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Enhancing food safety of pollen by means of irradiation

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Abstract

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Introduction. Bee pollen is widely used as a food additive, and also in the formulation of food products. Study is conducted to determine the feasibility of decontamination pollen by irradiation.

Materials and methods. In this experiment it was used the pollen from the Carpathian region of Ukraine in accordance with current regulatory documentation of Ukraine. Experimental samples were irradiated with UV rays at a dose of 2-8 kGy, and then analyzed the chemical composition, microbiological safety indicators, and determines the degree of lipid oxidation using the classical methods. All studies were performed with a three-fold repetition. Statistical analysis of the experimental data was carried out using Excel, the confidence level of $P \leq 0,05$.

Results and discussion. Increasing doses of pollen radiation reduces the number of colony forming units of microbial populations. 2 kGy destroy on 45,5% – total aerobic microorganisms, more than 40% - yeast and 41,5% – mold. 4kGy leads to almost complete disappearance of unwanted microflora and remains less than 25% of aerobic microbes from their original number, above 4 kGy reduces the number of viable cells to 99,9%. An increase in dose of pollen treatment from 2 to 8 kGy has no negative effect on the content of the main components of pollen. But at a dose of 2 kGy malondialdehyde increased by 3,35%, and at 4 kGy – at 5,86% in comparison with the control sample. The dose of 2 and 4 kGy reduces of flavonoids availability in tests to 3% and 5 %. These interventions also decrease the β -carotene in tests 1,2% and 2,7%, respectively.

Conclusions. Gamma irradiation provides a number of benefits and sterility assurance. Using this type of treatment pollen in order to increase its level of microbiological safety and the possibility of further usage in the production of fermented drinks it is possible to obtain the level of purity 99,9% and thus, save up to 95±1,5% essential material.

Introduction

Bee pollen (pollen) is widely used as a food additive (CN Patent № CN102106497 (A), Compound combined rape pollen chewable tablets and preparation method thereof, 2011; BG Patent № BG1733 (U1), Biostimulating composition from bee products, 2013; LT Patent № LT5811 (B), Production of the food additive with bee products and blue-green algae, 2012) as a component of medicines [1-3] and cosmetic medicine (CN Patent № CN103893101 (A), Ganoderma lucidum and pollen anti-aging cream, 2011). Also pollen is widely used in the formulation of food products (CN Patent № CN104000086 (A) – Coarse cereal vermicelli cake, 2014; UA Patent № UA35283 (U), Formulation for making coated cooked cakes "bdzhilka" (small bee), 2008), but less frequently as a component of dairy products (UA Patent № UA96209 (C2), Method for making butter with filler, 2011; CN Patent № CN101623033 (A), Liquid dairy product containing honey and bee pollen and production method thereof, 2013; UA Patent № UA72689 (A), Ice-cream "lisnyipodarunok" (forest gift), 2005).

In the practice of Ukrainian dairy industry pollen was not widely used in the production of fermented milk products.

In our previous studies, it was found that pollen stimulates the dairy process [4], has a positive effect on the chemical composition and nutritional value of yogurt [5].

Bee pollen, unlike honey and royal jelly, requires pre-treatment (sterilization) to prevent it from making unwanted microflora in pasteurized milk [6].

Currently, there are a lot of different (thermal, chemical, electrical, radiation) sterilization of food raw materials, as well as a combination of techniques developed with various combinations of the above mentioned methods. [7].

Heat treatment of pollen more effective at 40 °C. In this mode it possible to achieve the desired moisture content of raw 3 ± 1%, and thus to preserve a biologically active substance [8]. But this method does not provide an adequate level of microbiological purity of raw materials and in the future it will have a negative impact on the quality of prototypes yogurt [6].

Influence of chemical disinfectants is potentially dangerous because they can change the physical, chemical and biological properties of the treated pollen [9].

History of radiation processing of food products has more than 60 years [10]. Food and Agriculture Organization (FAO UNO) and the World Health Organization (WHO) approved the use of ionizing radiation for the treatment of food with the purpose of sterilization and preservation of radiation [11, 12].

It was obtained important knowledge concerning the future usage of ionizing radiation, for example, for inhibiting sprouting in potatoes and onions while storing, prolongation of storing meat and fish in a frozen state, disinfestations of grain and vegetables, sterilized meat and meat products for the purpose of storage in the unfrozen state, etc. Food irradiation is permitted in more than 60 countries [11].

