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Research into Gas Chromatography–Mass Spectrometry (GC-MS) for Ensuring the Effect of 1 MeV-Accelerated Electrons on Volatile Organic Compounds in Turkey Meat

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Abstract: One of the most important tasks in the food industry is the search for alternative biochemical markers of radiation treatment in dietary, chilled meat products such as chicken and turkey. Major organic volatile chemicals found in meat products can be precisely identified using gas chromatography coupled with mass spectrometry. In the response to the needs of the food industry, our research team conducted a series of experiments involving the irradiation of chilled poultry meat using an electron accelerator. The experiments showed that the concentration of pure volatile organic compounds in saline solution dropped exponentially with an increase in the irradiation dose, which proves that these chemicals decomposed when exposed to ionizing radiation. However, when turkey meat was exposed to an electron beam with doses up to 1 kGy, the concentration of alcohols, aldehydes, and ketones peaked, only to decrease with an increase in the irradiation dose up to 2 kGy, and then went up slightly when the irradiation dose was within the range from 2 kGy to 10 kGy. To determine the reason behind the nonlinear dependencies of organic compound concentrations in turkey meat on the irradiation dose, we developed a mathematical model that acknowledges the presence of two opposing processes, those of decomposition and accumulation of organic compounds as a result of the decomposition of other compounds that can be found in turkey meat.

Keywords: gas chromatography–mass spectrometry (GC-MS); volatile organic compounds; food irradiation; electron accelerator; turkey meat; mathematical model; decomposition and accumulation processes

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1. Introduction

Food irradiation is an effective method of suppressing microbial contamination and increasing the shelf life of products without using chemical compounds, canning, and additional heating or freezing of the processed object [1–4]. For each type of product, it is necessary to select the range of radiation doses [5–8], the radiation dose rate [9], the irradiation method, which includes the choice of the source of ionizing radiation and its operation mode [10], the relative position of the source and the processed object, and the container for placement-processed products, packaging, etc. [4,11–13]. Noncompliance with the technological regimes of irradiation leads to chemical changes in the product, and, as a result, to a change in its organoleptic, nutritional, and structural properties.

There are a number of techniques used to record and control chemical changes in food that occur after radiation treatment [14]. The method of electron paramagnetic resonance (EPR) is actively used for dry foods of plant origin, products containing calcium, cellulose, crystalline sugar, and others [15–17]. The thermoluminescence (TSL) and photoluminescence (PL) methods are used for foods that contain silicon, such as seafood, potatoes, onions, and beets [18,19]. For food products with a high fat content, the 2-thiobarbituric acid method (TBARS) is used for monitoring the aldehydic end products of lipid peroxidation [20–22]. At present, the most sensitive method for assessing the chemical changes in meat and fish products is the method of gas and liquid chromatography in combination with mass spectrometry (GC-MS and LC-MS) [23–27].

One of the main tasks of modern research in the field of irradiation of food products is the search for specific biochemical markers in the product. According to literature sources, volatile organic compounds, such as aldehydes, ketones, alcohols, and sulfur-containing and carbonyl compounds, etc., could be potential markers for meat and fish products. However, at the moment, there is no description of a general pattern in the behavior of various organic compounds depending on the dose of irradiation of food products, which could be used in the preparation of recommendations for determining the optimal modes of technological radiation treatment of meat and fish products, as well as poultry meat.

The purpose of this work was to identify the dependences of the concentrations of volatile organic compounds in poultry meat on the radiation dose when exposed to a beam of accelerated electrons with an energy of 1 MeV.

2. Materials and Methods

Chilled turkey fillet, stored in a refrigerator at a temperature of 2 °C for one day after slaughter, was chosen as the object of the study. It is difficult to assess the change in the concentration of a particular volatile compound as a result of exposure to ionizing radiation when a large number of chemical compounds that can react with each other can be found in turkey meat. Therefore, to analyze the change in the concentrations of volatile substances under the influence of ionizing radiation, a model experiment was carried out using standard samples of organic compounds: aldehydes (2-methylbutanal, pentanal, hexanal), ketones (butanone-2, pentanone-2), and alcohols (pentanol-1, hexanol-1) (Sigma-Aldrich, Burlington, MA, USA), each of which had been immersed in a saline solution of 0.9% NaCl at a concentration of 1 mg/L.

