

PHYSICOCHEMICAL, MICROBIOLOGICAL AND SENSORY EVALUATION OF FREEZE-DRIED EGGS STORED AFTER IRRADIATION

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Abstract

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An experiment with freeze-dried whole hen's egg has been carried out to study the efficacy of the combination of two methods used for the preservation of foodstuffs – freeze-drying and irradiation with optimal dose of gamma rays, in order to prolong its shelf-life as well as to guarantee its microbiological safety. The physicochemical, microbiological and organoleptic characteristics of the freeze-dried egg mēlange were determined. During the 4-year period of storage at room temperature of the freeze-dried (control group) and the freeze-dried and irradiated with a dose up to 3.5 kGy melange no significant changes were found into the sensory characteristics till the 28th month, while the physicochemical ones stayed unaltered. The quantity of thiobarbituric acid reactive substances (TBARS, meaning milligrams malondialdehyde equivalent per kilogram) in the irradiated samples was in parallel increase with the dose till the 6th month of the time of storage, while after that it was decreasing. The microbiological features of the irradiated freeze-dried eggs have been improved during storage. The mathematical processing of the data showed that the time of storage (F_A) was the main factor for the slight changes established, while gamma irradiation had no essential influence.

Key words: freeze-dried egg melange, gamma-rays treatment, storage, thiobarbituric acid reactive substances (TBARS), sensory evaluation, physicochemical characteristics

Introduction

The raise in the demand for foodstuffs that keep for a longer period their specific nutritious and taste characteristics has imposed the development of adequate methods of preservation that are able to meet a number of up to date requirements. Along with freeze-drying as a method of microorganism inactivation in foodstuffs, the method of treatment with ioniz-

ing radiation has been used during the last two decades (Prachasittisad et al., 1984; Klinger et al., 1986; Beuchat et al., 1993). The application of that method is perspective concerning the wider spread of salmonellas and other pathogenic microorganisms in eggs that present an essential risk factor in human diseases (Roberts, 1996).

The maximum dose of 10 kGy of gamma irradiation allowed by WHO/FAO/IAEA (1989) are justi-

fied with respect to microbiological requirements, but worsen the sensory features of foodstuffs. They result mainly in the changes of smell, taste or colour (Farkas, 1987; Katuzin-Razemet et al., 1989). That fact requires that irradiation must be used in combination with other methods in order to reduce the dose applied.

The purpose of the present study is to investigate the efficiency of the two methods combination – freeze drying of egg melange followed by treatment with a minimum dose of gamma radiation, in order to prolong its shelf life as well as to guarantee its microbiological safety.

Material and Methods

Fresh hen's eggs from a licensed poultry farm were washed and disinfected with a 1% solution of chloramine and then the whole egg's melange was homogenized in a laboratory blender. Bread yeast was used to separate the carbohydrates so as to prevent Maillard reaction during which the product gets brown (Handa and Kuroda, 1999). Fermentation was carried out for 5 to 6 h at 32–35°C (pH 5.5–6.0 by means of a 10% solution of citric acid). At the end of fermentation the pH was brought to its initial value (7.5) by means of a 12.5% solution of NH_4OH . The egg mëlange was statically pasteurized at a temperature of 65°C for 3.5 min and then immediately cooled down to 7–10°C and freeze-dried within an installation TG 16.50 (Vakutec–Germany), at the following temperature parameters: freezing at minus 30°C at an average rate of 2–3°C/min, sublimation at minus 30° to minus 25°C and further drying at 30° to 35°C under pressure of $2,5 \cdot 10^4$ Pa for 8–10 h. The so freeze-dried egg melange was then packed in a three-layer aluminum folio.

The so prepared samples were distributed in three experimental groups: 1st group – freeze-dried egg melange (control); 2nd group – freeze-dried egg melange irradiated with 2.0 kGy; 3rd group – freeze-dried

egg melange irradiated with 3.5 kGy.

The samples of groups 2 and 3 were treated in a gamma installation “Gamma-1300” with ^{137}Cs source at an average absorbed dose of 2.0 or 3.5 kGy with maximum errors of $\pm 10\%$. Atmospheric oxygen was not excluded during irradiation. The maximum temperature increase in the samples during treatment was about 15°C. The dose 3.5 kGy corresponds also to the theoretically calculated one, which fully guarantees decontamination of pathogens, according to the conception of 7 D_{10} (Farkas, 1987; Roberts, 1996).