Depending on the intensity of the radiation treatment in order to sterilize IAEA proposed technical terms [13] as follows:

- 4 to 6 kGy - radiation processing in order to suppress selectively a specific type of microorganism (e.g., Salmonella, Trichinella and etc.);
- 6 to 10 kGy - radiation processing of food products in order to increase the duration of storage, in doses that lead to the suppression of limited human-pathogenic microorganisms;
- 10 to 50 kGy is carried out for industrial sterilization of foods under conditions precluding the repetition of infection by microorganisms.

General standard for Irradiated Foods (CAC/RCP 19-1979, Rev. 2-2003) indicate that for radiation processing of food products it is permitted to apply the installation with the following types of ionizing radiation [12]:

- a. Gamma rays from the radionuclides ^{60}Co or ^{137}Cs ;
- b. X-rays generated from machine sources operated at or below an energy level of 5 MeV;
- c. Electrons generated from machine sources operated at or below an energy level of 10 MeV.

For many types of products it was organized optimal regimes of radiation treatment, conducted the long-term studies in order to indicate their suitability and safety of usage, and created radiation equipment [11, 12]. For example, in Poltava University of Economics and Trade it was demonstrated the results of bulk food ultraviolet radiation, indicated the advantages of UV radiation over other methods, proposed the method and equipment for microbicidal decontamination of powdery products, made necessary calculations associated with UV irradiation [14]. In the same time, data on the effect of radiation on the overall chemical composition, microbiological, carbohydrate and lipid powder bee pollen among domestic sources were not found.

The purpose of this work is to determine experimentally optimal doses of ultraviolet radiation to destroy $\geq 90,0\%$ of unwanted microflora in samples with minimal loss of food and biological value of raw materials.

Materials and methods

In this experiment it was used the pollen from the Carpathian region of Ukraine in accordance with current regulatory documentation of Ukraine. Samples were dried to moisture content of $3 \pm 1\%$, and stored at room temperature conditions, directly prior to the experiment these samples were pulverized. Experimental samples O1, O2, O3 and O4 were irradiated with UV rays at a dose of 2, 4, 6 and 8 kGy, respectively. The control sample (K) was not subject to treatment. Dose was determined photodetectors 8026 (Hamamatsu Photonics KK), head is H 8025 – 222.

The number of microorganisms in the samples mentioned above were determined based on the standard or conventional techniques. Moisture of the samples was determined by the mass difference between the initial sample and the dried at 105°C during 4 hours in the weighing bottle. Ash content was determined by combustion pollen powders in a muffle furnace in an air atmosphere in a porcelain dish at 900°C to constant weight. Crude protein was determined by Kjeldahl method. Lipid content was determined by extraction in Soxhlet apparatus. Reducing sugar content was determined by reaction with 3,5-dinitrosalicylic acid using Spectrocolorimeter Shimadzu 1600 (Japan). The absorbance of the solution is determined at a wavelength of 550 nm.

In order to determine the mass fraction of flavonoid it should be measured the absorbance of pollen extract on fotoelektrokolorimetre at a wavelength of 400 nm filter № 3. The amount of carotenoids is determined method of Ferreira I.C.F.R. (2009) [15].

Content of malondialdehyde in the pollen was assessed by the method of Jo C. (2000) [16]. Prepared biological material in the buffer solution of 2.0 ml were placed in a centrifuge tube and precipitated with 1 ml of protein solution 17% trichloroacetic acid. The resulting precipitate was separated by centrifugation for 10 minutes at 400 g (TSUM centrifuge - 1). 2 ml of supernatant was transferred to a test tube, then it was added 0.8 ml of 1% thiobarbituric acid solution and samples were placed for 10 min. in a boiling water bath. In order to control the process it was used samples containing buffer solution in place

of the supernatant. After the appearance of pink color samples are cooled to room temperature and measure the absorbance at 532 nm.

All studies were performed with a three-fold repetition. Statistical analysis of the experimental data was carried out using Excel, the confidence level of $P \leq 0,05$.

Results and discussion

Comparative analysis of the results of microbiological tests of all pollen samples is provided below (Table. 1).

Table 1
Microbial populations of studied bee pollen samples

Sample	Irradiation, kGy	Viable cell counts (log CFU/g)		
		Total aerobic	Yeast	Mold
K	0	4,4±0,01	1,7±0,02	2,0±0,01
O1	2	2,4±0,01	ND*	1,17±0,04
O2	4	1,1±0,04	ND	ND
O3	6	ND	ND	ND
O4	8	ND	ND	ND

* – viable colony was not detected at detection limit <10 CFU/g.