Samples of turkey meat (0.5 ± 0.1 g) and suspensions of organic compounds with a volume of 0.5 mL were placed in plastic microcentrifuge tubes with a volume of 2 mL (JSC "RZP", Rybinsk, Russia).

The samples were irradiated on a continuous-action electron accelerator UELR-1-25-T-001 (manufactured by SINP MSU jointly with JSC SPE "TORIY", Moscow, Russia) with an energy of 1 MeV, at an average beam current of 70 nA and ambient temperature of 20 °C. For each irradiation session, the studied samples in the amount of 8 pieces were laid out on a duralumin plate according to the method described in [9]. During each irradiation process, the charge Q (nC) absorbed by the duralumin plate was measured using an ADC (LLC "Production Association OWEN", Moscow, Russia), and the charge measurement error at a current of 50 nA was no more than 2%; other factors were the beam current I (nA) and exposure time t_{irrad} (s). The samples were irradiated at doses of 0.25 kGy, 0.5 kGy, 1 kGy, 2 kGy, 5 kGy, and 10 kGy.

To measure the dose absorbed by the samples, a Ferrous Sulfate (Fricke) Dosimeter was used, based on the transition of Fe^{2+} to Fe^{3+} ions and the change in the color of the solution after exposure to ionizing radiation. Test tubes with a $FeSO_4$ solution of 0.5 mL, poured into plastic microcentrifuge tubes, were laid out on a duralumin plate in the amount of 8 pieces in accordance with the method used for irradiating the studied poultry meat samples. The beam current was 50 nA. After irradiation, the solutions were transferred into a quartz spectrophotometric cuvette (Nuova Aptaca SRL, Canelli, Italia), and the transmittance τ of the solution was measured at a wavelength of 304 nm using a UV-3600

double-beam spectrophotometer (Shimadzu, Japan). Knowing the length of the optical path l = 1 cm, the molar concentration of Fe^{3+} ions in the solution was calculated using Equation (1):

$$M\left(Fe^{3+}\right) = \frac{\Delta S\left(Fe^{3+}\right)}{l\varepsilon} \quad (1)$$

where $\Delta S = 1/\tau$ is the optical density of the solution and ϵ = 2160 L/mol·cm is the extinction coefficient of Fe^{3+} ions, which characterizes the attenuation of the intensity of light fluxes passing through the solution. The dose absorbed by the solution was calculated by Equation (2):

$$D = \frac{kM\left(Fe^{3+}\right)}{\rho G\left(Fe^{3+}\right)} \quad (2)$$

where $k = 9.65 \times 10^6$ is the dimensionless coefficient, $M(Fe^{3+})$ is the molar concentration of Fe^{3+} ions, ρ = 1.024 g/cm^3 is the density of the dosimetry solution, and $G(Fe^{3+})$ = 15.6 ion/100 eV is the radiation chemical yield under the influence of accelerated electrons with energies up to 10 MeV.

During the experiment, the time of exposure and the charge absorbed by the duralumin plate, on which test tubes with a $FeSO_4$ solution were placed, were measured to determine the dose rate for the experiment (Table 1). Knowing the dependency of the dose absorbed by the solution on the exposure time at a fixed beam current of 50 nA, the dose rate absorbed by the dosimetric solution was calculated to amount to (1.56 ± 0.03) Gy/s. The dose rate and exposure time at a fixed current allowed us to determine the dose absorbed by the turkey samples.

Table 1. Dosimetric solution data.

№	Time of Exposure t_{irrad}, s	The Charge Absorbed by Duraluminium Plate Q, nC	Transmittance τ, rel.un.	Absorbed Dose D, Gy
1	34.6 ± 1	2390 ± 47	0.15 ± 0.01	40.8 ± 2.8
2	58.5 ± 1	4790 ± 95	0.28 ± 0.02	79.2 ± 5.5
3	78 ± 1	7380 ± 147	0.43 ± 0.03	120.3 ± 8.4
4	104 ± 1	9834 ± 197	0.57 ± 0.04	159.1 ± 11.1
5	130 ± 1	12,410 ± 248	0.71 ± 0.05	198.6 ± 13.9
6	201 ± 1	20,020 ± 400	1.16 ± 0.08	325.5 ± 22.8

To identify volatile compounds and evaluate their concentrations in turkey meat samples with a total weight of 4 g after exposure to radiation at various doses and to compare these with the concentrations of compounds in nonirradiated reference samples, a GC Agilent 8890 MSD 5977 E gas chromatography–mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) was used with a vaporous sample injection according to the method described in reference [27]. For each measurement, 2 g of homogenate turkey meat was used, two measurements were made on samples irradiated at the same dose, and two control measurements were made.

