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INFLUENCE OF HEAT TREATMENT TYPE ON THE FAT COMPONENT AND HETEROCYCLIC AROMATIC AMINES FORMATION IN THE CHOPPED POULTRY MEAT PRODUCTS

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Abstract

In this study the influence of heat treatment type on the change in the fatty acid composition, indices and parameters of fat oxidation, the level of heterocyclic amines formation in the chopped poultry ready-to-eat products enriched with ω-3 fatty acids and an antioxidant complex were studied. The composition of ingredients and recipes of ready-to-eat products are developed with consideration of the medical and biological requirements for the diets of oncological patients. These ready-to-eat products feature some antioxidant substances in their composition that can bind free radicals, and provide for a reduction in the risk of carcinogens formation during the manufacturing process. The heat treatment was run in several ways, also called as modes — cooking in a microwave oven (MW), cooking in a convection oven in the "convection" mode with preliminary short-term roasting, steam cooking. For comparison, the conventional, i. e. not enriched food sample was used as a control one. The introduction of ω-3 fatty acids into the food formulation made it possible to change the fatty acid composition towards increasing the proportion of polyunsaturated fatty acids (PUFAs). It was found that the profile of fatty acids was influenced by both the ingredients of the product and the type of its heat treatment. The joint use of a PUFA source and a complex of antioxidants made it possible to obtain a ready-to-eat product with a high level of ω-3; and to ensure the ratio of ω-6 group acids: ω-3 ratio amounts to 1–2:1. Heat treatment of enriched semi-finished products by microwave cooking and by steam cooking showed a lesser effect on the change in the content and composition of polyunsaturated fatty acids — the loss of fatty acids was 1.2% and 2.8%, respectively, while in the "convection" cooking mode with preliminary roasting this loss was equal to 3.5%. It was found that the antioxidant complex in the composition of the food product and gentle heat treatment methods cause less lipid peroxidation and the formation of carcinogenic heterocyclic aromatic amines (HAA) during the food manufacturing process.

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Introduction

In the modern world, among the non-communicable diseases, the oncological diseases now occupy a significant place. Among all carcinogenesis risk factors the share of nutrition is 35% [1, 2]. Quantitative and qualitative inferiority or, on the opposite, the excess of nutrients disrupts the normal metabolism, the functioning of organs and systems, and thus increases the risk of the emergence and development of malignant tumors. Diet is an important component in correcting the nutritional imbalances. The findings from Campbel et.al [3] showed that an increase of vitamin D content in blood serum and a decrease in the ratio of ω6/ω3 fatty acids due to dietary corrections and the inclusion of vitamin supplements into the diet affected the biology of prostate cancer cells in men and caused a decrease in the prostate-specific antigens level (PSA). The patients' diet consisted mainly of fruits, vegetables, turkey, chicken breast and cold-water fish, along with the following food supplements: ω3 PUFAs, curcumin, vitamin D3, and complex of B vitamins [3].

To compose a diet it is feasible to use the specialized and functional food. One of the main requirements for the composition of such food is the presence of antioxidants among the nutrients that can bind free radicals, as well as the absence of carcinogens formed due to the production process. The food products of animal origin are a source of complete proteins and fats, vitamins and minerals and should be included in a complete diet. In this regard, the creation of meat-based food (that could be included in the diet of people suffering from cancer) is one of the efficient ways to correct the structure of their diet.

Taking into account the biomedical requirements for the diet assigned for the cancer patients [4,5] in the Laboratory of "Functional and Specialized Nutrition" of the

V. M. Gorbatov Federal Research Center for Food Systems, recipes for chopped ready-to-eat products from poultry meat were developed. The implementation of medical and biological requirements was carried out through the choice of raw materials, ingredients and biologically active substances that have a beneficial effect on health. A reasonable approach in this case was the optimization of the macro- and micronutrients composition of the product, including the modification of the fat component, as well as the inclusion of ingredients with an antioxidant effect into the composition of the product. This will reduce the intensity of oxidative processes during the heat treatment of the food product and improve lipid metabolism, antioxidative action and vitamin status in patients.

In addition to carcinogens, the main nutritional factors that increase the risk of cancer are an excess of calories, fat, especially animal fat, cholesterol, mono- and disaccharides, a lack of complete proteins, essential amino acids, ω3 PUFAs, dietary fiber, polyphenolic compounds, deficiency of some micronutrients: vitamins C, E, B12, B9, carotenoids, flavonoids [6,7].

Reducing the pressure of carcinogenic risk on the human body and ensuring the optimal and balanced composition of all essential micro- and macronutrients in the diet will help increase the dietary anti-cancer resistance; that is reducing the risk of a tumor development by affecting the endogenous and exogenous reasons or factors of its development.

The current nutritional recommendations provide for reduced consumption of saturated fat by reducing and/or changing the quality of fat in the diet (partial replacement with unsaturated fats) [8].

To prevent the development of malignant neoplasms, nowadays physicians recommend limiting the consumption of red meat. Moreover, the meat of productive livestock animals features a high level of saturated fats; therefore, turkey meat was used as the basic raw material. Turkey lipids are rich in PUFAs, but contain predominantly ω-6 fatty acids.

