

# HETEROCYCLIC AROMATIC AMINES AS A SAFETY CRITERIA FOR MEAT AND FISH PRODUCTS

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**ABSTRACT.** *Against the background of adverse environmental conditions in Ukraine, experts in the field of medicine, food hygiene note the raising importance of various nutritional factors in increasing the risk of developing cancer. A number of compounds resulting from protein denaturation during the heat treatment of meat and fish products can pose a high risk. Such compounds include heterocyclic aromatic amines (HAA) i.e. substances that, in extremely low concentrations, can carry out mutagenic and carcinogenic effects on the human body.*

**Aim of the Research.** *To analyse studies from available sources of information about the likelihood and conditions of the formation of chemicals hazardous to human health in foods of animal origin during thermal cooking.*

**Methods and Materials.** *Review and analysis of scientific publications based on the results of experimental studies of European, American and Japanese scientists.*

**Results and Discussion.** *Studies have established that in the process of thermal cooking of food products of animal meat, poultry, fish, HAA are formed and accumulate as a result of complex multi-stage chemical reactions with the obligatory participation of amino acids, sugars, creatine and creatinine, which is its cyclic form. The formation of mutagenic HAA is affected by a number of conditions. For example, the accumulation of heterocyclic aromatic amines in fried meat products is most affected by the temperature of the surface of the pan and the outer layer of meat, the duration of frying, the degree of meat chopping, the presence of breading, pickling, the addition of onions to the chopped meat.*

**Conclusions.** *Taking into account that the diet of more than 40% of the population includes products containing HAA, the development and justification of safety criteria for meat and fish products concerning the content of heterocyclic aromatic amines as well as the development of ways to prevent their formation form an urgent problem.*

*The following tasks must be solved as priorities:*

- to develop and standardize methods for the determination of HAA in various types of food products;
- to conduct studies of the content of HAA in meat and fish products and in culinary products;
- to study the influence of various methods of thermal cooking on the accumulation of HAA in finished products made from meat and fish;
- to study the effect of the raw materials used and production technologies on the accumulation of HAA in food products;
- to optimize the formulae of meat and fish products and their production technologies in order to minimize the number of HAA formed in the finished product;
- to justify the acceptable levels of HAA in food products and to develop safety criteria for meat and fish products concerning the content of HAA on the basis of clinical and laboratory studies.

**Key Words:** *heterocyclic aromatic amines (HAA), meat products, fish products, thermal cooking, safety.*

**Introduction.** When studying the influence of environmental factors on human health, more attention is paid to the characteristics of his/her nutrition. With the development of analytical chemistry in food products, many chemical compounds are determined which are unfavourable in terms of their safety for humans. These include both chemical compounds present in food products prior to cooking, and chemicals that accumulate in food products during their cooking.

Since a significant part of the products that are in the human diet are meat and fish products containing protein components of animal origin, the special attention is paid to studying the processes of biochemical degradation of proteins, as a result of which heterocyclic aromatic amines are formed.

**Aim of the Research.** To analyse studies from available sources of information about the likelihood and conditions of the formation of chemi-

cals hazardous to human health in food products of animal origin during thermal cooking.

**Methods and Materials.** Review and analysis of scientific publications based on the results of experimental studies of European, American and Japanese scientists.

**Results and Discussion.** A number of compounds resulting from protein denaturation during the thermal cooking of meat and fish products can pose a high risk. Such compounds include heterocyclic aromatic amines (HAA) i.e. substances that, in extremely low concentrations, can carry out mutagenic and carcinogenic effects on the human body. Toxicological and hygienic studies of the HAA are actively conducted by Russian, American, European and Japanese scientists. The relevance of this problem is also recognized in Ukraine.

In vitro studies on microbiological objects showed HAA mutagenic activity, whereas animal experiments and epidemiological data confirm that HAA are carcinogens and contribute to the development of tumours of the gastrointestinal tract, liver and pancreas.

Indeed, in the United States for several years, large-scale studies have been conducted among the population, confirming the risk of developing cancer caused by food HAA. These data are in accordance with the findings of scientists from other countries [1-5, 10].

Considering that the diet of the majority of the population includes products containing HAA, scientists direct their efforts to establish the maximum acceptable levels of HAA in food products and to develop ways to prevent their formation.

Studies have established that in the process of thermal cooking of food products of animal meat, poultry, fish, HAA are formed and accumulate as a result of complex multi-stage chemical reactions with the obligatory participation of amino acids, sugars, creatine and creatinine, which is its cyclic form.

