

ASSESSMENT OF THE FATTY ACID PROFILE OF LYOPHILIZED BUFFALO YOGHURT AFTER GAMMA STERILIZATION

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ABSTRACT

The present investigation studies the fatty acid profile of buffalo yoghurt after lyophilization and treatment with doses of gamma rays – 2 kGy and 4 kGy, for prolonging shelf life and preserving the composition, the properties and the structure of the product. The extraction of the fat from the milk samples has been realized by the method of Roese-Gottlieb. The analysis of the fatty acids has been carried out with the aid of gas chromatograph Shimadzu 2010. The content of saturated fatty acids (SFA) in the treated samples does not change substantially, namely from 65.49 g/100 g fat for the control group to 66.27 g/100 g for the samples irradiated with 2 kGy and to 66.96 g/100 g fat at 4 kGy. The monounsaturated fatty acids (MUFA), including the oleic and vaccenic acids are characterized by comparatively constant quantities in the analyzed buffalo yoghurt after lyophilization and gamma-irradiation. The polyunsaturated fatty acids (PUFA) decrease from 4.70 g/100 g fat for the control group to 4.47 g/100 g fat for the samples irradiated with 2 kGy and 4.00 g/100 g fat at 4 kGy. The content of the conjugated linoleic acid (CLA) in the lyophilized buffalo yoghurt is low – 1.49 g/100 g fat and decreases to 1.43 g/100 g fat after irradiation with 2 kGy and to 1.23 g/100 g fat after irradiation with 4 kGy. On the other hand, the obtained lyophilizates are characterized by a very good proportion of the irreplaceable fatty acids: ω -3 : ω -6 fatty which varies from 2,14 to 2,72 and has a positive effect on human health.

Keywords: buffalo yoghurt, milk fat, fatty acid composition, lyophilization, gamma sterilization.

INTRODUCTION

The buffalo yoghurt is a biologically active food which is of increased researcher's and consumer's interest. It contains lactic acid, galactose, peptides, free amino acids, some vitamins /folic acid/, organic acids /succinic (amber), fumaric, benzoic/ and other components, that ensure a high biological sufficiency of its consumption and have a specific impact on human health. The presence of active lactic acid microflora is a prerequisite for improvement of the digestion, stimulation of the immunity and activation of the total metabolism [12].

The yoghurt milk fat is compounded of a specific mixture with a significant number of short, medium and long chain fatty acids in different proportions, giving it a specific aroma and marked nutritional value [8,12]. The saturated fatty acids predominate in its composition with an average content of 65-70 % of the total fat quantity. The palmitic, myristic and stearic fatty acids are in the

greatest quantities [11]. The content of unsaturated fatty acids in the milk fat is around 30-35 %, the oleic acid being in a predominant quantity [4]. The quantity of the biologically important polyunsaturated fatty acids (linoleic, linolenic and arachidonic) compared to their quantity in plant oils is small – around 3.5 %. The polyunsaturated fatty acids and their derivatives are united in two families – of the linoleic acid or ω -6 /sunflower oil/ and of the linolenic or ω -3 /walnuts, peanuts, almonds, hazelnuts and other nuts, fishes/. The main functions of the ω -3 and ω -6 fatty acids are: energy accumulation in the cell, maintaining of the body temperature, protection of the skin from drying, reproduction of certain hormones needed by the cells, the cell biochemistry and the energy metabolism; cardiovascular and immune health. The fatty acids of the ω -3 and ω -6 groups are a vital component of our nutrition. The balanced intake of ω -3 and ω -6 fatty these acids is considered more important than the quantity of the taken fatty acids [8, 9].

The milk lipids contain anticarcinogenic components as the conjugated linoleic acid (CLA), sphingomyelin and butyric acid. During storage the milk fats undergo physical and chemical changes, including autooxidation processes, in which trans-fatty acids are formed. The fatty acids in dairy products are in low concentrations because they are obtained as a result of biohydrogenation in the rumen of the ruminant but they can be also formed during heat treatment of the dairy products – roasting and frying. The trans-fatty acids are connected, by their biological effect and toxicological action, to coronary heart disease and disturbances in the essential fatty acids metabolism in the fetus, which leads to delay of growth [10]. The fatty acid profile influences the quality of the milk fat and contributes to its physical and organoleptic properties [7].

The modern scientific experience has proven that freeze-drying is the best method for quality preservation of fast spoiling products, including dairy products. By this method the end products feature preserved colour, aroma, nutritional properties, vitamins content, unchanged initial volume and quick rehydration [13].

The processing of foods by ionizing radiations is another modern method for preservation which opens up broad perspectives for the food industry taking into consideration its many-sided impact on the aroma, texture, colour, appearance and biochemical changes. Ionizing radiation is a powerful means for reducing and eliminating of the pathogenic microorganisms in foods during which insignificant changes occur in their composition. In case of a correctly conducted irradiation process the nutritional qualities of the products are preserved in the same way as in the traditional ways of foods preservation [3]. Sterilization of milk and dairy products by ionizing radiation with doses above 5 kGy and even 10 kGy, depending on the milk seeding, leads to strongly expressed changes in the taste, aroma and colour. The deteriorated organoleptic properties of the irradiated milk are a result of the changes in the fats, while milk darkening is due to changes in the hydrocarbon complex. By combination of the two technologies – lyophilization and gamma rays treatment with lower doses, unfavorable changes in the yoghurt technological and quality characteristics do not occur and a prolongation of its preservation term is achieved [2].

