USE OF LOW-ENERGY ELECTRON-BEAM IN THE TREATMENT OF SPECIAL FOOD PRODUCTS WITH A HIGH PROTEIN CONTENT

by

Vuk A. GAJIĆ [1], ***Nikola R. MIRKOVIĆ*** [2], ***Ivica T. VUJČIĆ*** [3], ***and Slobodan B. MAŠIĆ*** [3*]

[1] Department of Environment and Sustainable Development, Singidunum University, Belgrade, Serbia
[2]MDMG Invest-Chem, Šabac, Serbia
[3]Department of Radiation Chemistry and Physics, Vinča Institute of Nuclear Sciences, Nationaln Istitute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

Scientific paper
https://doi.org/10.2298/NTRP2202153G

Special high-protein foods suitable for diabetics must be treated to ensure the complete absence of microorganisms and bacteria. It is also important to achieve that this treatment does not change the nutritional value of the product. Among the new decontamination technologies, low-energy electron-beam treatment has proven to be an effective technique for inactivating bacteria with minimal impact on food quality. The paper aims to analyze the influence of low-energy electron-beam irradiation on the microbiological properties and nutritional value of high-protein foods.

Key words: low-energy electron-beam, gamma irradiation, high-protein food, microbiological decontamination, nutritional property

INTRODUCTION

There are about 60 million people with diabetes in Europe [1]. This is about 10.3 % of men and 9.6 % of women aged 25 years and over [2]. According to the Serbian Diabetes Registry, over 710 000 people in Serbia suffer from diabetes [3]. They need a special diet, with the lowest possible carbohydrate content [4]. Also, it is well known that proper and healthy food is a prerequisite for good health.

In co-operation with a local food manufacturer, we are developing high-energy products that would be ideal not only for diabetics but also for athletes, individuals on certain diets, and anyone who cares about health. Some of the high-protein products developed as part of this collaboration are original protein evening bread, protein hamburgers, protein crackers, protein chips, protein biscuits, cocoa cream without added sugar, protein bagels and scones, and protein tortillas and pancakes, protein pizza. These products are produced using innovative methods because they are not made with traditional raw materials, but with specially designed high proteins based on whey. Whey is a by-product of the dairy industry with high nutritional value. It is obtained during the cheese production process. Once the milk passes quality tests, enzymes are added to separate the curds from the liquid whey. The liquid whey is then pasteurized, and the protein is concentrated and isolated [5].

All the products are sugar-free. Firstly, the products were treated with gamma radiation, which guarantees the absolute absence of all microorganisms and harmful substances in said products [6] and significantly extends the shelf-life span [7]. Nevertheless, it has been determined that treatment with ionizing radiation can affect the change in the nutritional values of the product [8]. To avoid changes in nutritional value after irradiation, a low-energy electron-beam (LEEB) was used for the preservation of high-protein products [9]. Recent developments in LEEB technology have revolutionized aseptic packaging [10]. Advancements in electron-beam technology are shrinking the footprint of the devices used to generate ionizing radiation. With the relatively recent development of reliable, compact, cost-effective, LEEB a new class of in-line applications is now possible. The benefits of high-speed, high-efficacy treatments, with no chemicals and at room temperature, are now realized across a variety of packaging applications. Such developments are also attractive to the food industry [11].

The paper aims to analyze the influence of LEEB on the physical and chemical parameters of the preservation of high-protein foods suitable for diabetics.

MATERIALS AND METHODS

Sample collection

Samples of high-protein food (protein crackers and chips, evening protein bread, pizza base, and pancake mix) were collected by the manufacturer and delivered to the Radiation Unit at the Vinča Institute of Nuclear Sciences in Belgrade, Serbia, fig. 1. All these

* Corresponding author; e-mail: slobodan.masic@gmail.com

Figure 1. Samples of the high-protein food

products are low in carbohydrates and high in protein, and all are based on whey protein.

Gamma irradiation

Irradiation was performed in the Radiation Unit, using gamma rays from radioactive isotope ^{60}Co. Radiation doses of 1 kGy, 3 kGy, 5 kGy, 7 kGy, and 10 kGy were used. The average irradiation dose rate was 10 kGy h^{-1}. The delivered radiation dose's accuracy is controlled using the ECB/oscilloscope dosimetric system [12, 13]. Since the oscillotitrator's measurement [14] is highly temperature-dependent [15], all measurement of the absorbed radiation dose was performed at 20 °C. The homogeneity of product irradiation inside the package was verified by dose mapping [16].