Since HAA are present in products in very small quantities, their identification can be carried out only by modern and accurate analytical methods, using high-performance liquid chromatographic methods with ultraviolet, electrochemical and fluorescence detectors, liquid chromatography-mass spectrometry, gas chromatography, gas chromatography-mass spectrometry, electrophoresis and enzyme-linked immunosorbent assay [6].

According to the chemical structure all known HAA, which are dangerous to humans, are proposed to divide into 5 groups. The first group of "pyrolytic mutagens" includes pyrido-imidazoles and pyrido-indoles (Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, Phe-P-1, AaC, MeAaC), which are formed at cooking temperatures above 300°C and are found in trace amounts in finished food products. HAA of the second and third groups ("IQ compounds") – quinolines and quinoxaline (IQ, MeAQ, Igx, 4-MelQx, MelQx, 7,8-DiMelQx, 4,8-DiMelQx) are separated from traditional beef broth, from products made of minced beef and pork fried in traditional way, from products made of poultry and lamb and fried over charcoals, as well as from fried and smoked fish products. It has been established that free amino acids (proline, phenylalanine, serine, glycine, alanine, threonine), creatine (or creatinine), glucose, fructose take part in their formation. The most typical representative of the fourth group – of pyridines is 2-amino-1-methyl-6-phenylimidazo [4,5 b] pyridine (PhIP), found in fried meat and other food products. Creatine (or creatinine), phenylalanine (or leucine), and glucose are considered as its predecessor. The fifth group, i.e. furopyridines, includes compounds with an incompletely established structure separated from the products made of minced beef and pork fried in traditional way, as well as from meat juice obtained after frying meat products in the traditional way. In their mutagenic activity, they are similar to methylimidazofuropyridine (MeIFP).

Due to the diversity of the qualitative composition of raw products and the methods of thermal cooking, it is almost impossible to predict which HAA will be formed and accumulate in each particular case. Existing data show that HAA groups of quinolones, quinoxaline and pyridine are most often found. A quantitative assessment of carcinogenic HAA in various types of cooked food products showed that the level of 2-amino-1-methyl-6-phenylimidazo [4,5 b] pyridine (PhIP) was the highest (0.56-69.2 ng / g); that 2-amino-3,8-dimethylimidazo [4,5 f] quinoxaline (MeIQx) was the second largest (0.64-6.44 ng / g), and in other HAAs – 0.03-2, 50 ng / g [3].

The formation of mutagenic HAA is affected by a number of conditions. The largest amounts of mutagenic HAA appear in the crust of fried meat and fish (and much less frequently in the inner parts of finished products), as well as in meat juice, which flows into the pan when frying meat,

fish, poultry, thus the origin of HAA is associated with certain methods of thermal cooking of meat and fish products. Processing factors such as high temperatures and prolonged thermal cooking contribute to the formation of these compounds. It has been established that the accumulation of heterocyclic aromatic amines in fried meat products is most affected by the temperature of the surface of the pan and the outer layer of meat, the duration of frying, the degree of meat chopping, the presence of breading, pickling, the addition of onions to the chopped meat.

The greatest number of heterocyclic aromatic amines is found in fried products such as grilled meat, barbecue, as well as in portioned meat fried with a small amount of fat (in the traditional way), that is, under conditions that allow local burning (burn).

Comparative studies of various methods of thermal cooking have shown that frying in the traditional way, deep frying, and also frying on charcoal lead to the formation of substances of a much higher level of mutagenic activity than such methods as short-term roasting, braising, microwave irradiation [7-10].

Studies have established that substances that cause the appearance of HAA (HAA precursors) are formed and accumulate during autolysis while meat maturing. These are creatine, free amino acids and monosaccharides. This was confirmed by model experiments, during which a mixture of creatine, glucose and free amino acids (glycine, alanine) was exposed to high temperatures. Based on such data, Professor M. Jgerstad (Sweden) proposed a mechanism for the formation of HAA in the presence of creatine, hexoses, and free amino acids and showed that creatine content is a limiting factor in the formation of food mutagens. Further studies by M. Jgerstad showed that the cyclic form of creatine, i.e. creatinine, which is formed during the process of cooking, is reactive. During frying, various amounts of creatinine were created from creatine, and it was found that mutagenic activity was associated with creatinine levels.

It should be noted that not proteins, but free amino acids, are involved in the reactions of the formation of mutagenic HAA. It has been proven that serine, threonine, phenylalanine, alanine, leucine and tyrosine lead to the appearance of one of the known food mutagens 2-amino-3,8-dimethylimidazo [4,5 f] quinoxaline (MeIQx), 2-amino-1 methyl- 6-phenylimidazo [4,5 b] pyri-

dine (PhIP) or 2-amino-trimethylimidazopyridine (TMIP).