Increasing doses of pollen radiation reduces the number of colony forming units of microbial populations. Thus, 2 kGy destroy on 45,5% - total aerobic microorganisms, more than 40% - yeast and 41,5% – mold. Note, that 4kGy leads to almost complete disappearance of unwanted microflora and remains less than 25% of aerobic microbes from their original number. Irradiation above 4 kGy reduces the number of viable cells by 99,9%. This can be explained by the influence of short "tail" in the wavelength range 260-282 nm, which covers half of the first absorption peak of DNA and RNA. Photoproducts are formed, which cause either death or mutation of microorganisms [19]. The obtained experimental results confirm the data Kyoung-HeeKim with a team of co-authors [17].

Results of table.1 confirm the effectiveness of radiation treatment as technological methods of increasing the microbiological purity of bee pollen.

Further, it was determined the impact of fixed dose (2-4 kGy) on the overall chemical composition and content of the essential components of the raw materials. Comparative characteristics of a general chemical composition of the irradiated and not irradiated pollen are provided in Table 2.

Table 2
Overall chemical composition of bee pollen samples

Sample	Composition, %				
	Moisture	Ash	Crude fat	Crude protein	Carbohydrate
K	3,33±0,16	3,05±0,06	12,46±0,85	28,30±0,16	52,77±0,03
O1	3,30±0,06	3,00±0,01	12,45±0,77	28,36±0,08	52,77±0,03
O2	3,20±0,02	2,97±0,02	12,44±1,00	28,4±0,25	52,65±0,05
O3	3,33±0,15	3,00±0,03	12,39±0,91	28,41±0,29	53,31±0,03
O4	3,29±0,11	2,95±0,04	12,41±0,93	28,35±0,16	52,91±0,04

These data suggest that an increase in dose of pollen treatment from 2 to 8 kGy has no negative effect on the content of the main component of pollen. The average moisture ranges from 3.20 to 3.33%. The difference between the samples was, on average, 1.7%. The average ash ranges from 2.95 to 3.0% with a difference between the samples in an average of 2.3%. Crude protein, crude fat and carbohydrate in the irradiated pollen did not show any significant changes by irradiation. Namely, less than 0.5%. Perhaps, this is due to the fact, that under ultraviolet irradiation of solid particles processed only their superfine surface layer and the remaining substance do not experience any effect and, therefore, does not alter biochemical properties. This is the advantage of UV treatment compared with other methods of disinfection [14]. The World Health Organization states that process of irradiation in doses to 5 kGy does not lead to a loss of nutritional value of food products [18].

Lipid peroxidation causes changes in phospholipids. In connection locations peroxy radicals fatty acids are broken into fragments, which are located at the edges of the aldehyde groups having high reactivity. If the rupture occurred on both sides, it is formed malondialdehyde (MDA) [18]. Its amount in the experimental and control samples of pollen graphically shown in Drawing 1.

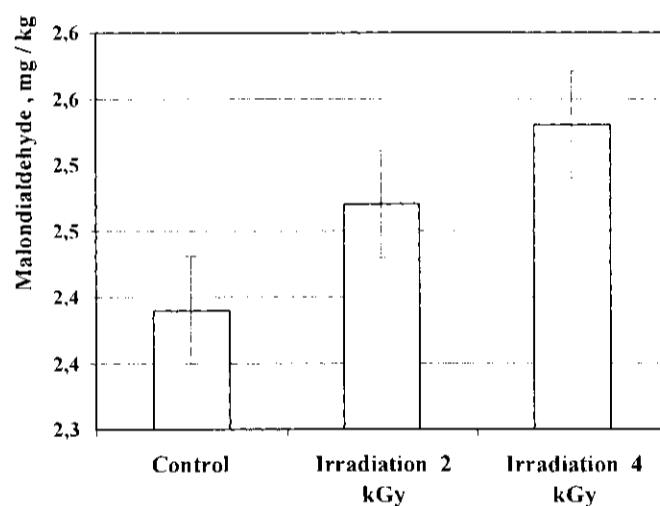


Fig. 1. Dynamics in the number of malondialdehyde included in irradiated

The higher the dose, the more dynamic is lipid peroxidation. At a dose of 2 kGy MDA increased by 3.35%, and at 4 kGy - at 5.86% in comparison with the control sample. This is because the fatty acids are broken into fragments attachment locations peroxy radicals. Aldehyde groups located on the edges of fragments having high reactivity. MDA is formed, if the break occurred on both sides [18].

It is now believed that flavonoids are essential components of human foods. They, like carotenoids act as antioxidants. In mammals flavonoids are able to alter the activity of many enzymes of metabolism [19].

The effect of radiation on flavonoids is indicated in Drawings 2.

