The influence of ionizing radiation on free protein content and microorganisms’ growth in oyster mushrooms

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Abstract. The article describes the relevance of finding new protein sources with high nutritional and biological value. For solving this problem, several methods of protein quality and digestibility analysis (PDCAAS, DIAAS etc.) in world practice are considered. The experimental researches included analysis of protein content and ratio of molecular masses of polypeptides depending on doses of ionizing radiation (3 kGy, 6 kGy and 9 kGy) of fresh and heat-treated oyster mushrooms (Pleurotus ostreatus) samples. It is defined that the protein content in heat-treated oyster mushrooms is significantly higher than in fresh mushrooms, so ionizing radiation can increase the biological value of mushroom free proteins and affect at molecular structure of product, its structural and mechanical properties through the destruction of proteins and their decomposition into amino acids. Ionizing radiation is also an instrument for mushroom products shelf life prolongation up to 21 days, which prevents microorganism vital activity and growth.

1. Introduction

One of the most actual global problems are climate change and unsustainable nutrition. They lead to such deplorable results as changes in national food systems, food security threats, irrational consumption of food resources. About one billion of people in the world are underfed. On the other hand, the same amount of people is overfed.

To achieve the goals of sustainable development by hunger elimination and worldwide protection from climate change effects, the radical transformations of food systems are required. One of the main tasks in this field is determining of future protein sources. Very often this problem is considered as a forecasted sharp increase in animal protein demand due to the growth of peoples’ incomes and population. In turn, the growth of animal protein production is high-risk and leads to further climate changes, since it’s a very resource-intensive process. Thus, the problem of protein deficiency is very important.

The insufficiency of animal protein is caused not so much by its quantitative lack, as by its quality, i.e. by the essential amino acids content, which is distinguished from amino acid content in vegetable proteins. It is known, that processed plant raw materials can be the rich source of proteins [1]. From this point of view, the actual problem is obtaining of easy-to-digest protein from mushrooms, as well as their cultivation with further processing into food production. Using of modern technologies, such as ionizing
radiation, allows to increase the amount of free protein and reduce microorganisms content in obtained production.

2. The formulation of the problem

According to the Liebig’s law of minimum, the functioning of living organisms is determined by the indispensable substance that is presented in the smallest quantity and considered as limiting. It should be noted that the product (or diet) must contain a strictly defined norm of food nutrients (vitamins, macro-, microelements and amino acids). When the element content is below or above the acceptable rate, it indicates that the food product (or dish) is imbalanced. Each food product should contain enough essential amino acids in optimal concentrations and ratios for the normal course of protein synthesis processes in human organism. The dependence of the organism functioning on the number of essential amino acids is used in determining of proteins’ biological value. Even the lack of one essential amino acid in food leads to incomplete digestion of others.

In world practice, the quality indicators and biological value are also widely used for evaluation and analytical procedures in comparing various sources of food protein. In 1993, PDCAAS was adopted as the preferred method for determining protein quality by the United States Food and Drug Administration (FDA) and the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) [2]. PDCAAS protein digestibility-corrected amino acid score is a method of the protein quality assessing, which is based on the human needs in amino acids and their digestibility.

In March 2013, a new definition method, the digestible indispensable amino acid score (DIAAS), was proposed to replace the current FAO protein ranking standard (PDCAAS). Unlike PDCAAS, which was based on the assessment of determined throughout the digestive tract raw protein digestibility, DIAAS determines the digestibility of amino acids at the end of the small intestine, which provides a more accurate estimating of amino acids amount digested in the organism [3].

Thus, the diversity of protein quality assessments confirms only that the value of the protein for the human organism is determined by following main parameters:

- balance on the essential amino acids content;
- correlation with the FAO reference protein;
- exchange efficiency, availability of digestion and utilization of the protein by human organism.

The protein digestibility depends both on the amount/ratio of amino acids and on the chemical composition of their sources (food products). Thus, both proteins with different amino acid profiles and products with different chemical composition will be assimilated in human organism differently [4, 5]. The progress in protein balance assessment systems has led to the development of a whole complex of mathematical dependencies reflecting individual qualitative assessments of the multi-component foods nutrient balance [2–4]. These dependencies are taken as the basis for modeling products based on cultivated mushrooms and mycoprotein.