It is concluded that creatine and free amino acids are the main reagents of mutagen-generating reactions that occur during meat frying. Creatine is a necessary part of all these reactions, and what specific compounds are formed in each case depends on the content of certain free amino acids in meat and their interaction with other, as yet unknown, compounds in meat.

Studies show that in natural meat, the ratio of creatine, free amino acids and monosaccharides is optimal for the formation of HAA [11-13].

The mechanism of formation of heterocyclic aromatic amines is currently presented as the result of the interaction of the products of carbonyl-amine reactions (Maillard) and creatinine (creatine anhydride).

In food industry and public catering 75-85% of products are the products which have necessarily been cooked with thermal processing. The low molecular weight nitrogenous substances of food products undergo significant changes during thermal cooking, entering into chemical interactions with each other (as well as with reducing sugars, which are normally present in food products), resulting in the formation of mutagenic and carcinogenic substances that pose a potential danger to humans.

Therefore, researchers, chemists, doctors, technologists, developers and designers are constantly working to improve and intensify the methods of thermal cooking of products. At the same time, it is relevant to create combined technological processes, which, in addition to thermal ones, include various methods of pre-treatment of food raw materials (mechanical, microbiological, chemical, and the use of enzyme preparations). Food products that are new in chemical composition differ from traditional ones. As recent studies show, essentially harmless chemicals can have a negative effect when combined with each other or participate in the formation of harmful substances. In products made of meat and fish, heterocyclic aromatic amines should be included in the group of toxic substances; therefore, a number of problems with their identification, establishment of acceptable levels in food products, development of technologies, and minimization of the formation of HAA should be solved.

Against the background of adverse environmental conditions in Ukraine, experts in the field

of medicine, food hygiene note the raising importance of various nutritional factors in increasing the risk of developing cancer. Unfortunately, food heterocyclic aromatic amines as potential mutagens and carcinogens in our country have not been studied to date. There are also no national programs on this subject, which coordinated full-scale research by specialists in the field of toxicology, medicine, food hygiene and food technology. In 2004-2006, studies were carried out at Kharkiv State University of Nutrition and Trade to study the influence of technological factors on the formation and accumulation of HAA in culinary products made using fermented meat raw materials and various methods of thermal cooking [14-16].

Conclusions. Ecological analysis of heterocyclic amines is important for maintaining human health, since all these compounds are powerful mutagens, and many are carcinogens in experimental animals.

The above facts confirm the relevance of the development and justification of safety criteria for meat and fish products concerning the con-

tent of heterocyclic aromatic amines.

The following tasks must be solved as priorities:

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### **ГЕТЕРОЦИКЛІЧНІ АРОМАТИЧНІ АМІНИ ЯК КРИТЕРІЙ БЕЗПЕЧНОСТІ М'ЯСНИХ ТА РИБНИХ ПРОДУКТІВ**

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**Резюме.** На тлі несприятливих екологічних умов в Україні фахівці в галузі медицини, гігієни харчування наголошують на зростаючому значенні різних факторів харчування щодо підвищення ризику розвитку онкологічних захворювань. Ряд сполук, що утворюються в результаті денатурації білків у процесі теплової обробки м'ясних та рибних продуктів, можуть становити високий ризик. До таких сполук слід віднести гетероциклічні ароматичні аміни (ГАА) – речовини, які вкрай в низьких концентраціях можуть здійснювати мутагенну та карценогенну дію на організм людини.

**Мета роботи.** Провести аналіз досліджень із доступних джерел інформації щодо ймовірності та умов утворення небезпечних для здоров'я людини хімічних речовин у харчових продуктах тваринного походження в процесі термічної кулінарної обробки.

**Матеріали та методи.** Огляд та аналіз наукових публікацій за результатами експериментальних досліджень європейських, американських та японських вчених.

**Результати.** Дослідженнями встановлено, що в процесі теплової кулінарної обробки продуктів із м'яса тварин, птиці, риби, ГАА утворюються та накопичуються в результаті складних багатоступінчастих хімічних реакцій із обов'язковою участю амінокислот, цукрів, креатину і його циклічної форми креатиніну. На формування мутагенних ГАА впливає ряд умов. Наприклад, на накопичення гетероциклічних ароматичних амінів у смажених м'ясних виробах найбільше впливають температура поверхні пательні та поверхневого шару м'яса, тривалість смаження, ступінь подрібнення м'яса, наявність панірування, маринування, додавання до подрібненого м'яса ріпчастої цибулі.