The aim of this work is to carry out investigations on the changes in the fatty acid composition of buffalo

yoghurt after lyophilization and after gamma rays treatment for prolongation of the preservation term by combined application of the two technological approaches.

EXPERIMENTAL

Subject of the study

The investigations were carried out with natural buffalo yoghurt, lyophilized in Institute of Cryobiology and Food Technology (ICFT).

The samples were distributed in 3 groups as follows:

- No 1 - control group, not irradiated;
- No 2 - test group, irradiated with a dose 2 kGy;
- No 3 - test group, irradiated with a dose 4 kGy.

Technological approach

Two methods of high technology were applied – lyophilization and gamma rays irradiation.

The freeze-drying was done in a vacuum freeze-drying installation TG 16.50, produced by “Hochvacuum” company, Germany, with contact plates heating, and the following parameters: drying temperature -40°C , condenser temperature -65°C , total pressure in the vacuum chamber 0.20-0.35 mm/Hg and secondary drying temperature up to 30°C .

The irradiation with doses of 2 kGy and 4 kGy was realized in a gamma-irradiation installation, “Gama-1300”, with radiation source Cs^{137} and a dose power of 1.5 kGy/min. The extraction of total lipids was carried out by the method of Roese-Gottlieb [1] with diethyl and petroleum ether. The transesterification of the milk fat was done with sodium methylate (CH_3ONa , Merck, Darmstadt) and subsequent drying with $\text{NaHSO}_4 \cdot \text{H}_2\text{O}$.

The fatty acids methyl esters (FAME) were analyzed with a gas chromatograph Shimadzu-2010 (Kyoto, Japan) equipped with flame-ionization detector and automatic injection system (AOC-2010i). The analysis was made on a capillary column CP7420 (100 m x 0,25 mm i.d., 0,2 μm film, Varian Inc., Palo Alto, CA). As a carrier gas was used hydrogen and as a make-up gas – nitrogen. A regime of the oven was programmed: initial column temperature 51°C for 8 min, and a 4-level increasing of the temperature from 170°C to 240°C until the process ending.

The data from the investigation were statistically processed by the method of Student.

RESULTS AND DISCUSSION

The milk fat represents a mixture of short-, medium- and long-chain fatty acids, in different quantitative relations depending on the nutrition, lactation and the species specificity of the organism. The detailed investigation of the fatty acid profile of buffalo yoghurt is presented in Tables 1- 4.

The lyophilized buffalo yoghurt contains 65.49 g/100 g fat saturated fatty acids and their total quantity remains comparatively constant in the irradiated lyophilized buffalo yoghurt with 2 kGy and 4 kGy respectively, as follows: 66.27 g/100 fat and 66.96 g/100g fat.

The quantity of the short chain fatty acids (P4:0, P6:0, P8:0 and P10:0) increases in the process of irradiation. The butiric acid (C4:0) in the lyophilized buffalo yoghurt is 4.47 g/100 g fat and increases to 4.66 g/100 g fat and 4.71 g/100 g fat as a result of irradiation with 2 kGy and 4 kGy. Similar are the results for the capronic (C6:0) acid whose quantity changes from 2.49 g/100 g fat for the control group to 2.60 g/100 g fat after irradiation (2 kGy and 4 kGy). The caprylic (C8:0) acid retains a comparatively constant level, independent of the treatment. The content of the caprynic (C10:0) acid in the analyzed buffalo yoghurts increases insignificantly as a result of the applied irradiation – from 4.13 g/100 g

fat for the control group to 4.24 g/100 g fat at 2 kGy and 4.50 g/100 g fat at 4kGy. The capronic, caprylic and caprinic acids have a substantial effect on the organoleptic properties of milk and dairy products. It has been established that they lead to an increase of the blood LDL-cholesterol values and to an increase of cardio-vascular diseases in humans. The lauric (C12:0) acid level increases from 2.92 g/100 g fat for the control to 2.99 g/100 g fat for the samples irradiated with 2 kGy and 3.15/100 g fat at 4 kGy. The content of the miristine (C:14) is retained relatively constant (from 10.63 to 10.66). The palmitinic (C16:0) acid changes its quantity from 25.75 g/100 g fat for the control to 26.40 g/100 g fat and 26.30 g/100 g fat for irradiated milk with 2 kGy and 4 kGy respectively. The content of stearic acid is relatively constant (Table 1).

The total content of monounsaturated fatty acids is 29.42 g/100 g fat for the control and 29.73 g/100 g fat and 29.19 g/100 g fat for the irradiated yoghurts (Table 5). The monounsaturated fatty acids (MUFA) are presented in Table 2. From MUFA the cis- and trans-isomers of the C18:1 spectrum is the richest, with a predominant share of the oleic acid C18:1 cis 9 (about 20 g/100 g fat) and the vaccenic acid C18:1 trans 11 (from 3.63 g/100 g fat for the control group and 3.70 g/100 g fat and 3.55 g/100 g fat – for the samples irradiated with 2 kGy

Table 1. Saturated fatty acids, g/100g fat.