The LEEB irradiation

For irradiation of protein product samples with LEEB, the Laatu machine from producer Bühler Group was used. Laatu offers a chemical-free solution with reduced running costs thanks to its low energy consumption and minimal or no product waste. Table 1 shows the specification of the Laatu electron-beam. The irradiation dose was 10 kGy, which is the maximum allowed dose for food irradiation [17].

Microbiological analysis

In the accredited microbiology laboratory, the initial contamination and the number of microorganisms, total molds, and bacteria in the samples were examined. The method used to determine these parameters was Ph. Eur. 7.0 (2.6.12. – Microbiological examination of nonsterile products (total viable aerobic count), and 2.6.13. – Microbiological examination of non-sterile products (test for specified micro-organisms)) [18]. Microbiological analyses were performed before irradiation and after gamma irradiation with different radiation doses. Also, analyses were done after irradiation with LEEB. The used diluent (buffered peptone water) and nutrient media for the development of microorganisms (tryptone soy agar, Rose Bengal agar, iron sulfide agar) are following international standard ISO 11737-1: 2018 [19].

Table 1. Technical specifications of Laatu

Parameter	Value	
Voltage	With supply frequency 50 Hz	400Y/230 VAC*
	With supply frequency 60 Hz	400Y/230 VAC + 460 VAC
Power 30	30 kW	
Product throughput (product dependent)	Up to 1 000 kgh^{-1}	
Air exhaust (depending on installation)	Up to 8 000 m^3	
Ambient temperature	From +5 to +40 °C	
Relative humidity, non-condensing (during operation)	From 10 % to 70 %	

Nutritional properties

The samples of high protein products were analyzed to determine their content of total fat, protein, carbohydrates, sugars, and dietary fiber after gamma and LEEB irradiation. These analyses were performed before irradiation and after gamma irradiation with the highest used radiation dose of 10 kGy, and after LEEB irradiation with a dose of 10 kGy.

Determination of total fat content in high protein products

Determination of total fat content in the high protein products samples was performed by Weibull-Stoldt-Standard application [20]. The sample was hydrolyzed using the Hydrolysis Unit E-416. The Soxhlet extraction was performed with the Extraction Unit E-816. The calculation of the samples' total fat was realized by the gravimetric method after the extract has dried to reach permanent weight. This application was following official methods (EN 98/64/EG, AOAC 963.15, §64 /§35 06.00-6).

Determination of carbohydrates and sugars content in high protein products

Determination of total carbohydrates and sugars present in high protein products was performed using the phenol sulphuric acid method [21]. This method is often used to determine the carbohydrate content of food [22]. The method is based on the dehydration of glucose to hydroxymethyl furfural in a hot acidic medium. A yellow product with phenol is formed, which has a maximum absorption at 490 nm [23, 24].

Determination of dietary fiber in high protein products

An enzymatic-gravimetric method was used to determine the content of dietary fiber in high protein product samples. The samples were degreased and then treated with enzymes that mimic the process of digestion in the human small intestine. Digestible carbohydrates were then broken down into simple sugars, which were removed by precipitation and filtration. After that, only dietary fiber remains in the sample [25].

Determination of protein content

Protein content before and after irradiation was determined using a standard ISO procedure, ISO 1871:2009 [26]. This standard provides general guidelines for the determination of nitrogen by the Kjeldahl method. The standard applies to food and feed products containing nitrogen compounds that can be directly determined by the Kjeldahl method [27].

RESULTS AND DISCUSSION

Effects of gamma irradiation on microbiological properties

To eliminate microorganisms, total molds, and bacteria from special high-protein products, samples were treated with different doses of gamma irradiation, from 1 kGy to 10 kGy. Table 2 shows the microbiological results after treatment with different radiation doses.

From tab. 2, one can see that the radiation dose of 10 kGy is enough to eliminate the total number of microorganisms and molds below the permitted limit. On the other hand, a treatment of 3 kGy is enough to remove all bacteria from the sample.

Effect of gamma irradiation on nutritional values of samples

To determine the influence of gamma radiation on the nutritional properties of the samples, an analysis of the nutritional values was performed in the non-irradiated sample and the sample irradiated with the highest used dose of 10 kGy which guarantees the complete absence of microorganisms. The data are presented in tab. 3.

From tab. 3, it has been determined that treatment with ionizing radiation can affect the change in the nutritional values of the product. A decrease in fat content could be due to the action of high-energy radiation on lipid molecules causing lipid peroxidation [28]. The biggest problem is that the proportion of carbohydrates increases and the proportion of protein decreases after exposure to gamma radiation at a dose rate of 10 kGyh^{-1}. An increase in carbohydrate content was due to the breakdown of oligosaccharides when samples were irradiated [29].

