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SUBSTANCES ANALYSIS

Determination of Chemical and Microbiological Characteristics of Meat Products Treated by Radiation

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Abstract—Radiation treatment of food products carried out to increase their shelf life can result in chemical transformations initiated by free radicals. Volatile compounds (alcohols, aldehydes, ketones, etc.) formed, in particular, as a result of lipid oxidation, impair the organoleptic properties of products. Method of gas chromatography-mass spectrometry (GC-MS) makes it possible to identify the fact of food processing by detection of volatile marker compounds: in the case of meat products, the existing standard brings under regulation detection of 2-alkylcyclobutanones, however, the products with a reduced fat content, such as turkey and chicken, require an alternative marker. The results of GC-MS study revealed the dependence of microbiological parameters and the content of various volatile organic substances in chilled turkey meat on the dose of electron radiation. It is shown that the total amount of alcohols, ketones and aldehydes (11 compounds) decreases exponentially with an increase in the absorbed dose. An increase in the radiation dose leads to a higher content of carbonyl compounds (aldehydes and acetone), which results in a specific taste and smell of the irradiated products. At the same time, the acetone concentration increases linearly with the absorbed dose, which makes it possible to use acetone as a potential marker of the degree of irradiation of low-fat meat products. Irradiation in the "working" doses (0.5–1 kGy) significantly suppresses the pathogenic microflora and keeps the organoleptic properties of the product.

Keywords: radiation treatment of food products, meat products, electron accelerator, absorbed dose, dosage rate, radiological monitoring, chemical and microbiological analysis of food, headspace analysis, gas chromatography-mass spectrometry

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INTRODUCTION

According to the Food and Agriculture Organization of the United Nations (FAO), 30% of food and agricultural products is lost or wasted annually [1], which causes the need to increase the shelf life of foodstuffs. The current demand to prolong the lifespan of foods calls for irradiation processing to enhance food safety [2–4]. The research into food irradiation has become highly relevant in Russia due to the introduction of Russian state standards (GOST) and other regulations stipulating the procedures for the irradiation processing, ensuring the control over industrial irradiation of foods, and creating a legal framework for new industrial irradiation centers [5].

Each type of food requires the use of a specific range of irradiation doses to inhibit the growth of pathogenic bacteria without causing a significant change to the chemical and organoleptic properties of the foods. Dry foods (spices, grains, etc.) are irradiated with the doses ranging from 1 to 10 kGy [6, 7], while meat and fish should be processed with the doses ranging from 0.5 to 3 kGy [8–10].

Food irradiation should be performed not only to ensure microbiological safety of food product but also to limit the chemical transformations caused by radicals, which may have a negative effect on the energy value, as well as biochemical and organoleptic properties of the product as a whole [11–23]. For dry products, such as tea, spices, powders, etc., it is necessary to apply the EPR method to control the chemical changes caused by free radicals [12–15]. However, when it comes to foods high in moisture, detecting the fact of irradiation using the EPR method is difficult due to diffusion and further disappearance of free radicals [16].

It is known that the radicals that launch the reaction lipid oxidation, modification of amino acids, car-

bohydrates, and DNA, as well as formation of secondary free radicals, essentially changes the acid-base balance of products containing lipid and protein fractions [17]. In the presence of a large amount of fat in the products, the accumulation of hydroperoxides lipids that decompose with the formation of free new radicals occur [18].

A number of modern studies are devoted to the quantitative analysis of fatty acid and registration of volatile chemical compounds formed in the product [19–24]. As a result of lipid oxidation, volatile compounds of various classes: alcohols, aldehydes, ketones, carbohydrates, and organic acids are formed. Many of them are responsible for the formation in the product of foreign rancid aftertastes and specific odors.