In order to reduce the content of saturated fats and increase the share of ω-3 PUFA, the fat component of the food product was modified. The important role of ω-3 PUFAs in the correction of lipid metabolism disorders, their beneficial effect on the blood lipid profile has been confirmed and proven by numerous studies. It has been reported in the literature that ω-3 PUFAs provide a positive effect on improving the nutritional status of patients with tumors, increase the immune function of patients, and reduce the level of inflammatory cytokines [9, 10].

While developing modified fat composition of the ready-to-eat products, linseed oil was used as it is a source of ω-3 polyunsaturated fatty acids. Flaxseed oil contains about 44–61% alpha-linolenic acid (ω-3), 1–30% linoleic acid (ω-6), 13–29% oleic acid (ω-9). The share of saturated acids in linseed oil is 9–11%.

Broccoli was used as a source of plant antioxidants. Among other antioxidants, broccoli contains such substances as diindolylmethane, indole-3-carbinol and glucosinolates (glucoraphanin — a precursor of sulforaphane), which are effective for struggle against various types of cancer. Thus, preclinical studies have shown that the antiestrogenic activity of indole-3-carbinol (I3C) and diindolylmethane (DIM) can help reduce the risk of hormone-dependent cancers. It was found that I3C and DIM modulate the expression and activity of biotransformation enzymes that are involved in the metabolism and excretion of many biologically active compounds, including steroid hormones, drugs, carcinogens and toxins [11]. There is confirmed evidence that glucosinolates are powerful compounds that interact with the epigenome to restore the normal epigenetic environment within the malignant cells [12]. Sita et al. [13] reported on the antitumor action of sulforaphane (SFN) used as an auxiliary method together with pre-existing therapies. Several reviewed studies underline the potent activity of SFN to kill tumor cells. In vitro studies have shown the role of sulforaphane in the inhibition of bladder cancer cell lines, cell cycle arrest and induction of apoptosis [13]. As biologically active substances with a proven antioxidant status, we used a complex of vitamins — B9, B12, C, D3. The levels of content and application of the antioxidant complex are set according to the current requirements. According to the requirements, the level of the added vitamins should guarantee their content in the enriched product in amount sufficient to meet at least 15% of the average daily requirement when consuming 100 g of the product, taking into account the inevitable losses of vitamins during the technological process. The antioxidant effectiveness of the vitamin complex in the product has been confirmed by studies. The introduction of antioxidants in the form of a vitamin complex into experimental samples of ready-to-eat products, regardless of the method of their processing, contributed to maintaining the high activity of the enzymatic antioxidant system, which was expressed by a significant increase in superoxide dismutase (SOD) and catalase (CAT) and led to the neutralization of peroxidation products [15].

Certain processes for the manufacture of meat products, including heat treatment, are one of the factors affecting the formation of carcinogens, due to the impact on the fat and protein components. In this regard, it is necessary to recommend those methods of heat treatment that will minimize the risk of their formation.

The fatty acid composition of the fat component is a key element that determines the stability of lipid oxidation during heat treatment. The higher the unsaturation of fatty acids, the more oxidative degradation occurs during heat treatment [16]. In the literature there are numerous results of studying the effect of heat treatment on the change in the fatty acid composition of various types of animal and vegetable fats. PUFAs are the most susceptible to high temperatures, and increasing the amount of ω-3 PUFAs in foods can

increase the food susceptibility to lipid oxidation. During the heat treatment of oils rich in polyunsaturated fatty acids, their oxidation leads to the formation of various radicals, which subsequently can form malondialdehyde [17].

The oxidation of fats is accompanied by a deterioration in their organoleptic properties and the formation of various oxidation products — peroxides at first, and then toxic polymeric compounds. It should be noted that the type and temperature regime of heat treatment plays a key role in managing the direction of oxidative processes.

It is also known that during the heat treatment of high-protein products of animal origin, the potentially carcinogenic compounds are formed, among which heterocyclic aromatic amines (HAA) are that very chemical compounds that have at least one aromatic ring and one amino group in their structure [18]. A row of scientific works prove the relationship between the consumption of products containing HAA and the manifestation of oncological diseases [19]. Therefore, having sufficient information about heterocyclic aromatic amines will help reduce the health risks associated with the above-specified compounds [20].

The process of HAA formation depends on various factors, including temperature, cooking time, fat content, and the presence of precursors. Additional factors that can affect HAA formation are pH, type of meat and ingredients added during cooking such as antioxidants, amino acids, fat, and sugars. Various ways to minimize HAA formation during heat treatment include reformulation of ingredients, modification of heat treatment modes, and the addition of natural and artificial antioxidants [21].

The authors of the article [22] provide data on influence of fat composition on HAA formation. Replacing animal fat with vegetable oils led to HAA level decrease in pork cutlets. At different cooking temperatures, all cutlets with modified fat featured significantly lower amount of MeIQ, 4,8-DiMeIQx.

Taking the above into consideration, it can be assumed that gentle cooking methods and the addition of a complex of antioxidants will minimize the risks of carcinogens during heat treatment.

The purpose of this research is to study the change in the fatty acid composition, fat oxidation parameters, the level of heterocyclic amines formation in ready-to-eat poultry meat products enriched with ω-3 PUFA and with a complex of vitamins during heat treatment, designated for the diets of cancer patients.

Objects and methods

The samples of chopped food products composed of turkey meat, broccoli, rice flour, onion, garlic and enriched with ω-3 fatty acids (linseed oil 2%) with the addition of an antioxidant vitamin complex (vitamins B9, B12, C, D3 intended for functional nutrition of cancer patients) were used as the experimental material. The control sample was composed without the addition of linseed oil and antioxidant vitamin complex. To level the fat content a mixture of vegetable oils (sunflower and rapeseed) was added to the control sample.

