X-2019-4-4-17-22

[2] Antipova L V, Popova Y A. and Cherkasova A V 2019 Products from rabbit meat for a healthy diet: the creation of assortment lines, nutritional and biological value Proceedings of Voronezh State University of Engineering Technologies 81 (1) 225-231. (In Russ.) https://doi.org/10.20914/2310-1202-2019-1-225-231

[3] Khairullin M F, Koval E A., Levitskaya I Y, Gadjiev M G and Sultonov B A 2019 Development of technology of preparation of pork semi-products with the application of low-temperature treatment. Proceedings of Voronezh State University of Engineering Technologies 81(2) 250-256. (In Russ.) https://doi.org/10.20914/2310-1202-2019-2-250-256

[4] Vostrikova N L, Kuznetsova O A, Kulikovskii A V and Minaev M Yu 2017 Formation of the scientific basis of meta-data associated with estimates of «onco-»risks linked to meat products Theory and practice of meat processing 2 (4) 96-113 https://doi.org/10.21323/2414-438X-2017-2-4-96-113

[5] Kulikovskii A V, Utyanov D A, Vostrikova N L and Ivankin A N 2018 Accumulation of carcinogenic substances in fat cocktails depending on the temperature treatment Vsyo o myase 2 30-33 (In Russ.) https://doi.org/10.21323/2071-2499-2018-2-30-33

[6] IARC 1993 monographs of the evaluation of the carcinogenic risk of chemicals to humans. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins (World Health Organization. Intl. Agency for Research on Cancer)
[7] Ferlay J 2012 Cancer Incidence and Mortality Worldwide (IARC Cancer Base No. 11)
[8] Solaykov A A 1998 The influence of the thermal treatment and preparation methods of semifinished products for the content of heterocyclic aromatic amines in fried meat food products, Diss. Abstract Candidate of Technical Sciences, Moscow, G. V. Plekhanov’s Russian University of Economics
[9] Alaejos M S and Afonso A M 2011 Factors that affect the content of heterocyclic aromatic amines in foods Comprehensive reviews in food science and food safety 10 (2) 52–108 https://doi.org/10.1111/j.1541–4337.2010.00141.x
[10] Buła M, Przybylski W, Jaworska D and Kajak-Siemaszko K 2018 Formation of heterocyclic aromatic amines in relation to pork quality and heat treatment parameters Food Chemistry 276 511–519 https://doi.org/10.1016/j.foodchem.2018.10.073