At present, it is important to establish the methods capable of detecting the use of irradiation to increase the shelf life of food products. For example, the role of aldehydes in products as indicators of the fact of irradiation is discussed in [25]. It is also known that the Kreis test [26], which includes a colorimetric analysis of the intensity of the red color formed as a result of the reaction of phloroglucinol with epihydrin aldehyde and other lipid oxidation products, as well as the test using 2-thiobarbituric acid (2-TBA) (TBARS method) can be used to detect the lipid oxidation. However, the disadvantage of these methods is their low sensitivity [27].

Currently, one of the promising methods for assessing changes in the content of chemical compounds is the method of gas chromatography in combination with mass spectrometry (GC-MS), which makes it possible to identify volatile substances that occur in irradiated products containing moisture, fat and protein fractions. It is based on gas chromatographic separation of volatile compounds followed by mass spectrometric detection. The GC-MS method has high sensitivity and selectivity, and also allows high-efficiency separation and determination of compounds with similar molecular weights. The comprehensive libraries of mass spectra (in the case of electron ionization) make it possible to identify unknown components by comparing the experimentally obtained mass spectra with library data [28]. In the case of determining non-volatile and temperature-unstable compounds (pigments, sugars, peptides, etc.), highly efficient liquid chromatography (HPLC-MS) should be used.

The use of these chemical analysis methods triggers an increased interest in universal markers which would make it possible to identify the fact of food irradiation treatment [29–34]. The current Russian standard GOST 34131-2017 establishes GC-MS method for the detection of 2-alkylcyclobutanones formed as a result of exposure of meat products to ionizing radiation. However, the detection of this chemical compound in low-fat meat products, such as turkey and chicken, is difficult and requires an alternative marker.

It is also important to establish a correspondence between the dose at which significant changes in the taste and smell of products occur, the dose at which the amount of various volatile compounds after ionizing radiation differs significantly from the indicators of the control non-irradiated samples, as well as the dose at which the total bacterial contamination of food is reduced from 10 to 100 times compared to the non-irradiated product.

The purpose of this work is to study the dependency of microbiological parameters and the content of various volatile organic compounds in chilled turkey meat on the dose of ionizing radiation.

RESEARCH METHODS

Electron Beam Irradiation

The object of the study are chilled turkey carcasses stored in a refrigerator at a temperature of 2°C for no more than two days from slaughter. To assess the microbiological and chemical parameters, turkey samples weighing (0.5 ± 0.1) g were placed into 2-mL plastic microcentrifuge tubes. Meat samples were distributed uniformly to ensure an even thickness of layer not exceeding 3 mm.

The samples were irradiated using continuous electron accelerator UELR-1-25-T-001 with a maximum energy of 1 MeV with maximum average beam power of 25 kW at an average beam current of 600 nA at an ambient temperature of 20°C in accordance with the irradiation scheme described in [35].

The control of the dose absorbed by the samples was carried out using the ferro sulfate dosimetry solution (Fricke dosimeter), based on the change in the valence of iron during irradiation, which leads to a change in the optical density of the dosimetry solution. The dose rate of electron beam irradiation during the experiment was $P_{elect} = (10 \pm 1)$ Gy/s.

Microbiological Analysis

The study of microbiological parameters of chilled turkey samples after electron beam irradiation was carried out in accordance with the procedure described in [35]. A turkey homogenate made from irradiated and control samples was diluted in ratios 1 : 2–1 : 10000 with saline solution to obtain isolated cell colonies in order to calculate their concentration in CFU/g. All measurements and seeding were carried out under sterile conditions at the temperature of 23°C.