The calculated values of the nutritional value of the samples were as follows: protein content 20.3 ± 1.6%; fat content — 7.2 ± 1.5%.

Heat treatment

The prepared semi-finished products were brought to ready-to-eat condition, and raw samples were also left as standards. For the experiment, gentle methods of cooking were chosen — in a microwave oven, by steaming and short-term roasting in a pan, followed by its bringing to complete readiness in a convection oven. In all preparation methods the readiness of the cutlet was determined by its final temperature — 85 °C (microbiological safety criteria) — inside the cutlet.

Microwave cooking

The samples were placed in an NN-C785JF microwave oven (Panasonic, Japan) and cooked at 1,000 W for 8 minutes in two heating cycles of 4 minutes each on each side until a core temperature of 85 °C was reached.

Cooking in a convection oven with pre-roasting

The samples were roasted in a hot frying pan with a Teflon coating on an electric stove, two-burner stove, EPK-27N (LLC "Elinoks", Russia) for 2 minutes on each side. Then they were placed in a steam convection oven Unox XVC304 series Chef Top LI1615AO (UNOX S.r.J, Padova, Italy) and brought to readiness (until 85 °C inside the cutlet) in the convection mode at a temperature of 180 °C. Total cooking time took 27 minutes.

Steam cooking

The samples were placed in an Unox XVC304 Chef Top LI1615AO steam convection oven (UNOX S.r.J, Padova, Italy) and brought to readiness (until 85 °C inside the cutlet) in the "steam" mode — 100%, at a temperature of 110 °C. Total cooking time took 29 minutes.

The final temperature of the samples cooking in the convection oven, both in the "steaming" mode and in the "convection" mode, was measured by inserting a temperature probe into the geometric center of each cutlet. The probe was built-in and connected to the temperature recorder of the steam convection oven. During heat treatment in a microwave oven, a portable RST07951 pro thermometer (with measurement range from –50 to +300 °C) (RST, Sweden) was used to measure the internal temperature.

After cooking, the samples were cooled down to room temperature and weighed to determine the yield of finished products.

Heat treatment losses (L_h) were calculated using the following formula:

$$L_h = (W_r - W_c) \times 100\% / W_r, \% \quad (1)$$

where

W_r — is the weight of the raw meat cutlets;

W_c — is the weight of the cooked meat cutlets.

Further, the samples were prepared for each analysis in accordance with the research procedure and method.

All studies were run at the Laboratory of "Scientific and Methodological Works, Biological and Analytical Research" of the Testing Center of the V. M. Gorbatov Federal Research Center for Food Systems (accreditation certificate No. RA.RU21PP69).

The experimental measurement equipment used in research was calibrated with an assessment of its accuracy and uncertainty in accordance with the requirements of ISO/IEC17025:2017[1].

All chemical reagents (salts and solvents of pure analytical grade) were confirmed by certificates of conformity and quality certificates; supplier is JSC "LenReaktiv" (St. Petersburg, Russia).

All aqueous solutions were prepared with deionized water from a MilliQDirect 8 system (France).

Study of indicators of oxidative stability of lipids

The acid value of the samples was determined by titration of free fatty acids with a solution of potassium hydroxide according to GOST R55480–2013[2].

The peroxide value of the samples was determined by the method according to GOST 34118–2017[3]. The method is based on the reaction of the interaction of the primary products of fat oxidation (peroxides and hydroperoxides) with potassium iodide in an acidic medium, followed by titration with sodium thiosulfate solution and the quantitative determination of released iodine.

Study of fatty acid composition

The composition of fatty acids was determined by gas chromatography according to GOST R55483–2013[4] on the Agilent 7890A automatic gas chromatograph (Agilent Tech., USA) with a flame ionization detector. To determine fatty acids, a Supelco SP 2560 100 m × 0.25 mm × 0.2 mkm chromatographic column (Supelco, USA) was used.

Determination of heterocyclic aromatic amines (HAA)

Instrumental determination and identification of HAA was run by high performance liquid chromatography [23]. The sample analysis was performed on an Agilent 1200 high performance liquid chromatography system (USA) with an Agilent 6410B triple quadrupole mass spectrometer. To determine HAA, a C18 chromatographic column, 4.6 × 50 mm, 1.8 μm (Agilent, USA) was used. The following were used as standard samples:

— 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%.

When choosing the conditions for chromatographic identification, acetonitrile for HPLC produced by Panreac (France) and formic acid Merck (USA) were used.

Statistical processing

All experiments were carried out with three parallel measurements. Quantitative data are presented as the arithmetic mean of three measurements ± standard deviation. Statistical analysis of the experimental data was run by the Kruskal-Wallis test, followed by pairwise comparison by the Mann-Whitney test using the STATISTICA 10.0 software. The significance level for all statistical tests was 5% ($p < 0.05$).

Results and discussion

Losses during ready-to-eat processing of the samples were: 22.0 ± 1.1% when cooked in a microwave oven; 29.0 ± 1.0% when cooking in a convection oven in the "convection" mode with preliminary short roasting and 8.0 ± 1.5% when cooking in a convection oven in the "steam" mode.