3. Results and Discussion

During the pilot study, dependences of the concentrations of volatile compounds on the radiation dose were obtained (Figure 1). The concentrations of pentanal (aldehyde), pentanol (alcohol), and pentanone (ketone) were decreased exponentially with the increase in the radiation dose. Similar dependencies were observed for all other organic compounds studied. Such dependencies occurred as a result of decomposition of the organic compounds caused by ionizing radiation with the doses ranging from 250 Gy to 10 kGy.

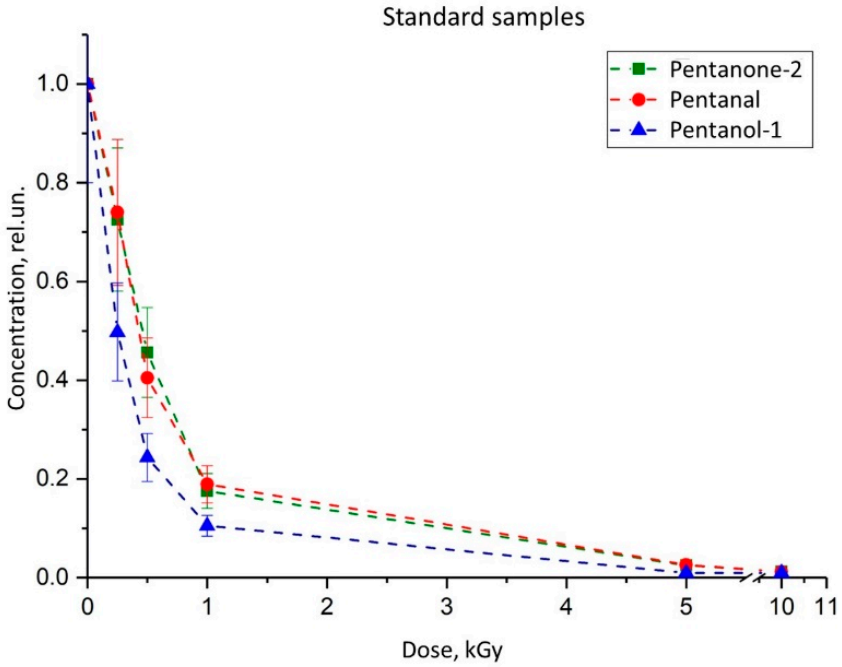
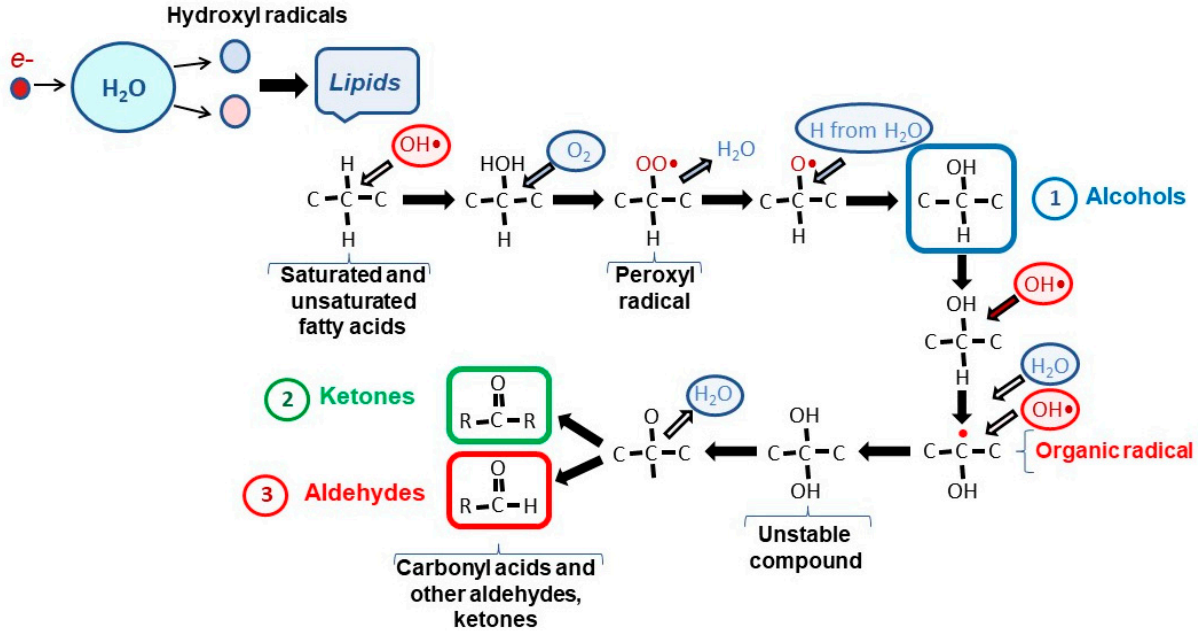