Chemical Analysis

Changes in chemicals in irradiated samples were studied using gas chromatography coupled with mass spectrometry. The test samples weighing 2 g were placed in vials for headspace analysis, 2 mL of a solution of sodium chloride (3 wt %) in distilled water was

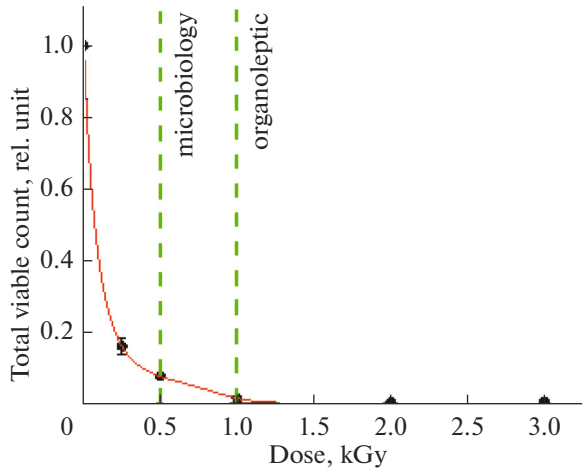

Fig. 1. Dependency of the relative concentration of viable cells in a turkey after irradiation on the dose. Dotted lines show the boundaries of the effective dose range.

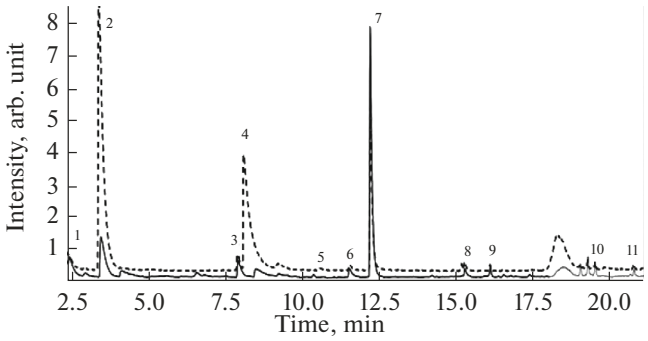

Fig. 2. Chromatograms of the turkey samples. The dotted chromatogram shows the control sample and the solid chromatogram represents the sample irradiated at the dose of 10 kGy.

added, hermetically sealed, and placed in an ultrasonic bath, where the compounds were extracted for 30 min. Then the samples were kept in a thermostat for 10 min at 95°C, following which 1 mL of the vapor phase of the sample was injected into the chromatograph.

A GC-MS Shimadzu GCMS-QP2010 Ultra equipped with an HT200H Headspace Autosampler was used to determine the components of volatile compounds. Data collection and chromatogram analysis were carried out using the GCMS solution software and the NIST/EPA/NIH Mass Spectral Library 2008 (NIST 08).

The separation of the volatile compounds was carried out using a CP-5 Sil capillary column with the dimensions 30 m × 0.25 mm × 0.4 μm. The temperature sequence for the separation of the components was as follows: the initial temperature—35°C was isothermally increased to 180°C with a rate of 5°C/min. Helium was used as the carrier gas. The helium flow through the column was 1 cm^3/min. Evaporator temperature was 200°C. The temperature of interface was 200°C. The energy of ionizing electrons was 70 eV. Quadrupole temperature was 200°C. The temperature of ion source was 230°C. Chromatograms were made for all ions in a scanning mode for m/z values ranging from 33 to 350 at a scanning rate of 3.3 scans/sec.

To determine the concentration of volatile compounds in turkey samples, standard samples were diluted in methanol in different concentrations. Next, the initial solutions of turkey homogenate were diluted with a 3% saline solution in deionized water as required. The concentration of the components analyzed during the experiment in the calibration solutions ranged from 0.025 to 1 mg/L. The resulting calibration solutions were analyzed under the same conditions as the test samples. The concentration of the components was calculated using external standard method factoring in the calibration dependencies, the peak area on the chromatogram as well as the weight of the initial sample.

RESULTS AND DISCUSSION

Figure 1 shows the dependency of the concentration of viable cells in turkey meat on the dose of electron beam irradiation relative to the concentration in control non-irradiated samples.

