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Effect of gamma irradiation on microbiological and nutritional properties of the freeze-dried berries

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Abstract. Lyophilization or freeze-drying is the technique of removing ice or other frozen solvents from a material through sublimation and the removal of bound water molecules through the process of desorption. Drying occurs in an absolute vacuum at temperatures from –40°C to –50°C. This technique is often used for the conservation of fruits, especially berries. During this process, the water changes from frozen to gaseous, with no thawing. Due to low temperatures and the high vacuum, most microorganisms are rendered inactive during the lyophilization process. However, if there is a necessity to destroy all microorganisms from treated food, subsequent irradiation with gamma rays is an appropriate method. This paper investigated the influence of different doses of gamma radiation on lyophilized berries' microbiological characteristics. It was shown that the radiation dose of 7 kGy is sufficient to eliminate the total number of microorganisms (excluding molds) to the extent that the number falls below the permitted limit according to the law on the microbiological safety of foodstuffs of the Republic of Serbia, and 5 kGy is enough for molds to be rendered inactive. It was also concluded that gamma irradiation does not affect the nutritional value of lyophilized berries.

Keywords: Freeze-drying • Gamma irradiation • Lyophilization • Microbiological properties • Nutritional properties

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Introduction

Lyophilization, also known as freeze-drying or cryodesiccation, is a low-temperature dehydration process [1] that involves freezing the product, lowering pressure, and then removing the ice by process of sublimation [2, 3]. Freeze-drying is most used to make instant coffee [4] but works exceptionally well on fruits such as apples [5] and berries [6].

The lyophilization process avoids product contamination because most bacteria and molds cannot survive this process. Freeze-dried fruit is an excellent addition to oatmeal, muesli, honey, teas, and many other healthy food products [7].

Freezing does not remove all microorganisms and bacteria from food. It may decrease the number of bacteria, but many harmful bacteria can generally still survive. Therefore, an additional gamma irradiation method can be applied to remove microorganisms from freeze-dried fruit [8].

In this paper, gamma irradiation's influence on reducing the total number of microorganisms, molds, and bacteria was examined. For this purpose, five types of berries were tested:
– *Rubus fruticosus* (blackberries)
– *Rubus idaeus* (raspberries)
– *Fragaria ananassa* (strawberries)

- *Vaccinium corymbosum* (blueberries)
- *Prunus cerasus* (sour cherries).

It was shown that the radiation dose of 7 kGy could eliminate the total number of microorganisms, molds, and bacteria below the permitted limit prescribed by the law on the microbiological safety of foodstuffs of the Republic of Serbia. These gamma irradiation doses do not affect the nutritional value of lyophilized berries.

Materials and methods

Sampling

Freshly harvested ripe fruits were purchased from the local green market in Belgrade, Serbia. The fruits were washed and dried at room temperature. The weight of each berry sample was 100 g. Dry samples of fruit were lyophilized.

Lipohylized berries were packed into sterilized zip bags and irradiated with different gamma radiation doses in the facility for radiation sterilization and conservation of the Vinca Institute of Nuclear Sciences.

Lyophilization

For the fruit lyophilization, the Home Freeze Dryer, HarvestRight, the USA were used. The freezing was done at –42°C, and after freezing, the ice was vacuum pumped out in 24 h. The vacuum pressure during freeze-drying was 20 Pa.

After the lyophilization process, the following values of berry powders were measured (Table 1).

Gamma irradiation

During gamma irradiation, high-energy photons, generated from the radioactive source Co-60, penetrate through the product and destroy the DNA chains in living organisms that thrive in the product (insects, molds, yeasts, and bacteria). Radiation doses of 3 kGy, 5 kGy, and 7 kGy were used. The average dose rate was 10 kGy/h.

For the measurement of absorbed radiation dose, we used the ethanol-monochlorobenzene (ECB) dosimeter system [9, 10]. Measuring equipment for ECB is the instrument OK-302/2 type oscillotitrator of Radelkis (Budapest, Hungary). Since the oscillotitrator's measurement [11] is highly temperature-dependent [12], all dosimeters are heated to 20°C.

Microbiological analysis

The initial contamination and the number of microorganisms, total molds, and bacteria in the samples were examined in the accredited microbiology laboratory, using the methods Ph. Eur. 7.0 (2.6.12. – microbiological examination of nonsterile products (total viable aerobic count)) [13]. Microbiological analyses were performed before irradiation and after gamma irradiation with radiation doses of 3 kGy, 5 kGy, and 7 kGy.

