Monitoring studies of the content of heterocyclic aromatic amines in second-course meals with chilled side dishes sold in the Russian Federation

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Abstract. The research carried out allowed establishing that during the industrial preparation of second-course meals with garnish in the meat components, heterocyclic aromatic amines are formed. Heterocyclic aromatic amines were found in all samples tested. However, the lack of information on the preparation technology of the selected samples does not allow a complete analysis of the results obtained. However, it is worth noting that the largest amount of heterocyclic aromatic amines was formed in samples with chicken meat cooked at the highest temperatures relative to other samples, judging by its appearance. The presence of heterocyclic aromatic amines in all studied samples indicates the potential harm of consuming such products for human health.

1. Introduction

Heterocyclic aromatic amines are relatively little studied to date carcinogens formed in food products during thermal processing. Many clinical trials in laboratory animals show that heterocyclic aromatic amines have carcinogenic and mutagenic activity [1-7]. Heterocyclic aromatic amines are formed in food products during high-temperature processing. High-temperature heat treatment methods are roasting, roasting over an open fire, grilling. The most considerable amount of heterocyclic aromatic amines is formed in meat products due to the high content of creatine and creatinine in it. Creatine and creatinine are the primary reagents in forming heterocyclic aromatic amines [8, 9]. Previous studies [10-13] have shown that the temperature and duration of heat treatment are the main factors influencing the formation of heterocyclic aromatic amines in the product.

Based on the previous, it is reasonable to assume that the main products that pose a risk of human consumption of heterocyclic aromatic amines are homemade dishes. However, the safety management of such products is not possible. However, lately, the market for second-course meals has been actively developing, especially in large cities. The reason is that the active rhythm of city dwellers' lives does not leave time for cooking homemade food. Today, both deliveries of ready-made meals to home a few days in advance and the sale of chilled and frozen second courses in stores are common. Moreover, in such products, the risk of forming heterocyclic aromatic amines in them is possible by technological methods. However, before that, large-scale monitoring studies are needed to collect data on the formation of heterocyclic aromatic amines in second lunch dishes.

In this regard, the purpose of this work was to collect data on the formation of heterocyclic aromatic amines in chilled second lunch dishes sold in stores. The most popular meat raw material for...
such products is chicken meat, the next most popular is beef. Information on the technology for preparing samples is not available, except for two samples. The manufacturer states in the marking that the heat treatment of the products is carried out by prolonged soaking at a temperature of no more than 95°C.

2. Materials and research methods

The objects of research were (names are given following the labeling):
- Sample 1: "Azu from beef with pasta";
- Sample 2: "Azu from beef with mashed potatoes";
- Sample 3: "Azu from beef with rice";
- Sample 4: "French chicken meat with browned potatoes";
- Sample 5: "Chicken cutlets with browned potatoes";
- Sample 6: "Chicken schnitzel with mashed potatoes";
- Sample 7: "Pilaf with chicken";
- Sample 8: "Pilaf with chicken";
- Sample 9: "Turkey with rice and vegetables";
- Sample 10: "Chicken fricassee with buckwheat";
- Sample 11: "Chicken in Thai style with rice and vegetables";
- Sample 12: "Pasta carbonara" (meat component – pork);
- Sample 13: "Turkey with rice and vegetables";
- Sample 14: "Pasta in the Navy";
- Sample 15: "Beef wok";
- Sample 16: "Cutlet with creamy sauce".

Only the meat component was investigated in all samples, except for sample 4 and sample 6. For investigation, the meat component was manually separated from the garnish, homogenized. And then, 3.00 ± 0.20 g of meat was taken for further extraction of heterocyclic aromatic amines. Sample 6: the meal was prepared from meat and bread due to the impossibility of separating the breading from the meat product. Sample 4: the sample also examined the "coating" of the meat product from mushroom mayonnaise (sample 4msg). In addition, the upper and lower layers of a meat product with a thickness of ≈1 mm were examined, but in this case, 1.00 ± 0.20 g each meal was taken (samples 1c and 1n, respectively).

For the determination of heterocyclic aromatic amines in meat products, the following were used as standard samples:
- a standard sample of 2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline (MeIQx) manufactured by Toronto Research Chemicals (Canada) with a basic substance content of at least 99.0%;
- standard sample of 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP) manufactured by ChemCruz (USA) with a basic substance content of at least 95.0%.