Mycoprotein is widely spread and has high consumer rating [6–9]. Mycoprotein is a form of unicellular protein sold in Europe and North America, as quorn produced from Fusarium venenatum [10]. In the 1960s, the Rank Hovis McDougall company (Great Britain) identified F. venenatum as a potential source of protein. F. venenatum was one of more than 3000 fungi species tested over a three-year period in terms of low costs, nutrition and taste. The fungus is grown in vats using glucose syrup as a nutrition basis. Today, F. venenatum is the only source of mycoproteins that can produce high percentage of protein biomass and is the most thoroughly tested food product on the European market for more than 12 years.

Among the most perspective objects of modern biotechnology, a special place is given to mycelial mushroom producers, which are the basis for obtaining a wide range of biopharmaceutical preparations, food additives and healthy food products. However, the use of mycoprotein on Russian market is still not justified in terms of consumer readiness, because products based on grown biomass are perceived by most buyers negatively. The mentality of the local consumers preferably refers to the possibility of consumption of cultivated mushrooms (such as oyster mushroom, champignons, honey agaric, shiitake) both in natural and in processed forms. All these factors define the solution of two main tasks:
development of processing technology and formulations of semi-finished products from cultivated mushrooms;

optimization of methods and technologies for cultivated mushrooms processing in order to improve the quality of the contained protein based on optimization of the amino acid profile and chemical composition of developed food products.

Today, 69 countries process more than 80 types of food products, which are treated with electron beam sterilization. The most perspective are technologies based on electronic, ionizing and $\gamma$-radiation. These methods are more environmentally friendly than chemical treatment. For many types of products, optimal irradiation modes have been determined, long-term studies of their suitability and safety of use have been conducted, and irradiation equipment has been developed. It has been proven that irradiating any food products with doses of up to 10 kGy is advisable, because it doesn’t cause toxic effects on them [11]. Higher doses can cause destruction of irradiated materials [12]. It is also known that ionizing radiation can decrease microbiological contamination and eliminate pathogenic microorganisms contained in food products [13, 14].

For fresh oyster mushrooms pretreatment, the ionizing irradiation method is proposed. It has several advantages, such as:

- increasing of product shelf life;
- the possibility of using vacuum packages for processing product in them;
- decrease of product losses;
- long-term preservation of products’ properties;
- lack of chemicals use;
- structural and mechanical changes in the product.

Cold sterilization of semi-finished products for improving the products safety is a part of the technological process. This method allows to increase the biological activity of nutrients contained in products.

The recommended dose of ionizing radiation for extending the shelf life of fresh mushrooms in several countries (Argentina, China, Croatia, Hungary, Israel, Korea, Mexico, Poland and the United Kingdom) is 1…3 kGy, and the recommended dose for disinfecting dried mushrooms used as seasonings, is 10…50 kGy. With electron beam sterilization doses of 6…10 kGy, the following changes in oyster mushrooms are expected:

- increased amino acid activity;
- increased activity of vitamins D, E, B-group;
- destruction of glucose to $\beta$-glucans and $D_3$-glucosides [10, 11].

Since the cell walls of oyster mushrooms contain chitin, which does not decompose in the gastrointestinal tract, mushrooms must be treated with radiation to free the cells contents maximally. Even low doses of radiation affect changes in product molecular structure and its structural and mechanical properties, which cause amino acids breakdown and decomposition. Using the ionizing radiation in mushroom semi-finished products obtaining improves the digestibility of protein substances of fungi, represented by complex and hardly soluble structural compounds, and it also improves the solubility of ready product.

It has been proven that $\gamma$-irradiation and electron beam irradiation are advisable for extending the shelf life of fresh mushrooms. $\gamma$-irradiation inhibited the opening of the cap and darkening of mushrooms’ fruiting body, while the microbial contamination level of mushrooms was reduced [15–17].