Since the fat component of the studied product contains a combination of animal fat and vegetable oils, we analyzed the fatty acid profiles of semi-finished and finished ready-to-eat products cooked in various ways (Table 1).

The fatty acid profile was influenced by both the composition of the product and the type of heat treatment. As can be seen from the above data, the addition of linseed oil instead of a mixture of sunflower and rapeseed significantly changed the ratio of ω-6/ω-3 fatty acids in the experimental semi-finished product by increasing the level of fatty acids of the ω-3 family. The ratio was 1:1.1, which indicates a high nutritional value of the experimental samples and allows considering this meat product as a source of ω-3 fatty acids.

During the heat treatment of samples with various methods until ready, the changes in the distribution of fatty acids was recorded. In quantitative terms, the main saturated fatty acids found in the fat of raw and cooked samples were palmitic acid (C16:0) and stearic acid (C18:0). Heat treatment led to an increase of saturated fatty acids level, both in control and experimental samples. Changes in the quantitative content of ΣSFA were largely caused by an increase in the content of palmitic acid (C16:0). In the steamed test sample, the content of stearic acid did not

[1] ISO/IEC17025:2017 "General requirements for the competence of testing and calibration laboratories". Technical Committee: ISO/CASCO Committee on conformity assessment, 2018. Retrieved from https://www.iso.org/standard/66912.html Accessed December 15, 2022.

[2] GOST R55480–2013 "Meat and meat products. Method for determination of acid value". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200103311 Accessed December 15, 2022. (In Russian)

[3] GOST 34118–2017 "Meat and meat products. Method for determination of peroxide value". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200146654 Accessed December 15, 2022. (In Russian)

[4] GOST R55483–2013 "Meat and meat products. Determination of fatty acids composition by gas chromatography". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200103852 Accessed December 15, 2022. (In Russian)

Table 1. Fatty acid profiles of raw and cooked poultry cutlets and broccoli

Parameter		Mass fraction, % of the total fatty acids			
		Raw	Microwave	Roasting + "convection"	"Steam cooking" mode
SFA					
Palmitic $C_{16:0}$	contr	10.14 ± 0.16 [a]	11.40 ± 0.19 [b]	15.94 ± 0.17 [c]	12.15 ± 0.18 [d]
	exper	6.27 ± 0.18 [a]	7.96 ± 0.16 [b]	9.70 ± 0.19 [c]	7.04 ± 0.15 [d]
Stearic $C_{18:0}$	contr	3.51 ± 0.14 [a]	3.73 ± 0.07 [b]	5.11 ± 0.14 [c]	3.59 ± 0.04 [a]
	exper	3.08 ± 0.14 [a]	3.39 ± 0.06 [b]	3.29 ± 0.04 [b]	3.01 ± 0.16 [a]
ΣSFA	contr	13.65 ± 0.14 [a]	15.13 ± 0.16 [b]	21.05 ± 0.14 [c]	15.74 ± 0.15 [d]
	exper	9.35 ± 0.13 [a]	11.35 ± 0.12 [b]	12.99 ± 0.13 [c]	10.05 ± 0.14 [d]
MUFA					
Palmitooleic $C_{16:1}$	contr	1.56 ± 0.07 [a]	1.75 ± 0.09 [b]	1.27 ± 0.02 [c]	ND
	exper	ND	ND	ND	ND
Oleic $C_{18:1}$	contr	46.21 ± 0.07 [a]	44.98 ± 0.25 [b]	40.62 ± 0.19 [c]	46.08 ± 0.03 [d]
	exper	40.09 ± 0.26 [a]	36.92 ± 0.18 [b]	35.48 ± 0.23 [c]	38.76 ± 0.27 [d]
Gondoinic $C_{20:1}\omega_9$	contr	0.69 ± 0.09 [a]	0.70 ± 0.07 [a]	0.49 ± 0.08 [b]	1.35 ± 0.19 [c]
	exper	0.62 ± 0.08 [a]	1.25 ± 0.04 [b]	1.09 ± 0.04 [c]	1.17 ± 0.03 [d]
ΣMUFA	contr	48.46 ± 0.09 [a]	47.43 ± 0.21 [b]	42.38 ± 0.16 [c]	47.43 ± 0.15 [b]
	exper	40.71 ± 0.25 [a]	38.17 ± 0.15 [b]	36.57 ± 0.21 [c]	39.93 ± 0.24 [d]
PUFA					
ΣPUFA ω-6	contr	31.84 ± 0.18 [a]	31.29 ± 0.29 [b]	32.04 ± 0.21 [a]	32.07 ± 0.18 [a]
	exper	26.12 ± 0.16 [a]	26.55 ± 0.12 [b]	32.22 ± 0.34 [c]	26.19 ± 0.17 [a]
ΣPUFA ω-3	contr	6.05 ± 0.17 [a]	6.15 ± 0.16 [a]	4.53 ± 0.11 [b]	4.76 ± 0.08 [c]
	exper	23.82 ± 0.21 [a]	23.93 ± 0.17 [a]	18.22 ± 0.17 [b]	23.83 ± 0.06 [a]
ΣPUFA	contr	37.89 ± 0.14 [a]	37.44 ± 0.18 [b]	36.57 ± 0.12 [c]	36.83 ± 0.13 [d]
	exper	49.94 ± 0.19 [a]	50.48 ± 0.18 [a]	50.44 ± 0.27 [a]	50.02 ± 0.27 [a]
ΣPUFA / ΣSFA	contr	2.78 ± 0.03 [a]	2.47 ± 0.05 [b]	1.74 ± 0.03 [c]	2.34 ± 0.04 [d]
	exper	5.34 ± 0.02 [a]	4.45 ± 0.02 [b]	3.88 ± 0.04 [c]	4.98 ± 0.03 [d]
ω-6 / ω-3	contr	5.26 ± 0.03 [a]	5.09 ± 0.02 [b]	7.07 ± 0.03 [c]	6.74 ± 0.02 [d]
	exper	1.10 ± 0.02 [a]	1.11 ± 0.03 [a]	1.77 ± 0.03 [b]	1.10 ± 0.04 [a]