The analysis was performed on an Agilent 1200 high-performance liquid chromatography system (USA) with an Agilent 6410B three quadrupole mass spectrometer. For determining heterocyclic aromatic amines, a C18 chromatographic column, 4.6 x 50 mm, 1.8 μm (Agilent, USA), was used. When selecting the conditions for chromatographic identification, the following reagents were used: acetonitrile for HPLC, manufactured by Panreac (France), Merck formic acid (USA), deionized water obtained on a MilliQDirect 8 system (France). Table 1 and Table 2 show MRM ion exposure parameters and gradient elution conditions.
Table 1. Parameters of exposure to ions in MRM mode and conditions of ionization by sputtering in an electric field (ESI) with registration of positive (+) and negative (-) ions

| Analyte | Molecular ion, m / z | Daughter ions, m / z | Fragmentor voltage (Frag), V | Dissociation energy (CE), V |
|---------|---------------------|---------------------|-----------------------------|-----------------------------|
| MeIQx   | 214.6 (+)           | 199.5               | 130                         | 30                          |
| PhIP    | 225.6 (+)           | 210.5               | 130                         | 30                          |

Table 2. Gradient Elution Conditions

| Time, min | Acetonitrile, % | Water, % | Flow rate, µL / min |
|-----------|-----------------|----------|---------------------|
| 0         | 10              | 90       | 400                 |
| 3         | 40              | 60       | 400                 |
| 4         | 60              | 40       | 400                 |
| 6         | 90              | 10       | 400                 |
| 8         | 90              | 10       | 400                 |
| 8.1       | 10              | 90       | 400                 |
| 12        | 10              | 90       | 400                 |

The detection limit under the selected conditions for both analytes was 0.1 ng / g.

Sample preparation. A sample of the analyzed product weighing (3.00 ± 0.20) g was placed in a round-bottom flask with a thin section with a capacity of 250 cm³ and added 50 cm³ of a solution of 1 M sodium hydroxide in ethanol. The flask was connected to a reflux condenser, placed in a water bath, and heated at a temperature of (80 ± 2)°C for 30 min or until the sample was dissolved entirely, periodically stirring the flask contents with a borosilicate glass rod. After that, the contents of the flask were cooled to room temperature.

The resulting hydrolyzate was transferred into a separating funnel with a volume of at least 250 cm³, and 10-15 cm³ of distilled water was added for cooling. Then, 25 cm³ of diethyl ether was added to a separatory funnel, and the mixture was allowed standing for 1-5 minutes until the layers were separated. After that, the lower layer was poured off and poured into another separatory funnel with a volume of at least 250 cm³ for repeated extraction. To it was added 25 cm³ of diethyl ether and left to separate the layers. After that, the lower layer was poured off, and the ether layer obtained after the first extraction was added to the upper ether layer.

The resulting ether was washed 2-5 times with distilled water in portions of 25-30 cm³ to cleanse the sample from alkali. Then the ether layer was passed through a membrane filter with 15-20 g of sodium sulfate for dehydration. The resulting ether was evaporated to dryness at a temperature not exceeding 40°C. 1 cm³ of acetonitrile was added to the dry residue. They were placed for 5 min in an ultrasonic bath until the residue was completely dissolved. The solution was passed through a membrane filter with a pore diameter of 0.45 µm into a chromatographic vial with a capacity of 2 cm³ for HPLC-MS / MS analysis.

3. Research results
The studies were carried out in two samples with three parallel measurements in each. The arithmetic mean of three parallels in the first sample was taken as the final result. The Student's test of two samples for each analyte to be determined in each sample did not exceed the table value at n = 3 and a confidence level of p = 0.95; the differences between the samples are statistically insignificant. Table 3 and Figure 1 show the study results of the second-course meals. Figure 1 shows information only about MeIQx.
Table 3. The results of studies of the meat component in the second-course meals with a side dish

| Sample No. | MelQx       | PhIP         |
|------------|-------------|--------------|
| 1          | 11.50±4.02  | Less than 0.1|
| 2          | 3.17±1.11   | Less than 0.1|
| 3          | 2.43±0.85   | Less than 0.1|
| 4          | 6.11±2.44   | Less than 0.1|
| 4msg       | 0.84±0.34   | Less than 0.1|
| 4v         | 7.66±3.06   | Less than 0.1|
| 4n         | 1.55±0.62   | Less than 0.1|
| 5          | 47.40±18.96 | Less than 0.1|
| 6          | 24.62±9.85  | Less than 0.1|
| 7          | 0.54±0.22   | Less than 0.1|
| 8          | 0.21±0.08   | Less than 0.1|
| 9          | 0.29±0.12   | Less than 0.1|
| 10         | 0.18±0.07   | Less than 0.1|
| 11         | 0.21±0.08   | Less than 0.1|
| 12         | 0.17±0.07   | Less than 0.1|
| 13         | 10.41±4.16  | Less than 0.1|
| 14         | 1.71±0.69   | Less than 0.1|
| 15         | 0.96±0.38   | Less than 0.1|
| 16         | 0.45±0.18   | Less than 0.1|