According to the problem situation, the purpose of the work was the researching of electronic ionizing radiation influence on free protein content and microorganisms’ growth in fresh and heat-treated oyster mushrooms.
3. Materials and methods

3.1. The organization of the experiment
Fresh oyster mushrooms (Pleurotus ostreatus) were purchased from commercial production enterprise Lukoshko, LLC based in Novosibirsk region, Maslyanino village. The following samples from the mushrooms were obtained:

- sample № 1 – fresh oyster mushrooms, not irradiated (control sample);
- sample № 2 – fresh oyster mushrooms irradiated with a dose of 3 kGy;
- sample № 3 – fresh oyster mushrooms irradiated with a dose of 6 kGy;
- sample № 4 – fresh oyster mushrooms irradiated with a dose of 9 kGy;
- sample № 5 – heat-treated oyster mushrooms, not irradiated (control sample);
- sample № 6 – heat-treated oyster mushrooms irradiated with a dose of 3 kGy;
- sample № 7 – heat-treated oyster mushrooms irradiated with a dose of 6 kGy;
- sample № 8 – heat-treated oyster mushrooms irradiated with a dose of 9 kGy;

For obtaining of samples №№ 1–4, fresh oyster mushrooms were preprocessed, washed, chopped on Robot Coupe R2 cutter and packed into vacuum packages using JEJU JDZ-260/PD vacuuming machine.

For obtaining of samples №№ 5–8, fresh oyster mushrooms were preprocessed, washed, chopped on Robot Coupe R2 cutter, heat-treated in A0S061EAA1 combi oven, cooled and then packed into vacuum packages using JEJU JDZ-260/PD vacuuming machine.

Samples №№ 2, 3, 4, 6, 7, 8 were irradiated in pulsed linear electron accelerator ILU-10 (developed by Budker Institute of Nuclear Physics, Novosibirsk).

After that, all samples were kept in the cooling cabinet at 0…6 °C.

Protein content analysis was held after 1 day of samples’ shelf life, microbiological evaluation – after 1, 7, 14 and 21 days of shelf life.

3.2. Protein content and microbiological research
The analysis on protein content was performed by the Bradford method. Protein mixtures in extracts, their content and ratio of molecular masses of polypeptides extracted into the solution were compared using electrophoresis method in polyacrylamide gel according to Laemmlli method [18].

Qualitative and quantitative amino acid content in fresh and heat-treated oyster mushrooms was determined using amino acid analyzer LA8080 (AminoSAAYA) Hitachi Hi Tech.

The microbiological indices were determined according to Russian national standards GOST 10444.15-94 “Food products. Methods for determination of quantity of mesophilic aerobes and facultative anaerobes”, GOST 10444.12-2013 “Microbiology of food and animal feeding stuffs. Methods for the detection and colony count of yeasts and moulds” and GOST 31747-2012 “Food products. Methods for detection and quantity determination of coliformes” according to the requirements of “Technical regulation of Customs union on the safety of food production” (TR TS 021/2011): not more than 1.0×10^4 CFU/g for mesophilic aerobes and facultative anaerobes, not more than 1×10^2 CFU/g for yeast and moulds, coliforms are not allowed in 1 g of product.

4. Results and discussion

4.1. Protein content analysis
The results of the protein content analysis in the samples are shown in figure 1.

According to the obtained data, it can be concluded that the protein content in heat-treated oyster mushrooms is significantly higher than in fresh mushrooms. More protein is transferred to mushrooms extract, which apparently may indicate a partial destruction of cells and cell walls during the heat treatment process.
Figure 1. Protein content in fresh and heat-treated irradiated oyster mushroom samples depending on the dose of ionizing radiation

The results of definition of protein mixtures in extracts, their content and ratio of molecular masses of polypeptides extracted into the solution are presented in figure 2. In the figure, line 1 shows control protein sample (bovine serum albumin, molecular weight 60kDa), line 6 – reference proteins (molecular weights 94.6 kDa, 60.0 kDa, 45.0 kDa, 31.0 kDa, 21.5 kDa and 14.4 kDa), lines 2, 3, 4, 5 – extracts from raw mushrooms (from left to right – samples №№ 1, 2, 3, 4), lines 7, 8, 9, 10 – extracts from heat-treated mushrooms (from left to right – samples №№ 5, 6, 7, 8).

Figure 2. Electropherogram of polypeptides extracts in oyster mushrooms samples

It is seen from the figure, that there are no significant changes in molecular masses of proteins and polypeptides in mushroom extracts after their irradiation, what can be explained by low irradiation doses. At the same time, the samples №№ 1–4 and №№ 5–8 differ from each other by polypeptide content. The samples №№ 5–8 show significant number of polypeptides with molecular weights 14.4 kDa and less and 45.0…50.0 kDa among with decrease of polypeptides with molecular weights 55.0…60.0 kDa.