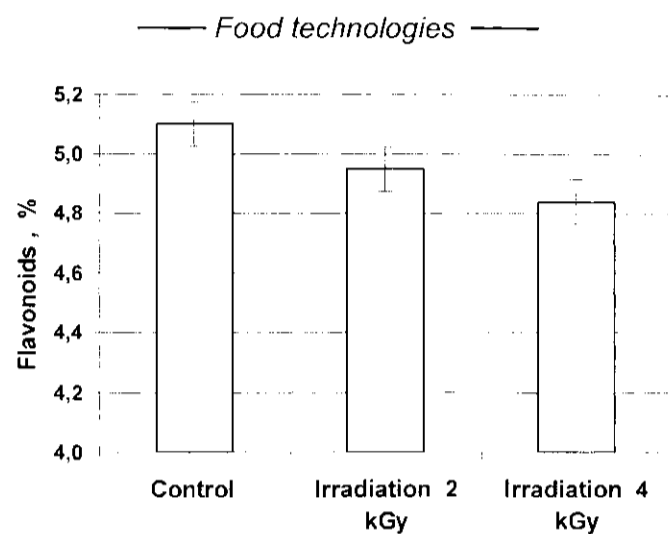


Fig. 2. The content of flavonoids in pollen samples with different intensity of treatment

The intensity of the radiation has a direct impact on the content of flavonoids in the pollen. The dose of 2 kGy reduces their availability in tests 2,9%. Further increasing the dose to 2 times reduction of flavonoids 5,1 as compared with the control sample and 2,2% in comparison with the sample O1. Dose treatment is recommended to determine given the benefits and disadvantages of this method of sterilization – 2kGy.

The effect of radiation on amount of carotenoids in content of pollen into the β -carotene is indicated in Drawings 3.

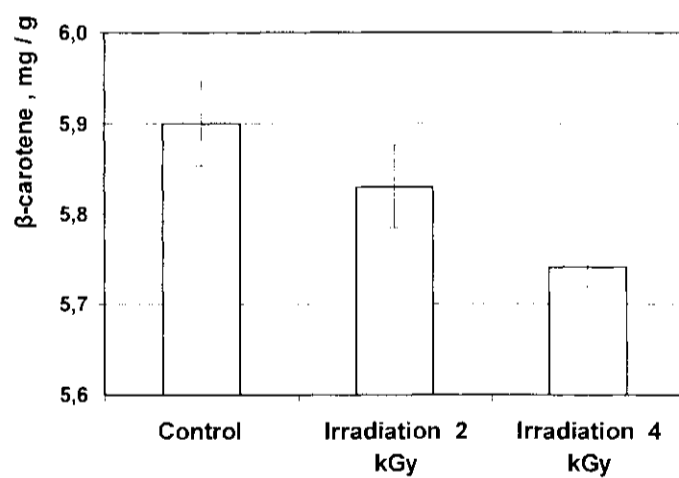


Fig. 3. Total content of carotenoids in samples of bee pollen.

The intensity of the radiation has a direct impact on the content of β -carotene in the pollen. The dose of 2 kGy reduces their availability in tests 1,2%. Further increasing the dose a 50% causes reduction of β -carotene 1,5%, in comparison with the sample O1 or 2,7

- at: ftp://ftp.fao.org/codex/publications/Booklets/RU/Irradiated_Rus.ed_final.pdf
12. Codex General Standard For Irradiated Foods (2003), available at: [file:///C:/Documents%20and%20Settings/Admin/%D0%9C%D0%BE%D0%B8%D0%B4%D0%BE%D0%BA%D1%83%D0%BC%D0%B5%D0%BD%D1%82%D1%8B/Downloads/CXS_106e%20\(1\).pdf](file:///C:/Documents%20and%20Settings/Admin/%D0%9C%D0%BE%D0%B8%D0%B4%D0%BE%D0%BA%D1%83%D0%BC%D0%B5%D0%BD%D1%82%D1%8B/Downloads/CXS_106e%20(1).pdf)
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% in comparison with the control sample. A slight decrease in β -carotene is most likely associated with exposure to radiation sparing regimen. A stiffer treatment leads to a significant destruction of the substance [20].

Dose treatment is recommended to determine given the benefits and disadvantages of this method of sterilization.

Conclusion

Gamma irradiation provides a number of benefits and sterility assurance. Using this type of treatment pollen in order to increase its level of microbiological safety and the possibility of further usage in the production of fermented drinks it is possible to obtain the level of purity 99.9% and thus, save up to 95±1,5% essential material.

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