Nutritional properties

The samples of freeze-dried berries before and after gamma irradiation were analysed to determine their content of total fat, water, protein, carbohydrates, sugars, dietary fiber, and energy values. The total fat content was determined by Weibull–Stoldt-Standard application [14]. The berries' water content was analysed using an electronic water content analyser that works on drying with air circulation. Protein content was determined using a standard ISO procedure, ISO 1871:2009 [15], and the Kjeldahl method [16]. Determination of total carbohydrate and sugars present in freeze-dried berries was performed using the phenol sulfuric acid method [17]. An enzymatic-gravimetric method was used to determine the content of dietary fiber in freeze-dried berries samples.

Results and discussion

Weight loss

Some studies have shown that with the irradiation treatment, the berries lose weight [18]. Weight changes were not observed in our case, which shows that the weight lost during irradiation depends on the sample's water content. In this case, all the water was eliminated by the lyophilization process.

Effect of gamma irradiation on microbiological properties

To eliminate total microorganisms, molds, and bacteria from freeze-dried berries, they were treated with gamma irradiation doses of 3 kGy, 5 kGy, and 7 kGy. The measured absorbed radiation doses were (3 ± 0.12) kGy, (5.0 ± 0.22) kGy, and (7.1 ± 0.18) kGy. The uniformity of the delivered dose was 3.6%.

Table 1. Percentage of removed water during the process of lyophilization

Berry fruit	Weight before lyophilization process (g)	Weight after lyophilization process (g)	% of removed water
Blackberries	1000	121	87.9
Raspberries	1000	142	85.8
Strawberries	1000	90	91.0
Blueberries	1000	163	83.7
Sour cherries	1000	180	82.0

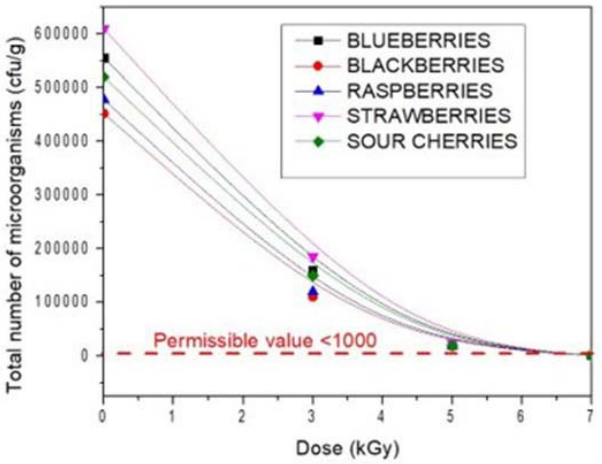

Fig. 1. Influence of different doses of gamma radiation on the total number of microorganisms in samples of freeze-dried berries. The red line represents the limit of the permissible value.

The content of the total number of microorganisms was translated into a radiation dose curve, which represents a logarithmic variation of the total number of microorganisms as a function of the absorbed radiation dose (Fig. 1).

Figure 1 shows that the radiation dose of 7 kGy is enough to eliminate the total number of microorganisms below the permitted limit. Similar results are shown in the literature [19].

Table 2 shows that the radiation dose of 5 kGy is enough to eliminate the total molds below the permitted limit, as reported in the previous research [20].

None of the six tested bacteria (*Salmonella sp., Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus, Sulfitoreducing clostridia*) were found in the samples, either before or after irradiation.

Effect of gamma irradiation on nutritional values of samples

Several studies have confirmed that doses up to 10 kGy do not cause any toxicological hazards or nutritional and microbiological problems in fresh berries [21]. To determine the gamma irradiation influence on the nutritional properties of freeze-dried berries, we performed an analysis in the non-irradiated samples and the samples irradiated with the irradiation dose of 3 kGy, 5 kGy, and 7 kGy. The data are shown in Tables 3–7.