The irradiated mushroom proteins are exposed to direct and indirect effects of ionizing radiation. When protein macromolecules are in a liquid solution state, direct effects can be neglected, while indirect effects predominate. However, proteins in solid state are ionized mainly by the direct interaction. During irradiation, the generation of primary water-free radicals (hydrated electron, hydrogen atom and hydroxyl radical) reacts very efficiently with proteins. The splitting and aggregation of proteins, which occur during irradiation, are associated with impaired secondary and tertiary protein structures that cause reactive groups for the action of free radicals (hydrated electrons, hydrogen atoms and hydroxyl radicals) as a result of water radiolysis (figure 3).
Radiation + $\text{O}_2$ $\rightarrow$ $\text{HO}^\bullet + \text{e}^-_{\text{aq}} + \text{H}^\bullet + \text{H}_2 + \text{HO} - \text{OH} + \text{H}_3\text{O}^+$

**Figure 3.** Water radiolysis

Therefore, various forms of protein modifications are expected, for example, polymerization (dimerization) and fragmentation. It is assumed that these reactions are the main mechanism underlying the physico-chemical changes in protein products. Various factors, such as protein structure and state and the conditions of irradiation (for example, dose, dose rate, temperature, presence of oxygen), affect chemical reactions that occur during protein irradiation.

Irradiation causes the coagulation of peptide chains, intramolecular disulphide chains, and secondary binding forces (such as hydrogen bonds, hydrophobic bonds, ionic bonds, or bonds that contain several subunits together as a functional protein). The irradiation mechanism includes a series of reactions that largely lead to the formation of ionic and free radical intermediates and ultimately creates stable products.

Irradiation of proteins can lead to certain permanent changes, such as deamination, decarboxylation, reduction of disulphide bonds, oxidation of sulphhydryl groups, modification of amino acid residues, valent change of coordinated metal ions, cleavage of the peptide chain and aggregation.

The results of analysis of essential amino acids content in heat-treated oyster mushroom protein (sample № 5) are shown in table 1. Changes of essential amino acids content in samples №№ 5, 6, 7, 8 are shown in table 2.

| Table 1. Content of essential amino acids in heat-treated oyster mushroom protein in comparison with FAO reference protein |
|---------------------------------------------------------------|
| **Amino acids** | **Amino acid content, g/100 g of raw protein** | **Amino acid content, g/100 g of FAO reference protein** | **Amino acid score, % to FAO reference protein** |
|-----------------|-----------------------------------------------|------------------------------------------------|-----------------------------------------------|
| Isoleucine      | 2.3±0.04                                      | 4.0                                            | 57.5                                          |
| Leucine         | 2.9±0.03                                      | 7.0                                            | 41.4                                          |
| Lysine          | 7.1±0.03                                      | 5.5                                            | 129.1                                         |
| Tryptophan      | 1.3±0.02                                      | 1.0                                            | 130.1                                         |
| Threonine       | 3.1±0.04                                      | 4.0                                            | 77.5                                          |
| Valine          | 2.9±0.03                                      | 5.0                                            | 58.0                                          |
| Methionine + cystine | 3.4±0.04                                        | 3.5                                            | 97.1                                          |
| Phenylalanine + tyrosine | 5.2±0.03                                          | 6.0                                            | 86.7                                          |