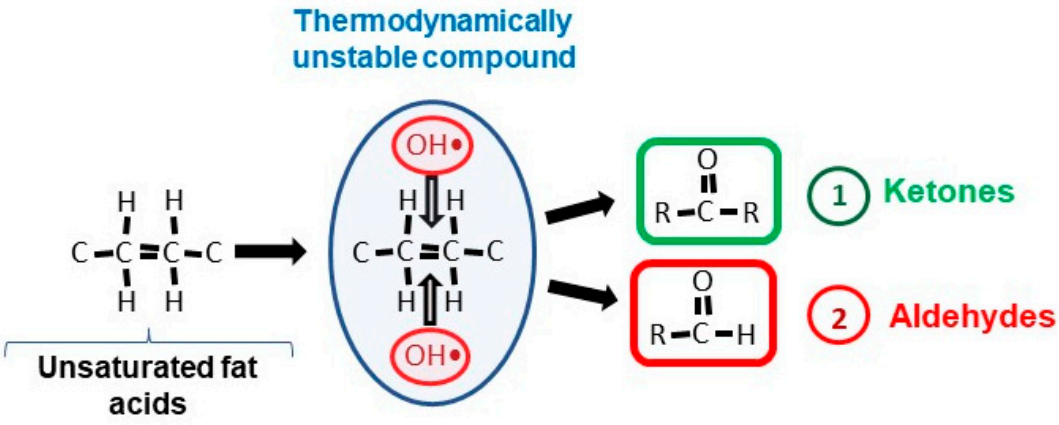
Figure 1. The dependencies of the concentrations of Pentanone-2, Pentanal, and Pentanol-1 in saline solution on the irradiation dose.

It has been scientifically proven that lipids are the key targets of irradiation [28]. Meat and fish products have a high concentration of lipids which contain fatty acids. During irradiation, free radicals induced by radiolysis oxidize fat acids that may decompose into volatile organic compounds [29,30]. The change in the concentrations of volatile organic compounds in the irradiated product containing fat acids is exemplified in Figure 2.



(**a**)

Figure 2. *Cont.*



(**b**)

Figure 2. Simplified diagrams of formation of aldehydes and ketones as products of alcohol oxidation (**a**), and by decomposition of thermodynamically unstable compound (**b**).

Hydroxyl radicals OH• may react with unsaturated fatty acids which are deficient in electrons in carbonyl groups and carbon–carbon double bonds (Figure 2a). After that, the resultant compound may react with the O_2 molecule, and this chemical reaction may cause H_2O and peroxyl radical OO• to form. Since this radical is unstable, the oxygen atom detaches from the radical, leaving the ion O• with one unpaired electron which can tear off a hydrogen atom from the H_2O molecule. The alcohols which appear as a result of this reaction may be oxidized when they react with OH• and OO• radicals. The organic radical formed as a result of this reaction may react with either the hydroxyl radical OH• or the H_2O molecule. The transient unstable compound which appears as a result of this process may release the H_2O molecule; and two carbon–carbon double bonds may form a C=O double bond. As a result, aldehydes or ketones can be formed. Thus, we illustrated one of the possible options for the formation of alcohols, which can further decompose into aldehydes or ketones (Figure 2a). Aldehydes are unstable compounds; therefore, they can decompose into carboxylic acids, transform into other aldehydes, or increase in number due to the breakdown of other substances. Ketones are stable compounds, so the likelihood of radical reactions with them is low.