A decrease in protein content with a gradually higher irradiation dose is because of a high rate of metabolic activities [30].

The measurement uncertainty was calculated based on 5 separate measurements.

Figure 2 shows the changes in the nutritional value of the product *Protein evening bread* depending on the radiation dose to which the samples were exposed.

Table 3. Nutritional values of samples before irradiation and after 10 kGy irradiation

Parameter	Non-irradiated	Irradiated with a dose of 10 kGy	Measurement uncertainty [%]
Total fat [%]	14.5	9.2	3.0
Carbohydrate [%]	31.3	32.0	5.8
Sugars [%]	3.9	4.0	5.8
Dietary fiber [%]	17.9	18.0	2.5
Protein [%]	40.0	41.3	4.0

Table 2. Total number of microorganisms, the total number of molds and different bacteria before and after the influence of different doses of gamma irradiation

Parameter	Dose [kGy]						Permissible value
	0	1	3	5	7	10	
Total number of microorganisms (cfu g^{-1})*	$5.6\cdot10^7$	$2.5\cdot10^7$	$4\cdot10^4$	$8\cdot10^3$	$2\cdot10^3$	< 1000	< 1000
Total number of molds (cfu g^{-1})	$5.4\cdot10^4$	$3.5\cdot10^4$	$5.6\cdot10^3$	130	< 100	< 100	< 100
Salmonella sp. (cfu g^{-1})	not present	not present	not present	not present	not present	not present	must not contain
E. coli (cfu g^{-1})	350	300	20	not present	not present	not present	must not contain
Staphylococcus aureus (cfu g^{-1})	150	90	15	not present	not present	not present	must not contain
Pseudomonas aeruginosa (cfu g^{-1})	not present	not present	not present	not present	not present	not present	must not contain
Bacillus cereus (cfu g^{-1})	5500	2100	300	<100	<100	<100	<100
Sulfite-reducing clostridia (cfu g^{-1})	10	not present	not present	not present	not present	not present	must not contain

*cfu – colony-forming unit

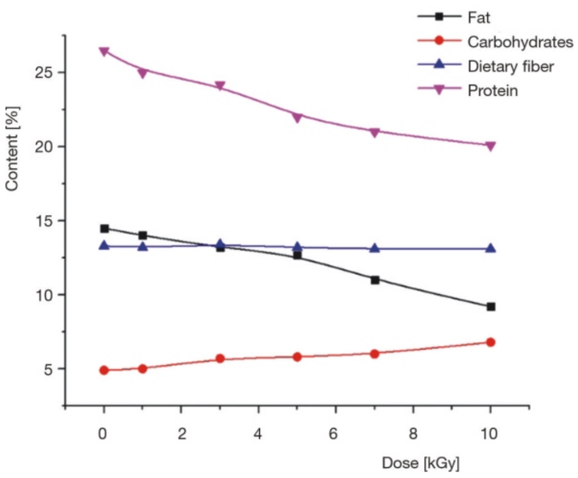

Figure 2. Changes in the nutritional value of the product *Protein evening bread*, depending on the radiation dose of gamma irradiation

Effect of LEEB irradiation on microbiological properties and nutritional values of samples

To avoid changes in nutritional value after irradiation, LEEB was used for the preservation of high-protein products.

The use of LEEB has advantages over the use of gamma rays or higher-energy electrons for the direct irradiation of food. These advantages arise from details of the interaction processes which are responsible for the production of physical, chemical, and biological effects. The main differences which favor the use of LEEB are depth of penetration, dose distribution, irradiation geometry, and costs.

Table 4 shows the microbiology and nutritional values of high-protein bread before irradiation and after LEEB treatment with a dose of 10 kGy.

Irradiation of the product surface with a LEEB appeared as a possible ideal solution. Such a treatment would neutralize the microorganisms. Microorganisms are located on the surface of the product and are formed mainly during the handling of the product. On the other hand, the change in the nutritional values of the product under the influence of high-energy ionizing radiation would be avoided.

Table 4. Microbiological properties and nutritional values of high-protein bread before irradiation and after LEEB treatment

Parameter	Non-irradiated	Irradiated with LEEB (10 kGy)
Microbiological properties		
Total number of microorganisms	52000 cfu g^{-1}	0
Molds	420 cfu g^{-1}	0
Nutritional values		
Fat	14.5 [%]	14.3 [%]
Carbohydrates	4.9 [%]	5.0 [%]
Sugars	1.6 [%]	1.6 [%]
Dietary fiber	13.3 [%]	13.3 [%]
Protein	26.5 [%]	26.4 [%]

CONCLUSIONS

Advancements in electron-beam technology are shrinking the footprint of the devices used to generate ionizing radiation. With the relatively recent development of reliable, compact, cost-effective LEEB, a new class of in-line applications is now possible. The benefits of high-speed, high-efficacy treatments, with no chemicals, and at room temperature, are now realized across a variety of packaging applications. Such developments are also attractive to the food industry.