Note: Different letters (a, b, c, d) denote a statistically significant difference between samples cooked by different methods at $p < 0.05$.

change significantly. ΣSFA of microwave-cooked sample increased by 10.8%; in the sample cooked by steam convection oven in the "convection" mode with preliminary roasting — by 54.2%; in the sample cooked a steam convection oven in the "steam" mode — by 15.3%. In the test samples, the increase in the percentage of ΣSFA in the test samples was as follows: in the microwave — 21.4%; in the "convection" mode with preliminary roasting — 38.9%; in the "steaming" mode — 7.5%.

The monounsaturated fatty acid profiles of the control and experimental samples were different and were represented mainly by oleic (C18:1) and gondoic (C20:1) acids before and after cooking.

Heat treatment led to a decrease in monounsaturated fatty acids amount in control and experimental samples for all methods of cooking. The decrease of ΣMUFA percentage in control samples after heat treatment was: microwave cooking — 2.1%; "convection" cooking mode with preliminary roasting — 12.6%; "steaming" cooking mode — 2.1%, in experimental samples — 6.2%, 10.2% and 1.9%, respectively. At the same time, the content of ΣMUFA decreased mainly because of decrease of oleic acid mass fraction, both in control and experimental samples in all methods of cooking.

In our experiment, the heat treatment led to a slight decrease in the amount of polyunsaturated fatty acids in control samples. ΣPUFA in microwave-cooked controls decreased by 1.2%; in the "convection" mode with preliminary roasting — by 3.5%; in the "steam" mode — by 2.8%. In the experimental samples, ΣPUFA did not change significantly in all methods of cooking.

Polyunsaturated fatty acids of the ω-3 group reacted differently to heat treatment in control and experimental samples. Cooking on the "convection" mode with preroasting led to a decrease in the concentration of ΣPUFA ω-3 in the control samples by 25% in the test samples — by 23.5%. When cooking in a steam convection oven in the "steaming" mode, the percentage of ΣPUFA ω-3 in the control sample decreased by 21.3%, while in the experimental sample it did not change significantly. The mass fraction of ΣPUFA of the ω-3 group did not change significantly when cooking the cutlet in a microwave oven, both in the control and in the experimental sample. The use of the "convection" mode with preliminary roasting significantly changed the ratio of ω-6 and ω-3 acids because of a significant decrease in their content in reference to the raw sample and samples cooked by other methods.

The addition of an antioxidant complex allowed ensuring a high level of added ω-3 fatty acids in the experimental sample after heat treatment. At the same time, the greatest safety was ensured with the use of microwave heating and steam cooking. Also, the presence of broccoli in the composition of the product could beneficially affect the high preservation of ω-3 fatty acids, both in the experimental and control samples. Cheng L. J. et al. [24] reported on the ability of various vegetables and, to a greater extent, broccoli to protect fats from oxidation and retained 99% of ω-3 fatty acids (eicosapentaenoic and docosahexaenoic acids) from their initial amount after 4 weeks of their storage at 40 °C in dry oil emulsions by adding the vegetable puree to tuna oil. Gheysen et al. [25] also found that broccoli in the emulsified state was found to be highly effective in protecting of ω-3 fatty acids against the oxidation in microalgae biomass.

Thus, the addition of linseed oil to the semi-finished turkey made it possible to achieve high values of the amount of PUFA by increasing the content of ω-3 fatty acids, improve the ratio of PUFA/SFA, as well as the ratio of ω-6/ω-3 fatty acids, which is one of the most important indicators of the nutritional value of lipids. In the control sample, the ω-6/ω-3 values ranged from 5.09 to 7.07 depending on the method of cooking. The highest value on those acids was observed for the sample cooked in the "convection" mode with preliminary roasting. The values of the ratio ω-6/ω-3 in the test samples were significantly lower than in the control ones, which is associated with the introduction of additional ω-3 fatty acids into the test samples and ranged from 1.1 to 1.77. Cooking in a microwave oven and in a steam convection oven in the "steaming" mode did not significantly affect the ratio ω-6/ω-3 in the test samples, and cooking in the "convection" mode with preliminary roasting led to an increase of this value.

Literature data on studies of the effect of heat treatment on the fatty acid profile of various types of meat and meat products, including poultry meat, are very contradictory.

Similar to our results in the studies of Wereńska M. et al. [26] showed an increase in ∑SFA of fatty acid profile of goose meat exposed to various heat treatment methods, while all considered types of heat treatment caused a significant decrease in the proportion of monounsaturated fatty acids (C16:1), however, in their work they did not consider the method of microwave cooking.

At the same time, studies by Nudda et al. [27] and Maranesi et al. [28] showed that none of the cooking methods affected the concentration of ∑MUFA in lamb samples.

