https://doi.org/10.46813/2025-157-134

MASS SPECTROMETRIC AND BIOLOGICAL STUDIES OF GLUCOSE/FRUCTOSE, IRRADIATED WITH PHOTONEUTRONS ON THE M-30 MICROTRON

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The processes of fragmentation of glucose and fructose molecules after neutron irradiation on the M-30 microtron were studied by mass spectrometry, and significant changes in the yield of their fragments fission after neutron irradiation were detected for the first time. It is shown that the interaction with neutrons primarily causes the breaking of essential carbon bonds and the deep destruction of molecules. This fact leads to an increase in the yield of a number of fragments, especially for m/z=18, 31, 43, compared to non-irradiated ones. The paper also presents temporal evolutionary changes in biological activity of the irradiated solutions and dry mixtures using as the example of test strains of *Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa.*

PACS: 539.1

INTRODUCTION

The study of the processes of fragmentation and transformation of biomolecules under external influence is essential for cell biochemistry, in particular, for explaining single- and double-stranded DNA breaks [1]. The study of these processes in monosaccharides and polyhydric alcohols is due to their crucial role as energy carriers and suppliers of structural components of living cells.

Glucose and fructose are widely used in medicine, food, and chemical industries. Despite having the same chemical formula ($C_6H_{12}O_6$), they differ in structure and properties, which makes them convenient objects for studying the fragmentation of bioorganic molecules after radiation exposure. When a nuclear particle collides with an organic molecule, it is ionized, excited, or destroyed, forming unstable molecular systems. These systems decompose into ionized structural fragments or neutral residues. Monosaccharides are characterized by the loss of water molecules (H_2O) and the release of carbon fragments (CH_2O) [2]. Interest in their fragmentation is also related to the role of low-energy electrons in radiation damage to DNA [3, 4].

Radiation technologies make it possible to change the structure of bioorganic compounds in a targeted manner and study their biological activity. For this purpose, flows of high-energy particles are used – electrons, gamma quanta, and neutrons. Neutrons have a high penetrating power. When they interact with a substance, elastic collisions occur, resulting in energy transfer maximized in collisions with light elements such as hydrogen, carbon, and oxygen, the main components of glucose and fructose. This fact causes the destruction of molecular structures, the detachment of electrons, or the ionization of atoms. The capture of a neutron is less likely with the formation of non-radioactive isotopes ^{13}C, ^{17}O.

Thus, unirradiated glucose/fructose samples are monomolecular compounds, while after neutron irradiation, they contain a combination of molecular and atomic fragments of different masses. Mass spectrometry allows us to study ion fragments and determine the channels of their formation.

This paper presents the results of mass spectrometric studies of the fragmentation of glucose and fructose molecules before and after neutron irradiation and the analysis of changes in biological parameters after such treatment. Photoneutron beams were generated on the M-30 microtron, and the samples were irradiated under the same conditions. The use of photon neutrons promotes more significant destruction of molecules and broadens the spectrum of fragment ions depending on the initial structure of the molecules. The study is a continuation of [5], where the mass spectra of bioorganic compounds after irradiation with fast electrons (12.5 MeV) were studied.

1. METHODS

The source of photoneutrons was the microtron M-30 electron accelerator of the Institute of Electronic Physics of the National Academy of Sciences of Ukraine as a result of two stages of nuclear particle transformations: electron beam into brake radiation and gamma-ray flux into neutrons by reaction (γ, n) on lead nuclei. For this purpose, a special converter assembly was placed at the electron outlet of the accelerator, which contained a 1 mm thick tantalum plate as a primary converter of electrons into brake radiation and a 1 cm thick lead plate as a photon source, behind which the samples were directly placed for irradiation. The thickness of the tantalum plate was optimal to obtain a high flux density of brake γ radiation with a maximum energy of 12.5 MeV. The interaction of gamma quanta with heavy metal elements leads to both their absorption and "evaporation" (86 %) of photoneutrons, as well as their knockout (14 %) in direct interaction with the nucleus. Under the conditions of this experiment, at an electron energy of 12.5 MeV, the energy of γ-quanta exceeds the reaction thresholds (γ, n) for natural lead isotopes $^{206\text{-}208}Pb$, which are in the range of

6.5…8 MeV, leading to efficient photon generation. The maximum energy of such photoneutrons can be up to 5 MeV, and the average energy can be estimated as 0.77 MeV for these experimental conditions [6, 7]. The photogeneration of the neutron flux during irradiation of glucose/fructose samples was carried out at an accelerated electron current of 2 μA, a temperature of 23 °C, and an irradiation dose of 3.3 kGy.

The study of molecular fragmentation channels was carried out on an installation with a monopolar mass spectrometer of the MX-7304A type [5]. The range of recorded masses of molecular fragments is 0…180 Da with a resolution not worse than ΔM = 1 Da. Molecular beams were formed by heating the starting substance near the temperature of 157 °C for glucose and 117 °C for fructose. A further increase in temperature can lead to their destruction [8, 9].