| Table 2. Content of essential amino acids in heat-treated oyster mushrooms depending on irradiation dose |
|---------------------------------------------------------------|
| **Amino acids** | **Amino acid content, g/100 g of raw protein** | **Sample № 5** | **Sample № 6** | **Sample № 7** | **Sample № 8** |
|-----------------|-----------------------------------------------|----------------|----------------|----------------|----------------|
| Isoleucine      | 2.3±0.04                                      | 2.5±0.02       | 2.7±0.02       | 2.5±0.03       |
| Leucine         | 2.9±0.03                                      | 3.0±0.02       | 3.3±0.03       | 3.1±0.02       |
| Lysine          | 7.1±0.03                                      | 7.1±0.02       | 7.3±0.02       | 7.2±0.03       |
| Tryptophan      | 1.3±0.02                                      | 1.4±0.02       | 1.6±0.03       | 1.4±0.02       |
| Threonine       | 3.1±0.04                                      | 3.3±0.02       | 3.5±0.02       | 3.3±0.02       |
| Valine          | 2.9±0.03                                      | 3.0±0.02       | 3.0±0.02       | 2.9±0.03       |
| Methionine + cystine | 3.4±0.04                                          | 3.5±0.02       | 3.5±0.02       | 3.4±0.02       |
| Phenylalanine + tyrosine | 5.2±0.03                                          | 5.3±0.02       | 5.5±0.02       | 5.2±0.02       |
According to obtained data and performed calculations, the oyster mushroom protein is limited mainly on leucine (41.4%), isoleucine (57.5%) and valine (58.0%). At the same time, it is rich in tryptophan (130.1%) and lysine (129.1%). At the irradiation doses up to 6 kGy there amino acid content in oyster mushrooms increases, and at 9 kGy decreases.

### 4.2. Microbiological research

The results of microbiological researches are presented in tables 3 and 4.

**Table 3.** Quantity of mesophilic aerobic and facultative anaerobic bacteria (QMAFAnM) in oyster mushroom samples

| Sample | 1 day of shelf life | 7 days of shelf life | 14 days of shelf life | 21 day of shelf life |
|--------|----------------------|----------------------|-----------------------|---------------------|
| № 1    | 1.2×10^2             | 9.6×10^2             | 5.4×10^2              | 2.2×10^4            |
| № 2    | 2.7×10               | 8.2×10               | 1.6×10^2              | 1.1×10^3            |
| № 3    | less than 10         | less than 10         | less than 10          | less than 10        |
| № 4    | not found            | not found            | less than 10          | less than 10        |
| № 5    | 5.4×10               | 3.8×10^2             | 1.6×10^3              | 1.1×10^4            |
| № 6    | 1.8×10               | 4.2×10               | 8.3×10                | 1.5×10^3            |
| № 7    | less than 10         | less than 10         | less than 10          | less than 10        |
| № 8    | not found            | not found            | less than 10          | less than 10        |

**Table 4.** Quantity of yeast and moulds in oyster mushroom samples

| Sample | 1 day of shelf life | 7 days of shelf life | 14 days of shelf life | 21 day of shelf life |
|--------|----------------------|----------------------|-----------------------|---------------------|
| № 1    | 4.2×10^1             | 6.2×10               | 2.3×10^2              | 4.4×10^2            |
| № 2    | 1.7×10               | 3.4×10               | 6.1×10                | 8.9×10              |
| № 3    | less than 10         | less than 10         | less than 10          | less than 10        |
| № 4    | not found            | not found            | less than 10          | less than 10        |
| № 5    | 2.5×10               | 5.1×10               | 1.3×10^2              | 2.6×10^2            |
| № 6    | 1.1×10               | 2.8×10               | 5.9×10                | 8.8×10              |
| № 7    | less than 10         | less than 10         | less than 10          | less than 10        |
| № 8    | not found            | not found            | less than 10          | less than 10        |

The obtained microbiological indices show that ionizing radiation influences on microorganisms’ growth. While increasing the irradiation dose from 3 to 9 kGy, the vital functions of microorganisms are significantly lowered during 21 days of irradiated oyster mushrooms’ shelf life.

Therefore, the microbiological indices of samples with 6 kGy irradiation dose don’t exceed allowable boundaries during this period. This boundary in not finite, so the shelf life period of oyster mushroom semi-finished products can be prolonged with increase of irradiation dose up to allowable 10 kGy.

The coliforms were not found in all oyster mushroom samples.

### 5. Conclusion

Ionizing radiation as a part of technological process of oyster mushrooms processing can increase the biological value of mushrooms’ free proteins. Irradiation doses from 3 to 6 kGy affect the molecular structure of product, its structural and mechanical properties. This causes the destruction of proteins and their decomposition into amino acids. Using ionizing radiation while obtaining semi-finished and finished products from cultivated mushrooms increases the digestibility of mushroom proteins, which are complex, sparingly soluble compounds, and improves the solubility of mushroom products.

Ionizing radiation is also an instrument for mushroom products shelf-life prolongation, which prevents microorganism vital activity and growth.
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