Fatty acids can also decompose in a different way. Hydroxyl radicals OH• may interact with unsaturated fatty acids, adding to carbon–carbon double bonds with the help of multiple bonds. As a result, this combination becomes thermodynamically unstable and breaks down into molecules from which aldehydes or ketones are formed (Figure 2b). Furthermore, OH• radicals can interact with C-H bonds and oxidize the fatty acid into parts from which alcohols are formed. Primary alcohols are oxidized to aldehydes, secondary alcohols to ketones. The reverse processes of the reduction of alcohols from aldehydes and ketones do not occur in radical reactions. Other multiple variants of chemical transformations are also possible.

To explain the behavior of the organic volatile compounds after exposure to ionizing radiation, we studied the conversion of alcohol hexanol into aldehyde hexanal in saline solution. With the irradiation doses were up to 100 Gy in a hexanol solution, the concentration of hexanal increased due to the decomposition of the corresponding alcohol hexanol (Figure 3). Furthermore, with an increase in the dose, the process of decomposition of hexanal itself prevailed over the process of its accumulation due to the decomposition of alcohol, and a decrease in the concentration of hexanal was observed.

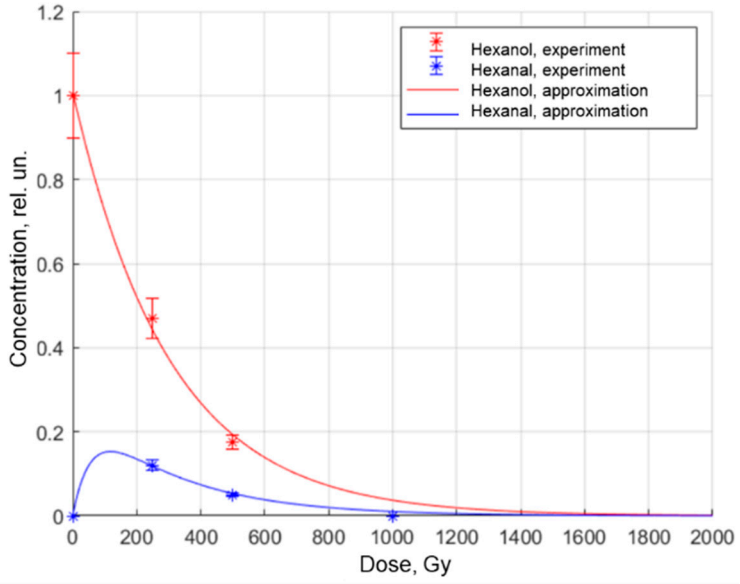


Figure 3. The dependencies of the concentrations of hexanol and hexanal on the irradiation dose obtained in the solution of hexanol alcohol.

Thus, changes in the concentrations of hexanol C_1 and its decomposition product hexanal C_2 can be described using differential Equation (3):

$$\begin{cases} \frac{dC_1}{dD} = -a_0 C_1 \\ \frac{dC_2}{dD} = -b_0 C_2 + c_0 C_1 \\ C_1(0) = 1, \ C_2(0) = 0 \end{cases}, \quad (3)$$

where C_1 is the concentration of hexanol (mg/L), C_2 is the concentration of hexanal (mg/L), a_0 is the decomposition constant of hexanol (Gy^{-1}), b_0 is the decomposition constant of hexanal (Gy^{-1}), c_0 is the decomposition constant of hexanol alcohol in aldehyde hexanal in physiological solution (Gy^{-1}), and $C_1(0)$ and $C_2(0)$ are the initial concentrations of hexanol and hexanal in the hexanol solution, respectively.

As a result of solving the system of differential equations, the dependences of the concentrations of the observed compounds on the radiation dose Equation (4) were obtained:

$$\begin{cases} C_1 = e^{-a_0 D} \\ C_2 = \frac{c_0}{a_0 - b_0} \times \left(e^{-b_0 D} - e^{-a_0 D} \right) \end{cases}. \quad (4)$$

In Equation (4), for the concentration of hexanol C_1, the function $e^{-a_0 D}$ denotes the decomposition of alcohol due to the action of ionizing radiation. For the concentration of C_2 hexanal, the first term in the function denotes the decomposition of aldehyde due to exposure to radiation, and the second term is responsible for its accumulation in the alcohol solution due to the decomposition of hexanol. Solid lines which are in Figure 3 are functions calculated by Equation (4).