It was found that the experimental dependency of the concentration of bacterial cells on the irradiation dose can be described using the following formula:

$$f(D) = e^{a+bD+cD^2+dD^3+o(D^3)},$$

where D is the absorbed dose of irradiation, a determines the position of the microorganism loss curve relative to the coordinate axes, while b, c and d reflect the rate of decrease in the number of viable cells in a turkey with an increase in the irradiation dose. The parameters of the function $f(D)$, calculated by the least squares method, were $a_{el} = (4.01 \pm 0.01) \times 10^{-7}$ (rel. units), $b_{el} = -10.7 \pm 0.3$ (Gy^{-1}), $c_{el} = 15.7 \pm 1.1$ (Gy^{-2}), $d_{el} = -9.3 \pm 0.9$ (Gy^{-3}) for electron beam irradiation. The correlation coefficient R_{el} was 0.999, which indicates that the proposed approximation is adequate.

Figure 1 shows that the irradiation with the doses exceeding 0.5 kGy at the dose rate of (10 ± 1) Gy/sec decreased the concentration of viable cells in turkey meat by more than 10 times. A further increase in the dose led to a significant decrease in the number of microorganisms. The dose of 3 kGy completely suppressed viable microorganisms. The analysis of changes in organoleptic parameters showed that a specific smell, taste and color appear at the doses of more than 1 kGy [36]. Figure 1 shows the boundaries of the effective dose range from 0.5 to 1 kGy, at which a significant decrease in pathogenic microflora occurs

Table 1. Identified compounds (peak number is indicated in brackets) in turkey samples (μg/kg; $n = 3$—number of repeats, $P = 0.95$—confidence level)

Compound	Time lapse, min	Irradiation dose, kGy			
		0	0.25	1	10
Acetone (1)	2.48	3.9 ± 1.0	7.1 ± 1.8	16 ± 4	39 ± 10
2,3-Butanedione (2)	3.435	3750 ± 938	1490 ± 373	978 ± 245	566 ± 142
Pentanal (3)	8.178	123 ± 31	154 ± 39	161 ± 40	163 ± 41
3-Hydroxybutanone-2 (4)	8.437	2117 ± 529	2178 ± 545	722 ± 181	237 ± 59
3-Methylbutanol (5)	10.62	11 ± 3	129 ± 32	56 ± 14	–
Pentanol-1 (6)	11.726	42 ± 11	54 ± 14	53 ± 13	52 ± 13
Hexanal (7)	12.315	1013 ± 253	1217 ± 304	1171 ± 293	1478 ± 370
Hexanol-1 (8)	15.35	49 ± 12	85 ± 21	50 ± 13	42 ± 11
Heptanal (9)	16.152	53 ± 13	48 ± 12	64 ± 16	90 ± 23
Octanal (10)	19.532	55 ± 14	52 ± 13	62 ± 16	98 ± 25
Nonanal (11)	20.63	65 ± 16	57 ± 14	84 ± 21	125 ± 31

without deteriorating organoleptic properties of turkey samples.

Figure 2 shows the chromatograms of the control sample and the sample treated at a dose of 10 kGy.

A significant change in both the position of the maxima in the chromatogram and the change in the areas of the peaks is observed. Table 1 shows the concentrations of volatile organic compounds identified in turkey samples.

Figure 3 shows the dependencies of the relative concentration of compounds in the turkey samples on the irradiation dose. All graphs also show the boundaries of the effective irradiation dose range applied to chilled turkey.

Eleven compounds identified in turkey samples belong to the 3 main classes of organic compounds: alcohols, aldehydes, and ketones. The highest content of carbonyl compounds 2,3-butanedione, 3-hydroxybutanone-2, and hexanal was detected in the non-irradiated samples. The content of 3-hydroxybutanone decreased with an increase in the irradiation dose, which can be caused by the oxidation of hydroxyl groups and the conversion of alcohols into aldehydes or ketones, depending on the structure of the compound (Fig. 3b). The most intensive oxidation of primary alcohols probably occurs at high radiation doses, such as 10 kGy. For example, the concentrations of 3-methylbutanol and hexanol-1 increased at doses of 0.25 and 1 kGy, as fat oxidation occurred. With a further increase in the dose from 1 to 10 kGy the content of 3-methylbutanol and hexanol-1 decreased, which can be caused by the conversion of alcohols into aldehydes (Fig. 3b). All aldehydes showed a linear increase in their content with an increase in irradiation dose, as it can be seen in Fig. 3c:

$$f(D) = A + BD,$$

where A is the initial concentration of compounds, B is the rate of increase in concentration with dose, R is the correspondence parameter. The values A, B and R are shown in Table 2.