The samples were stored under room temperature for 48 months and tested every 6 months and the following analyses were carried out of the all groups egg mëlange:

- **Physicochemical parameters:** Water content measured by means of a Sartorius thermo-control balance; Total protein by the Kjeldahl method (ISO 1871:2000); Total lipids by the Soxhlet method (AOAC, 1990); Minerals – by means of carbonizing in a muffle oven at 550°C (AOAC, 1990).

- **Secondary products of oxidation:** Thio-barbituric acid reactive substances (TBARS) expressed as malondialdehyde equivalent (MDA, mg/kg product), were defined by the Newburg and Concon method (1980).

- **Microbiological tests:** Salmonella – according to EN ISO 6579:2003; Coagulase-positive staphylococci – EN ISO 6888:2003; Sulphite-reducing clostridia – ISO 15213: 2003; *E. coli* – ISO 16649-2:2001; Coliform bacteria – ISO 4832:2002; Total aerobic and facultative anaerobic mesophilic bacteria – ISO 4833:2003; Total aerobic and facultative anaerobic psychrotrophic bacteria – ISO 17410: 2002.

- **Sensory evaluation** of the “tastiness” indicator of the spongy cake, produced from 171 cm³ rehydrated egg melange, 132 g sugar and 128 g wheat flour Type 500. The scale of multiplication comparison was applied using the 9-step hedonic scale of Larmond (1967). Tasting was carried out by a per-

manent group of 5 training persons.

• **Mathematical processing of the data**

The results were processed according to the dispersion method of Snedecor and Cochran (1980). An one factor-analysis was applied for each individual experimental group separately by defining the “storage time” factor – F_A , and reporting in % its power of influence. The two-factor analysis was applied by comparing the control group with the experimental ones. The reliability of all investigated factors F_x was defined, including “storage time” F_A , “irradiation” F_B and the interrelation of both factors - F_{AB} . The power of influence of each individual factor in percent was also defined.

Results and Discussion

Physicochemical characteristics

During the entire period of storage no changes were observed in the investigated indices in the three groups. Their water content was within 1.78 to 2.03 %, while the protein – from 56.03 to 56.88 %, total lipids – from 36.10 to 36.78 %, and minerals – from 2.85 to 3.05 % ($n = 5$).

Up to the 12th month of sample storage the MDA concentration (Table 1) varied within wide limits and the values in the irradiated samples were several times higher than those in the control ones. After the 24th month the concentration of MDA in all three groups was nearly equal. During the entire period of investigation the amount of MDA in all three groups of egg melange was considerably below the allowed limit for foodstuffs, which according to Wong et al. (1995) should not exceed 3.0 mg/kg. However those changes did not result in the worsening of the sensory features of the freeze-dried egg melange till the end of the period of investigation.

Microbiological characteristics

In the three investigated groups during the entire period of storage no pathogenic microorganisms were isolated, including salmonella bacteria at inoculation

of 25 g of the product, coagulase-positive staphylococci - of 1 g, sulphite-reducing clostridia – of 0.1 g and *E. coli* – of 1 g. According to those results the samples meet the requirements of FAO Codex Alimentarius for dried egg products and the Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs (O. J. No L 328/1/22.12. 2005). According to Farkas (1987), Grozdanov et al. (1993) and Roberts (1996) a dose of 3 to 5 kGy is sufficient for the devitalization of pathogens without any effect on the technological, nutritious and sensory features of the foodstuff, a fact also confirmed by the present investigations.

In all experimental groups a gradual reduction in the count of coliform bacteria to values below 10 cfu/g at the end of the storage was established (Table 2). There was a similar tendency concerning the total count of mesophilic and psychrotrophic bacteria, both aerobic and facultative anaerobic in all three groups and it was more clearly demonstrated in the treated samples.

By summarizing the microbiological results one may conclude that the irradiation of the concerned doses led to the reduction in the count of the mesophilic and psychrotrophic aerobic and facultative anaerobic microflora. The vegetative bacteria forms were the first to be devitalized, and that was a guarantee for the good microbiological status of the freeze-dried whole egg even after 48 months of storage at room temperature.

Sensory evaluation of the spongy cake

During the entire period of investigation the evaluations were within the limits of 5.13 to 4.82 for the control group, from 5.09 to 4.80 for the group irradiated with 2.0 kGy, and from 5.12 to 4.74 for the group - with 3.5 kGy. Those evaluations showed that no substantial organoleptic changes could be observed in result of the irradiation and storing of the freeze-dried egg melange.