It can be inferred that the changes in most nutritional were insignificant and within the limits

Table 3. Nutritional values of freeze-dried blueberries

Parameter/Dose	0 kGy	3 kGy	5 kGy	7 kGy
Total fat (%)	0.34	0.43	0.73	1.30
Water (%)	5.2	5.4	5.4	5.6
Protein (%)	6.0	6.1	6.2	6.2
Carbohydrate (%)	86	86	85	84
Sugars (%)	48.2	48.5	47.7	48.8
Dietary fiber (%)	10.9	11.3	11.1	10.8
Energetic value (kJ)	1593	1598	1594	1599

Table 4. Nutritional values of freeze-dried blackberries

Parameter/Dose	0 kGy	3 kGy	5 kGy	7 kGy
Total fat (%)	0.59	0.63	0.71	0.69
Water (%)	4.7	4.5	5.3	4.8
Protein (%)	6.0	6.1	6.3	6.2
Carbohydrate (%)	86	87	86	86
Sugars (%)	43.0	42.1	43.2	43.0
Dietary fiber (%)	14.1	14.3	13.9	15.1
Energetic value (kJ)	1603	1623	1611	1616

Table 5. Nutritional values of freeze-dried raspberries

Parameter/Dose	0 kGy	3 kGy	5 kGy	7 kGy
Total fat (%)	0.65	0.62	0.84	0.61
Water (%)	7.0	7.3	7.2	7.3
Protein (%)	7.1	7.1	7.2	7.3
Carbohydrate (%)	83	83	81	82
Sugars (%)	47.7	46.5	47.0	46.8
Dietary fiber (%)	19.8	20.1	20.1	20.6
Energetic value (kJ)	1572	1571	1547	1557

Table 6. Nutritional values of freeze-dried strawberries

Parameter/Dose	0 kGy	3 kGy	5 kGy	7 kGy
Total fat (%)	0.67	0.62	0.58	0.59
Water (%)	5.9	6.1	6.1	6.1
Protein (%)	5.9	6.0	6.1	6.0
Carbohydrate (%)	85	85	85	85
Sugars (%)	57.6	57.0	58.0	57.8
Dietary fiber (%)	9.7	10.1	9.8	9.9
Energetic value (kJ)	1587	1587	1587	1586

Table 7. Nutritional values of freeze-dried sour cherries

Parameter/Dose	0 kGy	3 kGy	5 kGy	7 kGy
Total fat (%)	0.22	0.33	0.21	0.48
Water (%)	7.8	7.9	8.3	8.1
Protein (%)	8.1	8.1	8.2	8.2
Carbohydrate (%)	82	81	81	81
Sugars (%)	52.4	50.0	50.0	51.0
Dietary fiber (%)	8.7	9.1	9.2	9.0
Energetic value (kJ)	1666	1643	1640	1660

Table 2. The total number of molds before and after the influence of different doses of gamma irradiation

Total mold ($cfu \cdot g^{-1}$)	Dose (kGy)				Permissible value
	0	3	5	7	
Blueberries	810	130	<100	<100	<100
Blackberries	740	110	<100	<100	<100
Raspberries	530	<100	<100	<100	<100
Strawberries	640	<100	<100	<100	<100
Sour cherries	550	110	<100	<100	<100

of measurement error. The exception is the total fat content in the samples. Its values vary and do not follow any trend; so, we can assume that their detection method is quite unreliable at such a low amount of total fat content in the samples.

Conclusions

Lyophilization is an effective method for preserving food. It is a widely used method to preserve berries. Exposure to low temperatures and vacuum during the lyophilization process removes most microorganisms from the berries. A highly effective method for the complete elimination of microorganisms from lyophilized fruit is high-energy gamma radiation. This study aimed to determine the radiation dose required to eliminate all microorganisms, molds, and bacteria from freeze-dried berries.

It was found that the process of lyophilization eliminates different percentages of water from berries, from 82.0% to 91.0%. The irradiation process does not affect the weight loss of the freeze-dried berries. As the radiation dose increases, the total number of microorganisms and molds in all tested samples decreases. A dose of gamma radiation of 3 kGy eliminates 85–89% of the total microorganisms in the samples, while a dose of 5 kGy is sufficient to eliminate over 97% of microorganisms. It was determined that a dose of 7 kGy is enough to eliminate all microorganisms.

On the other hand, a dose of 3 kGy destroys most mold, while 5 kGy is enough to eliminate all mold from freeze-dried fruit. The influence of gamma radiation on the change of nutritional values of samples was also investigated. It has been shown that gamma radiation does not affect the change in freeze dried berries' nutritional values. The only deviation was established in the percentage of fat. Still, their content in dried berries is small, and the differences in values can be attributed to measurement errors and uncertainty of the method.

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