Table 2. The values A, B and R for aldehydes

Aldehyde	A, rel. unit	B, rel. unit	R
Nonanal	0.968 ± 0.095	0.099 ± 0.032	0.83
Octanal	0.979 ± 0.035	0.082 ± 0.011	0.97
Heptanal	0.986 ± 0.068	0.074 ± 0.021	0.87
Hexanal	1.089 ± 0.060	0.038 ± 0.015	0.77
Pentanal	1.143 ± 0.098	0.020 ± 0.020	0.83

As it can be seen in Fig. 3, the content of 2,3-butadione decreased with an increase in the irradiation dose, which can be caused by the destruction of C–C bonds and the formation of other compounds with a smaller number of carbon atoms in the molecule, such as acetone, which showed a linear increase in concentration with an increase in irradiation doses (Fig. 3b). Such an increase in the concentration of acetone, can be explained by the fact that this saturated ketone is a very oxidation-resistant substance that accumulates with increasing absorbed radiation dose. Thus, acetone can be used as a potential marker for assessing the absorbed dose in irradiated low-fat meat products.

Figure 4 shows the dependency of total concentration of all compounds identified in turkey samples on the irradiation dose.

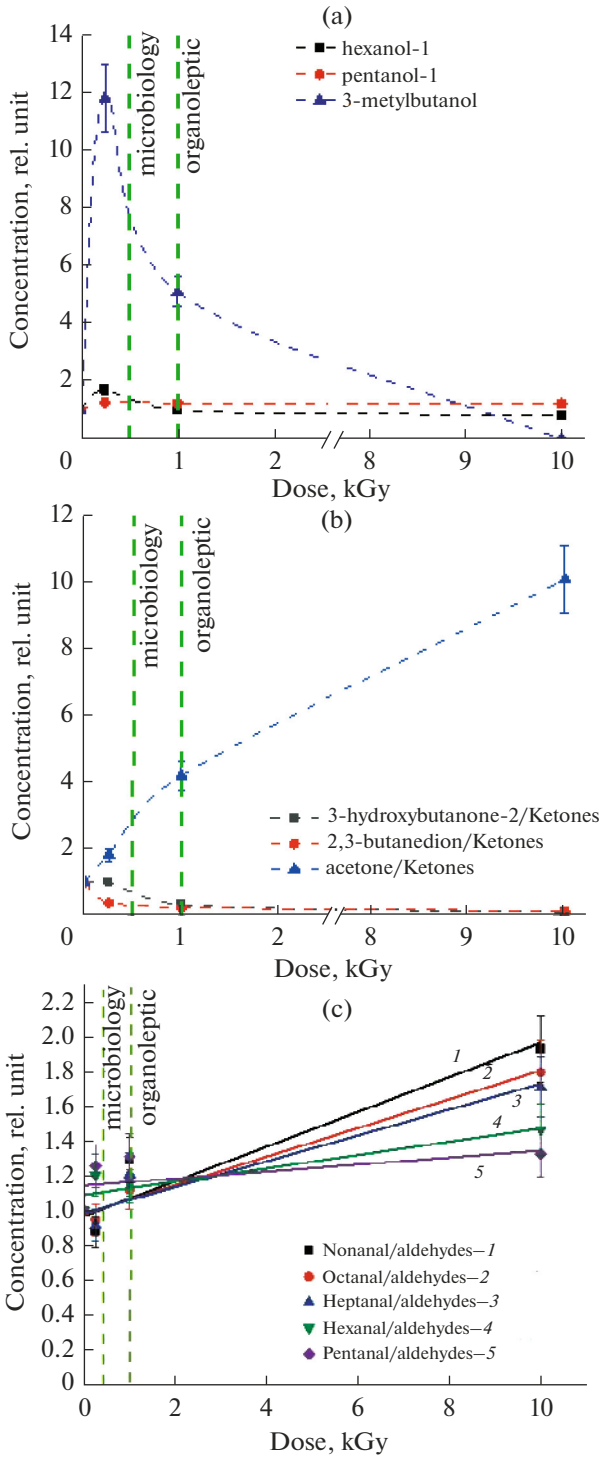

Fig. 3. Dependencies of the relative concentration of compounds identified in turkey samples on the irradiation dose: (a) alcohols, (b) ketones, and (c) aldehydes.

The analysis showed that the total amount of volatile compounds decreases exponentially with an increase in irradiation dose:

$$f(D) = A + Be^{-CD},$$

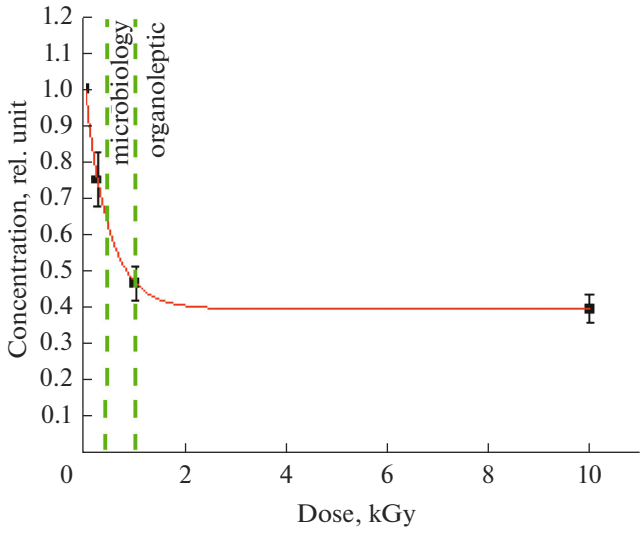

Fig. 4. Dependency of the relative total concentration of compounds identified in turkey samples on the irradiation dose.