Most studies declare that PUFAs are the most prone to oxidative degradation. Heat treatment reduces their concentration in the finished product; however, on the contrary, there are many studies that do not agree with these results. For example, according to Kouba et al. [29] and Gerber et al. [30] PUFAs play a role of structural lipids in muscle and are less susceptible to changes during cooking, and their proportional increase is expected in meat after heating, as observed in our study.

At the same time, Jiang et al. [31] and Campo et al. [32] also believe that unsaturated fatty acids, especially PUFAs, are less affected by cooking than SFAs; and explain this by the fact that PUFAs are more integrated into the membrane structure, while SFAs are more concentrated in the triglyceride fraction. The proportional change in fatty acid composition can be explained by the loss of fat during cooking, which affects mainly adipose tissue triglycerides with a relatively higher amount of SFA rather than unsaturated fatty acids.

In the studies of A. Nudda et al. the increase in PUFA content in cooked meat was recorded, partly due to an increase in linolenic acid (C18:3ω3) (+25.4%) and mainly due to an increase in the concentration of very long chain PUFA, especially EPA and DHA (+51.0%) [27].

In a study by Campo et al, no significant differences were found in either individual ω-3 fatty acids or ∑PUFA ω-3 in various lamb cooking methods like stewing, grilling, and roasting [32].

The heating processes of pork and lamb meat in the study by Janiszewski et al. [33], did not always lead to statistically significant changes in FA profile, and in many cases the values of the most commonly used indicators of the beneficial properties of fat remained unchanged, for example, the ratio ω-6/ω-3 remained below 4÷5, which corresponds to recommendations of EFSA (2012) and FAO/WHO (2008) [34,35].

Jiang et al. [31] found that grilling did not significantly affect the total content of fatty acid in beef steaks and the levels of most fatty acids, but the level of linolenic acid (C18:3ω3) increased.

Opposite results were obtained by Maranesi et al. In their study, lamb meat microwaved and roasted on a preheated electric grill showed decrease of ∑PUFA ω-3 and ∑PUFA ω-6 [28].

Janiszewski et al [33] found that the grilling process had almost no significant effect on the FA profile in lamb. In both raw and roasted lamb leg a similar ratio of individual acids was found, as well as equal proportional shares of SFA, MUFA and PUFA. At the same time, heat treatment of pork led to significant changes in its fatty acid composition and caused a significant increase in SFA and a decrease in individual PUFAs, especially PUFAs of the ω-3 group — eicosapentaenoic, docosapentaenoic and docosahexaenoic acids.

Campo et al. [32] found no significant difference in fatty acid profile between the raw and grilled lamb. The explanation for these results was based on the short grilling time and the fact that its final temperature was not very high (75 °C).

According to [36], heating reduced the PUFA/SFA ratio in beef, but did not change the ω-6/ω-3 ratio.

Emektar et al [37] showed in their studies that heat treatment of vegetable fats resulted in significant degradation of unsaturated fatty acids, mainly PUFAs. Oleic acid was practically stable in sunflower and soybean oils at all

temperatures, while it decomposed significantly in olive oil under the same conditions. High temperatures and PUFA content increase the rate of oxidation and produce more degradation products, like 2-dienals.

Mitrea et al [38] and colleagues reported that heating of liquid vegetable oils (sunflower, rapeseed and corn) up to 180 °C led to significant changes in the percentage of saturated fatty acids, as well as increased the acid and peroxide numbers, while the percentage of mono- and polyunsaturated fatty acids decreased.

Lipid oxidation

The resistance to fatty component oxidation in the samples, containing various types of vegetable oils was evaluated by changing the acid and peroxide numbers. The risk of the formation of peroxidation products increases along with an increase of unsaturated fatty acids content, while the presence of antioxidants can reduce it [39,40]. The chemical composition of products provided a significant influence on the rate of thermal oxidation of fat, which is explained, in particular, by significant amount of antioxidants in some of them. Thus, the proteins included in the products are capable to provide antioxidant effect; some substances, formed as a result of melanoidin formation, have an oxidation-reducing effect and can interrupt the chain of oxidative transformations.

While comparing the influence of different methods of cooking the enriched semi-finished products on the formation of oxidation products, it was found that no matter what methods of heat treatment in the finished product was used, the level of lipid oxidation products significantly increased in comparison with the parameters of the raw product. Heat treatment in the "convection" mode with pre-roasting showed the lowest oxidative stability of lipids, as evidenced by the higher values of lipid oxidation, both in the control and in the experimental sample compared to the other cooking methods, which was confirmed by the loss of polyunsaturated ω-3 fatty acids. At the same time, cooking in a steam convection oven in the "steaming" mode did not provide a significant effect on the peroxide value and acid value, both in control and experimental samples, and the parameters of samples cooked in a microwave oven took an intermediate position.