The molecular beam from the effusive source intersected at an angle of 90 degrees with an electron beam with adjustable energy from 5 to 70 eV, current in the range of 0.05…0.5 mA, and the minimum energy spread in the electron beam was ΔE1/2 = 250 MeV. The mass-spectrometric energy scale was calibrated with an accuracy of at least ±0.08 eV. The concentration of molecules in the zone of interaction with the electron beam was about 10^{11} cm^3.

The processes of electron interaction with glucose/fructose molecules have certain peculiarities since their energy is much higher than the binding energy of atoms. Therefore, in addition to the impact ionization of molecules or their structural segments, they can transition to different excited and ionized states, which leads to further fragmentation. At a constant vaporization temperature, the composition of the molecular beam will be determined by the state of the substance, in particular, before or after its radiation treatment.

The biological effects of the test objects were evaluated by the presence of antibacterial or stimulating impact on the test microorganisms we selected. Three strains of bacteria were used in the study: S*taphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa*, which are non-spore-forming opportunistic bacteria that differ in their phylogenetic affiliation and pathogenicity. Conditionally pathogenic microorganisms are represented here by gram-positive and gram-negative clinical isolates (staphylococci and pseudomonads) and a gram-negative strain of commensal bacteria – lactose-positive *Escherichia coli*. A bacterial suspension was prepared from the daily culture of the respective microorganisms according to the turbidity standard of 0.5 McFarland density units (1.5×10^8 CFU), which was determined using a densitometer (Den-1). The purity of the culture was determined bacterioscopically by the Gram's method using immersion microscopy and a light microscope (Primo Star iLED, Carl Zeiss) [10]. This work used selective chromogenic nutrient media CHROMagar™ Staph aureus, CHROMID® P. aeruginosa Agar, and MacConkey E. coli Agar (Liofilchem) to cultivate test microorganisms. Biological changes were determined immediately after irradiation and after the recovery of its physicochemical parameters 24 h after irradiation. The exposure time was 2 h in both cases.

2. RESULTS AND DISCUSSION

Fig. 1 shows the mass spectra of glucose (a) and fructose (b) before and after photon irradiation. The diagrams show the intensities of molecular fragment yields for unirradiated and irradiated samples. They demonstrate the peculiarities of fragmentation and intensity of fission fragment yields under the conditions of this experiment: the energy of the scanning electron beam was 70 eV, and the temperature of the molecule source was 430 K. It has been shown that for non-irradiated samples of glucose and fructose, their mass spectra demonstrate the dominance of a symmetric fission channel in the vicinity of *m/z* = 73 Da. The general characteristic of the obtained mass spectra is the demonstration of the ability of these molecules to deep fragmentation, both separately under the action of a beam of low-energy (70 eV) electrons and jointly by electrons and a beam of fast (up to 5 MeV) neutrons after irradiation.

However, the difference in their chemical structure leads to different degrees of molecular fragmentation. In unirradiated glucose, Fig. 1,a, five series of peaks of structural fragments in the vicinity of *m/z*= 15…20; 30; 45; 50…60 and 73 Da, corresponding to CH_3^+, H_2O^+, CHO^+, CH_3O^+, $C_2H_3O^+$, $C_2H_4O_2^+$, $C_2H_5O_2^+$, $C_3H_3O_2^+$, $C_3H_5O_2^+$, ions, and other peaks (side peaks) differ at ±1 Da, i.e., they are formed as a result of hydrogen atom migration.

The mass spectrum of non-irradiated fructose in Fig. 1b shows more significant fragmentation of the fructose molecule. Seven series of peaks can be distinguished here, some of which are also localized for glucose at *m/z*= 15…20, 30, 45, 50-60, 73 (see Fig. 1,a). However, there are also fragments with *m/z*= 85…90 and 105, possibly related to $C_4H_5O_2^+$, $C_3H_7O_3^+$ and $C_4H_7O_3^+$. The most intense (prominent) peaks in these series correspond to CH_3^+, CH_3O^+, $C_2H_3O^+$, $C_2H_4O_2^+$, $C_2H_5O_2^+$, $C_4H_5O_2^+$, $C_3H_6O_3^+$, $C_4H_7O_3^+$, ions, and peaks close to them formed by hydrogen atom migration.