Figure 1. The number of MelQx in main lunch dishes with chilled side dishes.
4. Discussion

The results showed that heterocyclic aromatic amines are formed in all studied samples. However, it should be noted that PhIP was not detected in any of the studied samples in quantities that would allow determining its concentration reliably.

The largest amount of heterocyclic aromatic amines was found in samples 5 and 6. The same manufacturer made both samples. Moreover, both samples are characterized by a fried "spot of contact" of the product with the heating surface. From this fact, it can be assumed that both products were prepared precisely by frying. Suppose this assumption is correct, and both products were prepared under approximately the same conditions. In that case, the use of breading reduces the amount of heterocyclic aromatic amines formed by almost half.

Samples 9 and 13 – Turkey with Rice and Vegetables. However, the research results showed that in sample 13, the amount of heterocyclic aromatic amines was more than 30 times relative to sample 9. This fact is most likely due to the preparation technology. As mentioned earlier, sample 9 was prepared using prolonged simmering at a temperature of no more than 95°C. The turkey in Sample 13 was most likely roasted. If this assumption is correct, it again suggests that the heat treatment temperature is one of the most significant factors in managing the risk of formation of heterocyclic aromatic amines in the product.

The research results for sample 4 showed the following results. In French meat, the largest amount of heterocyclic aromatic amines is formed in the upper part of the meat product. The traditional cooking technology of this dish is roasting with a whole product without turning it over during heat treatment. If this dish was cooked traditionally, then the lowest amount of heterocyclic aromatic amines was formed in the lower part of the product due to the vegetable oil, which was most likely applied to the heating surface. The effect of vegetable fats on the formation of heterocyclic aromatic amines in the product was considered in more detail in [14]. A much more significant amount of heterocyclic aromatic amines in the upper part of the product can theoretically be explained by the fact that the specific heat capacity of mayonnaise is much less than the specific heat capacity of meat. The reason is that, on average, 75% of mayonnaise is made from vegetable fats. Because of this feature, the heating of mayonnaise with mushrooms occurred faster than meat and mayonnaise with mushrooms. In this case, mayonnaise acts as a kind of heating surface with which the meat component of the product contacts. Either the formation of more heterocyclic aromatic amines in the upper part of the article is due to the following. During cooking, most likely, the meat product was fried on one side, after which the product was turned over and brought to culinary readiness, frying a little on the other side. In addition, the heterocyclic aromatic amines found in mayonnaise with mushrooms indicate the following. During the heat treatment, either migration of heterocyclic aromatic amines is possible, or the formation of heterocyclic aromatic amines is also possible in products with mushrooms.

5. Conclusions

Studies have shown that second-course meals, in theory, can negatively affect the human organism. Unfortunately, the current lack of information on the maximum permissible concentrations of heterocyclic aromatic amines on their toxic and lethal doses for human organisms does not allow an objective assessment of how dangerous their consumption by humans can be. However, based on the annually growing number of scientific publications devoted to heterocyclic aromatic amines, it can be assumed that such data will be available relatively soon, and by that time, it is necessary to collect as much data as possible on the content of heterocyclic aromatic amines in food. Further research is needed on their accumulation in second-course meals. However, other foods that may pose a risk of heterocyclic aromatic amine formation, such as burgers sold at fast-food restaurants, need to be investigated.

The results also made it possible to determine the vector of further research on managing the risk of heterocyclic aromatic amines formation in meat products. First of all, using the temperature of heat treatment, then using vegetable oils during heat treatment. Particular attention should be paid to
studies of the effect of breading on the amount of heterocyclic aromatic amines formed in meat products.

Acknowledgments
The research was carried out within the framework of the state assignment of the Federal Research Center of Food Systems named after V.M. Gorbatov RAS FNEN-2019-0009.

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