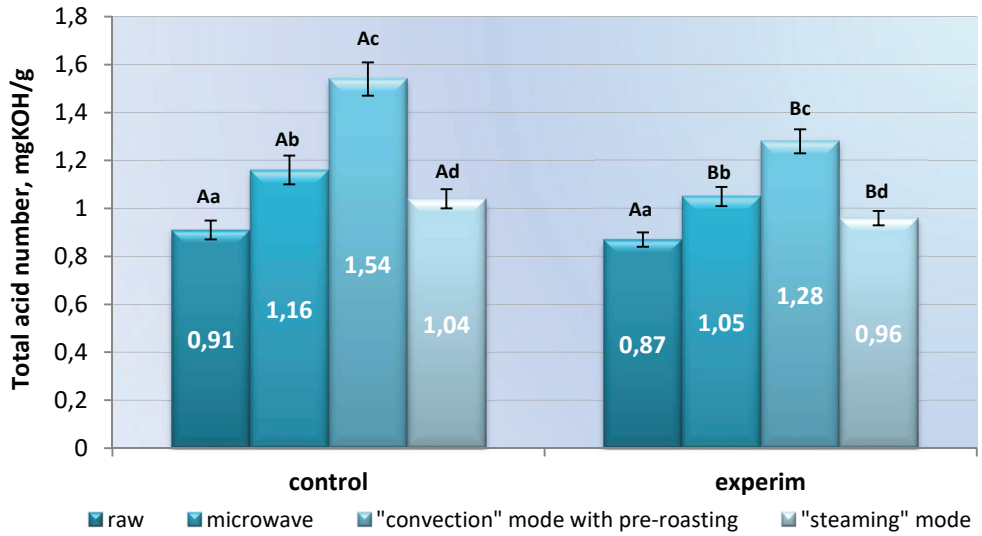
The influence of processing methods on the content of free fatty acids, expressed as a change in acid value, is shown below in the Figure 1.

The parameters of acid value in the cooked test samples in comparison with the raw sample increased: after microwave cooking by 20.7%; when cooking in the "convection" mode with preliminary roasting — by 47.1%; when cooking in a steam convection oven in the "steaming" mode — by 10.3%, while in control samples it increased by 27.5%, 69.2% and 14.3%, respectively.

The different dynamics of changes in acid values in the experimental samples and control samples may be associated with a different amount of included unsaturated fatty acids, which are more susceptible to oxidation, as well as a different content of antioxidant substances.

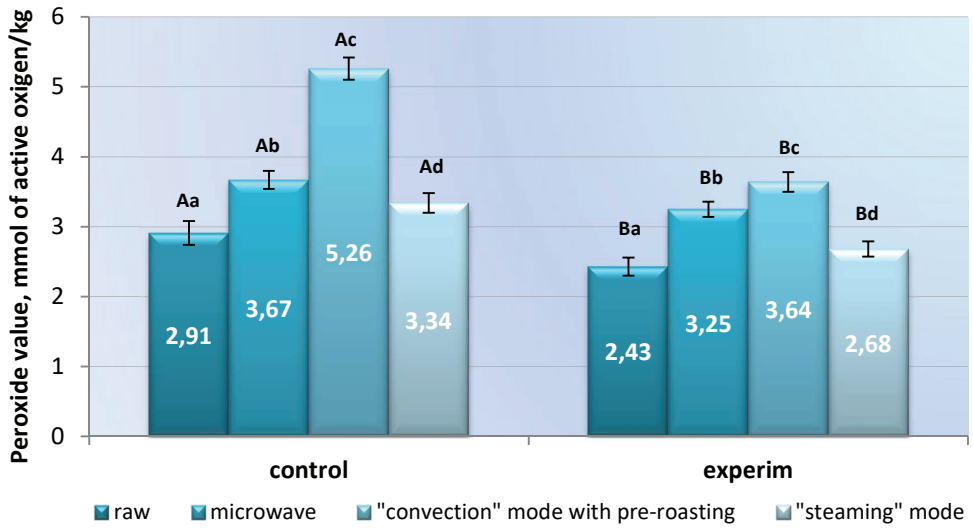
Free fatty acids are considered to be a good indicator of fat lipolysis, either due to the lipase and/or microbial lipase enzymes contained, or due to the effect of oxidation during heat treatment [41,42]. Sobral et al [43] reported that oven and microwave cooking equally increased lipid oxidation in chicken burgers, as evidenced by the loss of free amino acids and ω-6 PUFAs prone to oxidation. At the same time, the addition of oregano to burgers as a natural antioxidant significantly reduced lipid oxidation and maintained the content of ω-6 PUFA [43]. Scientists [44] showed that the cooking method (boiling in water, roasting with fat and without fat) provided a significant influence on the composition of fatty acids in the ready-to-eat products from mallard duck meat. The most favorable ratio ω-6/ω-3 was found in oil-fried products. The least changes in lipid oxidation was recorded in the process of roasting the skin-off products.

The peroxide values of the samples are shown below in the Figure 2.



Note: Different lowercase letters (a, b, c, d) denote a statistically significant difference between the samples cooked in different ways at $p < 0.05$; different capital letters (A, B) denote a statistically significant difference between control samples and experimental samples at $p < 0.05$.

Figure 1. The influence of various heat treatment methods on the acid value of ready-to-eat products



Note: Different lowercase letters (a, b, c, d) mean a statistically significant difference between samples prepared in different ways at $p < 0.05$; different capital letters (A, B) mean a statistically significant difference between control and experimental samples at $p < 0.05$.

Figure 2. The influence of various methods of heat treatment on the peroxide number of the ready-to-eat products

The peroxide values, similar to the acid value in the experimental finished samples, in comparison with the raw ones, increased: when cooked in a microwave oven — by 33.7%; when cooking in the "convection" mode with preliminary roasting — by 49.8%; when cooking in a steam convection oven in the "steaming" mode — by 10.3%, while in control samples it increased by 26.1%, 80% and 14.8%, respectively.