**Висновки.** Беручи до уваги, що раціон харчування понад 40 % населення включає продукти, що містять ГАА, констатуємо, що розробка та обґрунтування критеріїв безпечності м'ясних та рибних продуктів за вмістом гетероциклічних ароматичних амінів та розробка способів запобігання їх утворення – проблема актуальна.

Як першочергові необхідно вирішити наступні задачі:

- розробити та стандартизувати методи визначення ГАА в різних видах харчових продуктів;
- провести дослідження вмісту ГАА в м'ясних та рибних продуктах та в кулінарній продукції;
- вивчити вплив різних способів теплової кулінарної обробки на накопичення ГАА в готовій продукції із м'яса та риби;
- вивчити вплив сировини, що використовується, та технологічних процесів виробництва на накопичення ГАА в харчових продуктах;
- оптимізувати рецептури м'ясних та рибних виробів та технологій їх виробництва з метою зведення до мінімуму кількості утворюваних ГАА в готовій продукції;
- на основі клініко-лабораторних досліджень обґрунтувати допустимі рівні вмісту ГАА в продуктах харчування та розробити критерії безпечності м'ясної та рибної продукції за вмістом ГАА.

**Ключові слова:** гетероциклічні ароматичні аміни (ГАА), м'ясні продукти, рибні продукти, теплова кулінарна обробка, безпечність.

### **ГЕТЕРОЦИКЛИЧЕСКИЕ АРОМАТИЧЕСКИЕ АМИНЫ КАК КРИТЕРИЙ БЕЗОПАСНОСТИ МЯСНЫХ И РЫБНЫХ ПРОДУКТОВ**

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**РЕЗЮМЕ.** На фоне неблагоприятных экологических условий в Украине специалисты в области медицины, гигиены питания отмечают возрастание значения различных факторов питания в повышении риска развития онкологических заболеваний. Ряд соединений, образующихся в результате денатурации белков в процессе тепловой обработки мясных и рыбных продуктов, могут представлять высокий риск. К таким соединениям следует отнести гетероциклические ароматические амины (ГАА) – вещества, которые в крайне низких концентрациях могут осуществлять мутагенное и канцерогенное действие на организм человека.

**Цель работы.** Провести анализ исследований из доступных источников информации о вероятности и условиях образования опасных для здоровья человека химических веществ в пищевых продуктах животного происхождения в процессе термической кулинарной обработки.

**Материалы и методы.** Обзор и анализ научных публикаций по результатам экспериментальных исследований европейских, американских и японских ученых.

**Результаты.** Исследованиями установлено, что в процессе тепловой кулинарной обработки продуктов из мяса животных, птицы, рыбы, ГАА образуются и накапливаются в результате сложных многоступенчатых химических реакций с обязательным участием аминокислот, сахаров, креатина и его циклической формы креатинина. На формирование мутагенных ГАА влияет ряд условий. Например, на накопление гетероциклических ароматических аминов в жареных мясных изделиях наибольшее влияние имеют температура поверхности сковороды и поверхностного слоя мяса, продолжительность жарки, степень измельчения мяса, наличие панировки, маринования, добавление к измельченному мясу репчатого лука.

**Выводы.** Принимая во внимание, что рацион питания более 40 % населения включает продукты, содержащие ГАА, разработка и обоснование критериев безопасности мясных и рыбных продуктов по содержанию гетероциклических ароматических аминов и разработка способов предотвращения их образования – проблема актуальная.

Как первоочередные необходимо решить следующие задачи:

- разработать и стандартизировать методы определения ГАА в различных видах пищевых продуктов;
- провести исследования содержания ГАА в мясных и рыбных продуктах и в кулинарной продукции;
- изучить влияние различных способов тепловой кулинарной обработки на накопление ГАА в готовой продукции из мяса и рыбы;
- изучить влияние используемого сырья и технологических процессов производства на накопление ГАА в пищевых продуктах;
- оптимизировать рецептуры мясных и рыбных изделий и технологий их производства с целью сведения к минимуму количества образуемых ГАА в готовой продукции;
- на основе клинико-лабораторных исследований обосновать допустимые уровни содержания ГАА в продуктах питания и разработать критерии безопасности мясной и рыбной продукции по содержанию ГАА.

**Ключевые слова:** гетероциклические ароматические амины (ГАА), мясные продукты, рыбные продукты, тепловая кулинарная обработка, безопасность.

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