The gradual decrease in the evaluations for the indicator “tastiness” started after the 28th month and at the end of the storage the values compared to the

Table 1
Thiobarbituric acid reactive substances (TBARS) meaning malondialdehyde equivalent (mg/kg product) of freeze dried and irradiated egg mélange during storage (n = 5)

Experimental groups Storage time in months	№ 1 (control)	№ 2 (2.0 kGy)	№ 3 (3.5 kGy)
Start	0.081 ± 0.003	0.113 ± 0.003	0.393 ± 0.003
6	0.080 ± 0.002	0.267 ± 0.005	0.720 ± 0.002
12	0.262 ± 0.002	0.252 ± 0.006	0.273 ± 0.002
18	0.150 ± 0.003	0.066 ± 0.004	0.154 ± 0.004
24	0.140 ± 0.003	0.152 ± 0.003	0.128 ± 0.003
36	0.147 ± 0.004	0.156 ± 0.004	0.131 ± 0.004
42	0.161 ± 0.005	0.172 ± 0.005	0.141 ± 0.005
48	0.170 ± 0.005	0.181 ± 0.005	0.160 ± 0.004

initial ones in percents was: 3.6 for group 1, 4.0 for group 2, and 5.2 for group 3. That tendency could be explained with the decrease in the swelling capacity and solubility of the egg mélange at the end of the storage period, resulting in tougher and more rubbery consistency of the cake.

Mathematical processing of the data

During the one factor analysis made for each separate experimental group it was found that the values for all tested indices are reliable at $F_x \geq 99.9$. For that reason the “time” factor had considerable influence on the sensory features of the experimental groups with power of influence of the “storage time” factor above 70%. The two-factor analysis showed similar results.

In all tested indices the organized factors are of third degree reliability ($F_x \geq 99.9$). The “storage time”

factor F_A as well is of third degree significance ($F_A \geq 99.9$). The “irradiation” factor (F_B) and the interaction between the two factors (F_{AB}) are insignificant. This confirms the results of the one-factor analysis in the sense that storage time is the basic factor for the changes in the experimental samples.

Analyzing the results of the groups irradiated with 2.0 or 3.5 kGy it was established that the size of the dose of irradiation has no considerable effect on the investigated indices. The basic factor here also is the storage time with about 80% power of effect.

Conclusion

After a long period of storage (4 years) of lyophilized egg mélange as well as lyophilized and gamma irradiated mélange with doses up to 3.5 kGy, no sig-

Table 2
Microbiological indices of freeze dried and irradiated egg mélange during storage (n = 5)

Experimental groups	Storage time in months	Coliform bacteria, cfu/g product	Total count of mesophilic bacteria, cfu/g product	Total count of psychrotrophic bacteria, cfu/g product
№1 (control)	Start	$2.20 \cdot 10^2$	$1.25 \cdot 10^3$	$5.50 \cdot 10^3$
	12	$.10^2$	$1.03 \cdot 10^3$	$4.80 \cdot 10^3$
	36	$.10^2$	$0.88 \cdot 10^3$	$2.23 \cdot 10^3$
	48	>10	$0.54 \cdot 10^3$	$1.95 \cdot 10^3$
№2 (2. 0 kGy)	Start	$1.50 \cdot 10^2$	$1.10 \cdot 10^3$	$5.80 \cdot 10^3$
	12	$5 \cdot 10^1$	$0.56 \cdot 10^3$	$2.26 \cdot 10^3$
	36	$2 \cdot 10^1$	$0.40 \cdot 10^3$	$1.15 \cdot 10^3$
	48	>10	$0.35 \cdot 10^3$	$0.145 \cdot 10^3$
№3 (3. 5 kGy)	Start	$1.30 \cdot 10^2$	$1.23 \cdot 10^3$	$1.03 \cdot 10^4$
	12	$.10^2$	$0.68 \cdot 10^3$	$4.67 \cdot 10^3$
	36	$.10^1$	$0.45 \cdot 10^3$	$1.60 \cdot 10^3$
	48	>10	$0.21 \cdot 10^3$	$0.25 \cdot 10^3$

nificant changes have been occurred in its sensory properties until the 28th month, while the physico-chemical ones stayed unchanged during the entire period of testing. Up to the 12th month of storage the MDA concentration varied within wide limits and its values of the irradiated samples correlated with the dose. After the 24th month of storage the data of all three experimental groups got almost equal.

The microbiological status of the lyophilized egg mélange treated with gamma radiation stayed unaltered and improved during storage and was within the standard limits. Statistic processing of the data showed that the "storage time" factor (F_A) was the dominant one for the changes established, while gamma irradiation had no significant influence on the changes in the investigated indices.

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