Three groups of volatile compounds were identified in turkey meat samples after exposure to ionizing radiation, namely, alcohols, aldehydes, and ketones. The dependencies of the concentrations of aldehydes (nonanal, hexanal, octanal, heptanal, pentanal), alcohols (hexanol-1, pentanol-1) and ketone (3-methylbutanone-2) on the irradiation dose, divided by concentrations of compounds in control nonirradiated samples of turkey meat, had a similar character.

The behavior of volatile organic compounds as decomposition products of fatty acids in foodstuffs differed from the nature of the dependencies of the concentrations of volatile organic compounds in saline solution on the radiation dose. Under the influence of acceler-

ated electrons at a dose of 250 Gy, the concentration of aldehydes increased by 1.2–1.4 times compared to the reference values. The concentration of hexanal in turkey samples irradiated at a dose of 10 kGy turned out to be 1.2 times higher than the control values.

The concentration of alcohols (hexanol-1, pentanol-1) in turkey samples increased by 1.75–2 times in doses from 250 Gy to 1000 Gy. Further, with an increase in the dose, the concentration of alcohols fell exponentially, the concentration of alcohol thereafter increased at a dose of 10 kGy, and the values exceeded the reference values by 1.6–2.25 times. The dependence of the concentration of 3-methylbutanone-2 on the irradiation dose had the following form: the concentration increased threefold compared with the reference value upon irradiation at a dose of 250 Gy; thereafter, in the range from 250 Gy to 10 kGy, an exponential decline occurred in the values close to the reference values.

Thus, for all identified compounds, in the dose range up to 1000 Gy, a nonmonotonic nature of the dependence of concentrations on the irradiation dose was observed. A smoother form of these dependences was observed when the dose was increased.

The analysis of the dependencies of the concentrations of organic compounds in turkey samples on the radiation dose showed that two competing processes occur in all the samples: the breakdown of a chemical compound and the formation of molecules of this compound due to the breakdown of other compounds. Which of the processes will prevail is influenced by the structure of the molecules themselves, the presence of other compounds that make up the compound, and their concentrations, as well as the radiation dose.

Therefore, based on the functions (4), a function was proposed that describes the change in the concentration of this volatile compound in a poultry meat sample depending on the radiation dose. It consists of a decomposition function of a volatile compound $C_{dec}(D) = a \times e^{-dD}$ and a function of accumulation of molecules due to the decay of other volatile compounds $C_{acc}(D) = b \times \left(1 - e^{-cD}\right)$. It is also possible to accumulate molecules of this compound due to the decay of other organic molecules, which is proportional to the radiation dose. Thus, the dependence of the change in the concentration of a volatile organic compound in poultry meat has the following form:

$$C(D) = C_{dec}(D) + C_{acc}(D) = a \times e^{-dD} + b \times \left(1 - e^{-cD}\right) + k \times D, \quad (5)$$

where

a (mg/L) is a parameter denoting the initial concentration of the compound;

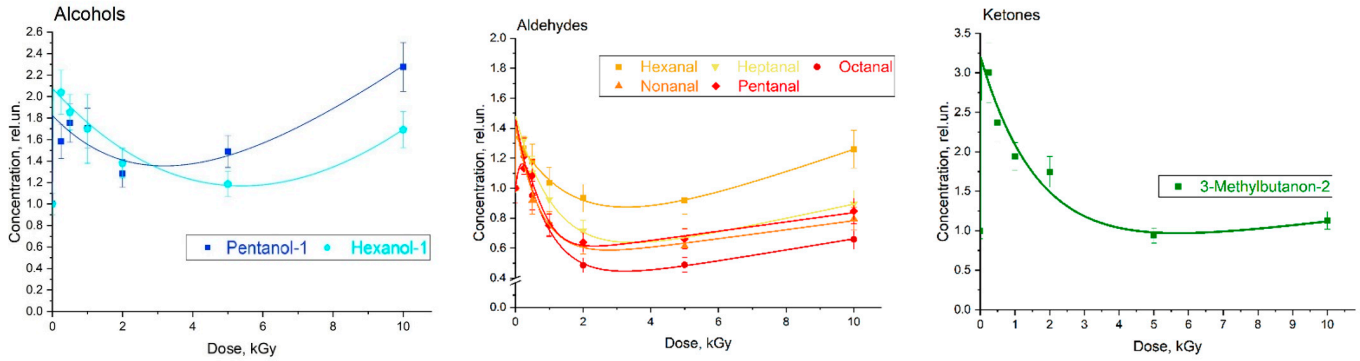
d (1/Gy) is a parameter characterizing the decay rate of a volatile compound after exposure to radiation;