where A is the initial concentration of compounds, B shows the maximum difference in concentration values of the compounds identified in turkey samples, C shows the rate of decrease in concentration with an increase in irradiation dose. The values represented in formula indicated above were $A = 0.397 \pm 0.001$ (rel. un.), $B = 0.603 \pm 0.001$ (rel. un.), $C = 2.127 \pm 0.003$ (Gy^{-1}), the correlation coefficient was 1.0.

Figure 4 shows that the total amount of volatile compounds decreased by more than 2 times in turkey samples irradiated with the dosed ranging from 0.5 to 1 kGy. The total content of volatile organic compounds decreases exponentially, and the change in the concentration of chemicals is due to radical oxidation of volatile compounds after exposure to ionizing radiation.

CONCLUSIONS

The study conducted by our research team shows that the GC-MS method allows to identify with high accuracy the volatile compounds which can be found in irradiated turkey samples and efficiently separate compounds with similar molecular weights.

During the research alcohols, aldehydes and ketones were identified in turkey meat before and after electron irradiation. It was established that the total amount of volatile compounds decreased exponentially with an increase in the irradiation dose turkey samples were exposed to.

The dependencies of the content of alcohols, ketones and aldehydes on the irradiation dose showed that the content of carbonyl compounds (all aldehydes and acetone) went up with an increase in the irradiation dose, which can account for a specific taste and smell of irradiated meat products.

In the absence of acetone in the control samples a linear increase in its concentration with a higher irradiation dose was detected in turkey samples. This proves that acetone can be used as a potential marker of the degree of irradiation in low-fat meat products.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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