Irradiation of glucose and fructose with neutron beams leads to a significant change in their mass spectra. Thus, irradiation of glucose samples increases the intensity of light fission fragments, especially in the mass range from 12 to 45 Da (see Fig. 1,a). This impact reduces the yield of some fragment ions, for example, such as *m/z*=47, 52, 56, 72, 74, 80, 98, 101…103, 131 Da. Nuclear particles do not significantly affect some low-intensity peaks of the mass spectra. It should be noted that the intensities of the peaks in the mass spectrum corresponding to these fragment ions are extremely low. This indicates a high probability of their further fragmentation and formation of ions in the mass range *m/z* = 0…75 of different intensities. A comparison of the mass spectra of the glucose molecule before and after irradiation (see Fig. 1,a) shows that the position and number of peaks of fragment ions do not change, but their intensities are significantly redistributed.

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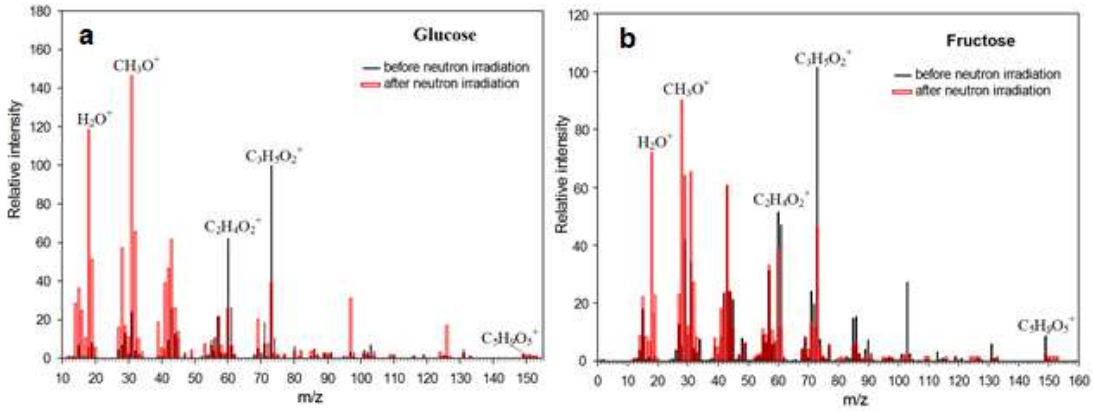

Fig. 1. Differential mass spectra of glucose (a) and fructose (b) molecules, considering the difference in their intensities before and after irradiation with photon neutrons of the M-30 microtron

The peculiarities of the destruction of these molecules in mass spectrometric studies are presented in Fig. 2 in the form of Fisher projections of variants of different fragmentation schemes. The presence of a hydroxyl group increases the possibility of dissociative decomposition of molecules during electron impact ionization. Schemes of Figs. 2,a-c demonstrate the dehydration of the molecule, with the loss of a water molecule in case (b) coming from the C2 and C3 atoms, and in case c) from the C5 and C6 atoms (see Fig. 2,a). At the second stage (II), the carbon backbone between C3 and C4 is broken as a result of the decay of the original molecule after the electron impact. The intensity of the $C_2H_5O_2$+ cation (*m/z*=73) in the mass spectrum of glucose and fructose is maximal, indicating the predominance of the second fragmentation channel (b). Thus, under electron impact conditions, the C4-C5 and C3-C4 bonds of the glucose molecular ion are broken first, resulting in the formation of fragments with the most intense peaks in the mass spectrum.

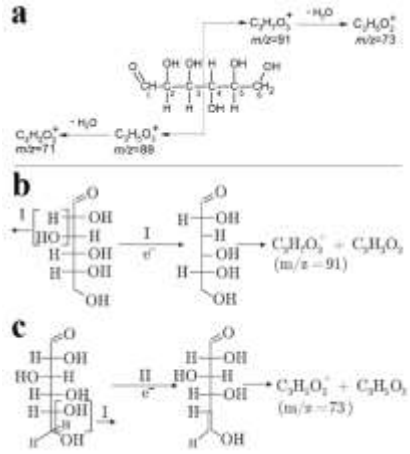

Fig. 2. Schemes of fragmentation channels of glucose and fructose molecules by electron impact: the main (a), probable (b, c)

A common feature of the mass spectrum of a fructose molecule after irradiation (see Fig. 1,b) is a significant redistribution of intensity, especially in the range *m/z*=12…50. Thus, the peak of the CH_3O+ ion (*m/z*=31) increased almost 8-fold compared to the non-irradiated one. As noted above, the irradiated sample contains intact molecules and fragments. These fragments were formed due to neutrons knocking hydrogen, carbon, and oxygen atoms out of the molecule and its subsequent dissociation. Carbon atoms form the molecule's backbone, so their removal causes the most significant damage. Thus, neutrons can cause the effective breaking of C2-C3, C3-C4, and C5-C6 bonds, which leads to an increase in the yield of fragments of *m/z*=18, 31, 43 (see Fig. 2,b).

Thus, these glucose/fructose degradation features under the influence of radiation factors may affect their biological activity. Irradiated samples containing both whole molecules, their fission fragments, and metastable structures formed during their ordering undergo different degrees of enzymatic cleavage in the food tract compared to non-irradiated samples. Therefore, studying their biological activity using common test microorganisms is relevant.