b (mg/L) is a parameter characterizing the maximum concentration of a volatile compound in a turkey sample during the decomposition of other volatile compounds;

c (1/Gy) is a parameter characterizing the rate of accumulation of a volatile compound due to the decomposition of other volatile compounds, e.g., the decomposition of alcohol into aldehyde;

k (mg/(L × Gy)) is a parameter describing the rate of formation of a volatile compound due to the decay of other types of organic molecules of compounds with an increase in the radiation dose.

The dependencies of the concentrations of organic compounds in turkey samples on the irradiation dose and the corresponding analytical dependencies calculated by Equation (5) are plotted in Figure 4.



(**a**) (**b**) (**c**)

Figure 4. The dependencies of the concentrations of alcohols (**a**), aldehydes (**b**), and ketones (**c**) in turkey samples on the irradiation dose and the corresponding functions, calculated by Equation (5).

The function parameters calculated by Equation (5) for each identified compound, as well as the correlation coefficients corresponding to them, are presented in Table 2. The values of the correlation coefficients indicate the adequacy of the proposed mathematical model for describing the behavior of organic volatile compounds in turkey meat after exposure to accelerated electrons in various doses.

Table 2. Function parameters for different organic compounds.

Organic Compound	The Function Parameters					
	a, mg/L	*d*, Gy^{-1}	*b*, mg/L	*c*, Gy^{-1}	*k*, mg/(L × Gy)	*R*
Hexanal	0.99 ± 0.04	0.45 ± 0.01	0.34 ± 0.01	470,317 ± 235	0.090 ± 0.001	0.97
Heptanal	1.01 ± 0.05	0.87 ± 0.01	0.49 ± 0.01	5485 ± 25	0.041 ± 0.002	0.97
Octanal	1.05 ± 0.05	1.05 ± 0.03	0.32 ± 0.01	593 ± 12	0.032 ± 0.005	0.95
Nonanal	0.99 ± 0.04	1.45 ± 0.07	0.49 ± 0.01	3.8×10^6 ± 257	0.034 ± 0.002	0.97
Pentanal	1.01 ± 0.04	1.35 ± 0.05	0.49 ± 0.02	8.12 ± 0.92	0.045 ± 0.001	0.93
Hexanol-1	17.07 ± 0.85	0.070 ± 0.015	1.08 ± 0.05	1230 ± 37	0.81 ± 0.01	0.95
Pentanol-1	2.23 ± 0.07	0.27 ± 0.01	0.83 ± 0.03	1264 ± 57	0.25 ± 0.01	0.97
3-Methylbutanone-2	43.72 ± 0.99	7.81 ± 0.85	42.27 ± 0.89	77,683 ± 210	−0.051 ± 0.002	0.98

4. Conclusions

During the study, we experimentally obtained the dependencies of a number of volatile organic compounds (alcohols, aldehydes, and ketones) in chilled turkey meat treated with 1 MeV-accelerated electrons in the irradiation dose.

The concentration of all volatile compounds identified in turkey meat was non-monotonous, with the values peaking at the doses up to 250 Gy and then decreasing with an increase in the irradiation dose up to 2 kGy, only to go up slightly when the irradiation dose was within the range from 2 kGy to 10 kGy.

The mathematical model proposed for describing the change in the concentration of volatile substances in turkey samples after exposure to accelerated electrons in the dose range from 0.25 kGy to 10 kGy confirmed our assumptions. Two competing processes play a role in the dependency of volatile organic compounds on the dose: decomposition and accumulation of volatile compounds caused by the decomposition of other compounds. The applicability and adequacy of this model for each type of identified volatile compound were demonstrated in the study.

The knowledge of behavior patterns of volatile organic compounds, being products of possible chemical transformations of fatty acids in turkey, after exposure to ionizing radiation helps us to understand the fundamental physical and chemical processes that occur in meat and fish products after irradiation.

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