The use of LEEB in the treatment of special high-protein products for diabetics has shown great potential for further development and application. A dose of 10 kGy proved to be sufficient for complete sterilization of the tested products in both cases, with gamma radiation, and with LEEB treatment. However, while with gamma radiation there are significant changes in nutritional values (–37 %, +3 %, +3 % for total fat, carbohydrates, and protein respectively), with LEEB treatment these changes are negligible.

ACKNOWLEDGMENT

The research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia and the IAEA.

AUTHORS' CONTRIBUTIONS

Sample collections and measurements were performed by V. A. Gajić and N. R. Mirković. The measurement setup was conceived and prepared by S. B. Mašić. The theoretical analysis was carried out by I. T. Vujčić and S. B. Mašić. All authors analyzed and discussed the results and reviewed the manuscript.

REFERENCES

[1] ***, Europe Diabetes report 2000 – 2045 (2022), IDF Diabetes Atlas, Brussels, Belgium

[2] Lopez Sanchez, F. G., *et al.*, Gender Differences in the Association Between Cataract and Mental Health in Adults with Diabetes, A Cross-Sectional Analysis from the Spanish National Health Survey 2017, Front. Public Health, 2021, London, UK

[3] ***, Incidence and Mortality of Diabetes in Serbia, Institute of Public Health of Serbia Dr Milan Jovanovic Batut, Belgrade, Serbia, 2021

[4] Tay, J., *et al.*, Nutritional Adequacy of Very Low- and High-Carbohydrate, Low Saturated Fat Diets in Adults with Type 2 Diabetes: A Secondary Analysis of a 2-Year Randomised Controlled Trial, *Diabetes Research and Clinical Practice,* (2020), Oct., 108501

[5] Ozel, B., *et al*., Challenges in Dried Whey Powder Production: Quality Problems, *Food Research International, 160* (2022), Oct., 111682

[6] Mazzuca, C., *et al*., A New Analytical Approach to Characterize the Effect of -Ray Sterilization on Wood, *Microchemical Journal, 143* (2018), Aug., pp. 493-502

[7] Francesco, E. R., *et al.*, X-Ray Irradiation as a Valid Technique to Prolong Food Shelf Life: the Case of Ricotta Cheese, *International Dairy Journal, 99* (2019), Dec., 104547

[8] Robichaud, V., *et al.*, Effect of É£-Irradiation on the Microbial Inactivation, Nutritional Value, and Antioxidant Activities of Infant Formula, *Lebensmittel-Wissenschaft und-Technologie, 125* (2020), Feb., 109211

[9] Aisala, H., *et al*., Comparison of Low Energy and High Energy Electron Beam Treatments on Sensory and Chemical Properties of Seeds, *Food Research International, 148* (2021), Jul., 110575

[10] Pillai, S., Pillai, E., Agriculture: Electron Beam Irradiation Technology Applications in the Food Industry, *Encyclopedia of Nuclear Energy,* (2021), Feb., pp. 313-329

[11] Schottroff, F., *et al*., Decontamination of Herbs and Spices by Gamma Irradiation and Low-Energy Electron Beam Treatments and Influence on Product Characteristics Upon Storage, *Journal of Radiation Research and Applied Sciences, 14* (2021), 1, Dec., pp. 380-395

[12] ***, International Organization for Standardization (2009) ISO/ASTM 51538 – Practice for Use of the Ethanol-Chlorobenzene Dosimetry System 2009, Geneva, Switzerland

[13] Kovacs, A, *et al*., Evaluation Methods of the Ethanol – Monochlorobenzene Dosimeter System, *Proceedings*, *6th Tihany Symposium on Radiation Chemistry, 2* (1987), pp. 701-709

[14] Kovacs, A, *et al*., Oscillometric and Conductometric Analysis of Aqueous and Organic Dosimeter Solutions, *Radiation Physics and Chemistry, 46* (1995), 4-6, pp. 1211-1215

[15] Vujčić, I, *et al*., Accuracy of Determining Absorbed Irradiation Dose at Different Temperature Measurements Using Ethanol-Chlorobenzene Oscillotitrator system, *Nucl Technol Radiat, 33* (2018), 4, pp. 363-368