This study studied the biological effect of glucose/fructose dry matter powders and their solutions before and after irradiation with nuclear particles, which are given in Table. As indicated above, the absorbed dose determined by the method of thermally stimulated luminescence was estimated at 3.3 kGy, which ensures sufficient destruction of glucose/fructose molecules into {Ri} fission fragments [11, 12]. The analysis of the data obtained should be carried out considering both the {Ri} arrays and the final products of their relaxation according to the Lego game principle. In aqueous solutions, it is also necessary to consider the characteristics of cation-anion water fragments that can form more complex metastable chemical compounds with excellent biological activity.

According to the results of the biological activity of *Staphylococcus aureus*, irradiated solutions of both sugars (glucose and fructose) inhibited the growth of this microorganism equally strongly, causing complete elimination of staphylococci immediately after irradiation and leading to a more significant effect (1×10^2 CFU/ml) compared to the impact of pure irradiated water (2.5×10^4 CFU/ml). For *Escherichia coli*. None of the solutions inhibited this bacterium completely when treated immediately after irradiation. The irradiated glucose solution and the solution made from dry irradiated glucose dissolved in non-irradiated water characterized the highest inhibitory effect.

Effect of irradiated solutions on the population of selected strains of microorganisms. The biological effect of non-irradiated starting substances (control) is characterized by a concentration of 1.5×10^8 CFU/mL

№	The name of the solution	Concentration, CFU/mL					
		Staphylococcus aureus		*Escherichia coli (lac+)*		*Pseudomonas aeruginosa*	
		(stage 1)	(stage 2)	(stage 1)	(stage 2)	(stage 1)	(stage 2)
1	The fructose solution is irradiated	NG	1×10^2	3×10^3	1×10^7	NG	NG
2	Glucose solution is irradiated	NG	1×10^2	1×10^3	4×10^3	NG	NG
3	Fructose in irradiated water	2×10^3	1.6×10^4	5×10^7	NG	1.9×10^4	10^4
4	Glucose in irradiated water	NG	1.4×10^4	1×10^8	NG	4×10^3	5×10^5
5	Dry irradiated fructosein non-irradiated water	NG	2.4×10^4	1.1×10^8	1.5×10^3	1×10^5	2×10^5
6	Dry irradiated glucose in non-irradiated water	NG	1×10^4	4×10^3	4×10^3	HP	5×10^1

NG – no growth.

It was also found that the irradiated samples had a more significant inhibitory effect on the *Pseudomonas aeruginosa* strain. It has been shown that irradiated fructose and glucose solutions added to suspensions of the tested pathogenic strain lead to the elimination of these microorganisms immediately and 24 h after irradiation. Dry irradiated glucose in non-irradiated water shows a complete absence of strain growth immediately after irradiation and 5×10^1 CFU/ml 24 h after irradiation. Still, a similar fructose solution does not show this effect.

Thus, it has been found that neutron irradiation of glucose and fructose powders leads to significant changes in the intensities of the mass spectra of molecules, namely, an increase in the intensity of light fragment ions, an increase in the intensity of some peaks, but some fragments become low-intensity. It should be noted that, in general, the number and position of fragment ion peaks in the mass spectra of irradiated and non-irradiated glucose/fructose samples do not change.

The established changes in biological parameters indicate the presence of a selective effect on test microorganisms from different phylogenetic groups. The biological (antibacterial) effects of irradiated solutions and dry substances of glucose and fructose differ significantly in direction and strength of manifestation, depending on the type of microorganism tested, the time after irradiation, and the method of solution preparation.

The work was carried out within the framework of the project supported by the National Research Foundation of Ukraine. State registration number – 2023.04/0083.

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Article received 14.04.2025

МАС-СПЕКТРОМЕТРИЧНІ ТА БІОЛОГІЧНІ ДОСЛІДЖЕННЯ ГЛЮКОЗИ/ФРУКТОЗИ, ОПРОМІНЕНИХ ФОТОНЕЙТРОНАМИ НА МІКРОТРОНІ М-30

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Мас-спектрометричним методом досліджено процеси фрагментації молекул глюкози та фруктози після опромінення нейтронами на мікротроні М-30. Вперше виявлено суттєві зміни виходу іонів-фрагментів після опромінювання. Показано, що взаємодія з нейтронами спричиняє, у першу чергу, розрив важливих вуглецевих зв'язків. Це призводить до зростання інтенсивності виходу низки фрагментів, особливо для m/z=18, 31, 43, як порівняти з неопроміненими.Також представлено часові еволюційні зміни біологічної активності опромінених розчинів та сухих сумішей на прикладі штамів тест-мікроорганізмів *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa.*