Heterocyclic amines

As the potentially hazardous compounds can accumulate during the technological processing of products, in particular xenobiotics of endogenous origin, i. e. heterocyclic aromatic amines, it is important to study the conditions for their formation during the heat treatment of ready-to-eat products, as well as the choice of a cooking method that minimizes HAA formation.

Table 2. Effect of cooking methods on mutagenic HAA content

Cooking methods		HAA, ng/g of the ready product		ΣHAA, ng/g of the ready product
		MeIQx	PhIP	
Microwave	contr.	2.15 ± 0.86^{a}	5.67 ± 2.27^{a}	7.82 ± 3.13^{a}
	exper	LoQ	$0.72 \pm 0.29^{*a}$	$0.72 \pm 0.29^{*a}$
"Convection" mode with pre-roasting	contr.	3.12 ± 1.25^{a}	8.13 ± 3.25^{a}	11.25 ± 4.5^{a}
	exper	1.73 ± 0.69^{a}	4.32 ± 1.73^{b}	6.05 ± 2.42^{b}
"Steaming" mode	contr.	2.21 ± 0.88^{a}	5.53 ± 2.21^{a}	7.74 ± 3.0^{a}
	exper	$0.2 \pm 0.08^{*b}$	$0.65 \pm 0.26^{*a}$	$0.85 \pm 0.34^{*a}$

Notes: * — statistically significant difference ($p < 0.05$) of the test samples relative to the control; a, b — different letters denote a statistically significant difference ($p < 0.05$) between samples cooked by different methods.

The scientific literature reports that MeIQx occurs more frequently than other HAAs in cooked meat products [45,46]. In our work, MeIQx was found in all the studied samples, except for the experimental sample cooked in a microwave oven. At the same time, the concentration of MeIQx in the test samples was significantly lower than in the control samples for all studied methods of heat treatment: in the "convection" mode with preliminary roasting — by 44.5%; in the steam convection oven in the "steaming" mode — by 91%. Convection cooking with pre-roasting resulted to the highest concentration of MeIQx in the samples compared to other cooking methods.

PhIP was found in all studied samples. Its maximum concentration was observed in the samples prepared in the "convection" mode with preliminary roasting. At the same time, in the experimental samples with the added antioxidant complex, the content of PhIP was significantly lower than in the control ones. The inhibitory effect of the antioxidant complex on PhIP amounted to 87%, 47%, and 88% in poultry cutlets with broccoli cooked in a microwave oven in the "convection" mode with pre-roasting and in a steam convection oven in the "steaming" mode, respectively.

Based on the obtained results, it can be concluded that the antioxidant vitamin complex added into the test samples provided an inhibitory effect on HAA formation, which is confirmed by some foreign studies, according to which the introduction of various antioxidants into the product reduces the level of dangerous HAA formation.

Comparison of various methods of cooking the semi-finished products until their readiness showed that cooking in microwave and by steaming forms less HAA compared to the convection method with pre-roasting.

Our results are consistent with data from Jamali et al., who state that the amount of HAA is directly proportional to losses during the meat cooking [47].

Keşkekoğlu et al [48] in their study of cooked beef and chicken meatballs reported that HAA formation depended on both the type of meat and method of the cooking. At the same time, the addition of a natural antioxidant (e. g. pomegranate seed extract) to minced meat significantly reduced the concentration of HAA in the finished product [48].

The amount of total HAA was significantly reduced in beef cutlets cooked at 200 °C for 3 minutes after the addition of water-soluble vitamins [49]. The decrease of total HAA content in marinated pork cooked for 1 hour was achieved by adding α-tocopherol [50].

The study [51] came to the conclusion that addition of L-ascorbic acid and α-tocopherol reduced mutagenicity in pork sausages [51].

The addition of oregano, as a natural antioxidant, significantly reduced the concentration of HAA at various cooking methods and at temperatures of 175 °C, 195 °C, and 225 °C [52].

Suleman R. et al noted that grilling lamb cutlets led to the higher total HAA than cooking them with both infrared and overheated steam [53,54].

Conclusion

Based on the requirements of the diet composed for the cancer patients, a recipe for ready-to-eat products from turkey has been developed for its inclusion into the complex therapy of patients. The addition of ω-3 fatty acids into the product composition made it possible to change the fatty acid composition towards the increasing of PUFA proportion. In order to enrich the diet of patients and to reduce oxidative processes during heat treatment and storage of the ready products, a complex of vitamins featuring an antioxidant effect was added to the recipe. The study of the influence of various methods of cooking the products until ready-to-eat condition on the composition of fatty acids and their oxidation showed that, depending on the composition of the product and the method of its heat treatment, the ratio of fatty acids and the intensity of oxidative processes varied. The addition of an antioxidant vitamin complex increased the survivability of added ω-3 fatty acids during the heat treatment of the product in a microwave and by steam cooking. Also, gentle heat treatment methods and application of antioxidants cause the formation of HAA in the product to a lesser extent, but it was not possible to completely block their formation. Taking into consideration the obtained results, for the cooking of a meat-based oncoprophylactic product, it is recommended to cook meat with a minimum heat load, i. e. on steam or with a minimum cooking time in a microwave oven. More research is needed for further investigation of the inhibition of HAA formation in ready-to-eat food products due to addition of the antioxidants.

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