[16] Rankovic, B. M., *et al*., Dose Mapping of Products with Different Density, Irradiated in 60Co Irradiation Facility of the Vinca Institute, Serbia, *Nucl Technol Radiat, 35* (2020), 1, pp. 56-63

[17] Morehouse, K., Komolprasert, V., Overview of Irradiation of Food and Packaging, ACS Symposium Series 875, Irradiation of Food and Packaging, 2004, Chapter 1, pp. 1-11

[18] ***, European Directorate for the Quality of Medicines & HealthCare, European Pharmacopoeia 7.0, 2011

[19] ***, International Organization for Standardization (2018) ISO 11737-1:2018 Sterilization of Health Care Products – Microbiological Methods – Part 1: Determination of a Population of Microorganisms on Products

[20] ***, Hydrolysis Unit E-416, Extraction Unit E-816 Soxhlet (2007), Fat Determination According to Weibull-Stoldt – Standard application, https://www.buchi.com/en/knowledge/applications/total-fat-determination-meat-products-according-weibull-stoldt-method

[21] Masuko, T., *et al*., Carbohydrate Analysis by a Phenol-Sulfuric Acid Method in Microplate Format, *Anal. Biochem., 339* (2005), 1, pp. 69-72

[22] Roberts, R., Elias R., Determination of Carbohydrate Using Phenol Sulphuric Acid Method, Food Analysis (4thEd), S. Nielson (ed.): Springer, New York, USA, 2011

[23] Sadasivam, S., Manickam, A., Phenol Sulphuric Acid Method for Total Carbohydrate, Biochemical Methods, New Age International (P) Ltd. New Delhi., 2005, p.10

[24] Agrawal, N., *et al*., Estimation of Total Carbohydrate in Flour of Different Types of Grain, International Journal of Researches In Biosciences, *Agriculture and Technology, II* (2014), 3, pp. 36-40

[25] Prosky, L., *et al*., Determination of Total Dietary Fiber in Foods and Food Products: Collaborative Study, *Journal of AOAC INTERNATIONAL, 68* (1985), 4, pp. 677-679

[26] ***, International Organization for Standardization (2009), ISO 1871:2009 Food and Feed Products – General Guidelines for the Determination of Nitrogen by the Kjeldahl Method

[27] Kjeldahl, J., Neue Methode zur Bestimmung des Stickstoffs in Organischen Körpern (New method for the Determination of Nitrogen in Organic Substances), *Zeitschrift für analytische Chemie, 22* (1883), 1, pp. 366-383

[28] Khan, Q., *et al*., Effect of Gamma Irradiation on Nutrients and Shelf Life of Peach (Prunus persica) Stored at Ambient Temperature, The Open Conference Proceedings Journal (Biological Sciences, Chemical Sciences, Physical Sciences, Medicine, Engineering & Technology), ISSN: 2210-2892 – 10, 2020

[29] Kumar Dixit, A., *et al*., Effect of Gamma Irradiation on Lipoxygenases, *Trypsin Inhibitor, Raffinose Family Oligosaccharides and Nutritional Factors of Different Seed Coat Colored Soybean (Glycine max L.)., Radiation Physics and Chemistry, 80* (2011), 4, pp. 597-603

[30] Yau-Hoong, K., *et al*., Radiation Processing Of Food Proteins – A Review on the Recent Developments, *Trends in Food Science & Technology, 30* (2013), 2, pp. 105-120

Received on July 27, 2022
Accepted on September 15, 2022

УПОТРЕБА НИСКОЕНЕРГЕТСКОГ ЕЛЕКТРОНСКОГ СНОПА ЗРАЧЕЊА У ТРЕТМАНУ СПЕЦИЈАЛНИХ ПРЕХРАМБЕНИХ ПРОИЗВОДА СА ВИСОКИМ САДРЖАЈОМ ПРОТЕИНА

Вук А. ГАЈИЋ, Никола Р. МИРКОВИЋ, Ивица Т. ВУЈЧИЋ, Слободан Б. МАШИЋ

Посебна високопротеинска храна погодна за дијабетичаре мора бити третирана на начин да се осигура потпуно одсуство микроорганизама и бактерија. Такође је важно постићи да овај третман не утиче на промену нутритивних вредност производа. Међу новим технологијама деконтаминације, употреба нискоенергетског електронског снопа зрачења показала се као ефикасна техника за инактивацију бактерија са минималним утицајем на квалитет хране. Циљ рада је анализа утицаја нискоенергетског електронског снопа зрачења на микробиолошка својства и нутритивну вредност високопротеинских намирница.

Кључне речи: нискоенергетски електронски сноп, гама зрачење, високопротеинска храна, микробиолошка деконтаминација, нутритивно својство