Fatty acid	Control	2 kGy	4 kGy
C-4:0	4.47 ± 0.05	4.66 ± 0.03	4.71 ± 0.03
C-6:0	2.49 ± 0.03	2.60 ± 0.02	2.60 ± 0.01
C-8:0	1.62 ± 0.02	1.65 ± 0.01	1.66 ± 0.01
C-10:0	4.13 ± 0.05	4.24 ± 0.03	4.50 ± 0.02
C-11:0	0.08 ± 0.00	0.06 ± 0.00	0.07 ± 0.00
C-12:0	2.92 ± 0.03	2.99 ± 0.02	3.15 ± 0.02
C-13:0	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00
C-14:0	10.63 ± 0.09	10.10 ± 0.07	10.66 ± 0.05
C-15:0	1.01 ± 0.01	1.00 ± 0.01	1.04 ± 0.01
C-16:0	25.75 ± 0.30	26.40 ± 0.19	26.30 ± 0.14
C-17:0	0.65 ± 0.01	0.66 ± 0.01	0.68 ± 0.00
C-18:0	11.18 ± 0.13	11.33 ± 0.08	11.06 ± 0.06
C-20:0	0.20 ± 0.00	0.20 ± 0.00	0.19 ± 0.00
C-21:0	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
C-22:0	0.12 ± 0.00	0.13 ± 0.00	0.13 ± 0.00
C-24:0	0.08 ± 0.00	0.08 ± 0.00	0.07 ± 0.00
C-26:0	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00

Table 2. Monounsaturated fatty acids, g/100g fat.

Fatty acid	Control	2 kGy	4 kGy
C-10:1	0.16±0.00	0.16±0.00	0.16±0.00
C-12:1n1	0.07±0.00	0.09±0.00	0.11±0.00
C-14:1n5	0.57±0.01	0.54±0.00	0.58±0.00
C-15:1n5	0.13±0.01	0.00±0.00	0.00±0.00
C-16:1n7	1.20±0.01	1.19±0.00	1.24±0.01
C-17:1n7	0.22±0.00	0.22±0.00	0.21±0.00
C-18:1t4	0.02±0.00	0.02±0.00	0.02±0.00
C-18:1t5/6/7	0.32±0.00	0.33±0.00	0.32±0.00
C-18:1t9	0.21±0.00	0.22±0.00	0.23±0.00
C-18:1t10	0.45±0.01	0.43±0.00	0.46±0.00
C-18:1t11	3.63±0.04	3.70±0.03	3.55±0.02
C-18:1c9/C-18:1t12/13/	20.28±0.24	20.51±0.15	20.08±0.13
C-18:1t15/C-18:1c11	0.67±0.01	0.71±0.00	0.68±0.00
C-18:1c12	0.13±0.00	0.14±0.00	0.14±0.00
C-18:1c13	0.02±0.00	0.07±0.00	0.06±0.00
C-18:1t16	0.49±0.03	0.56±0.00	0.54±0.01
C-18:1c14	0.00±0.00	0.00±0.00	0.00±0.00
C-18:1c15	0.44±0.05	0.50±0.00	0.48±0.01
C-20:1n9	0.04±0.00	0.05±0.01	0.04±0.00
C-22:1n11	0.02±0.00	0.02±0.00	0.00±0.00
C-24:1n9	0.10±0.00	0.01±0.00	0.00±0.00

and 4 kGy). Of interest for science is the vaccenic acid – C 18:1 trans 11, which is a substrate of the synthesis of the conjugated linoleic acids in the mammal gland by the action of $\Delta 9$ -desaturase enzyme. All trans-isomers except for C-18:1 trans 11 are considered “undesired” because of their different degree of carcinogenicity. Their portion in the total fatty acid spectrum of the analyzed milks varies from 2.16 to 2.26 g/100 g fat. For the vaccenic acid (C18:1 trans 11) and the oleic acid (C18:1cis 9) considerable changes as a result of the gamma irradiation have not been observed.

The polyunsaturated fatty acids decrease from 4.70 g/100 g fat for the control group to 4.47 g/100 g fat at irradiation with 2 kGy and 4.00 g/100 g fat at irradiation with 4 kGy (Table 5). For the alpha-linolenic acid (C18:3n-3) a decrease of the concentration from 0.85 g/100 g fat for the control up to 0.73 g/100 g fat (2 kGy) and 0.64 g/100 g fat (4 kGy), and a comparatively constant level of the gamma-linolenic acid (C18:3n-6) has been observed (Table 3).

The comparatively lower CLA content in the analyzed milk samples compared to the dairy products from sheep milk should be noted (Table 5). The total content of conjugated fatty acids decreases from 1.64 g/100 g fat

for the lyophilized buffalo yoghurt to 1.61 g/100 g fat for irradiated buffalo yoghurt with 2 kGy and 1.37 g/100 g fat for that irradiated with 4 kGy. CLA is a natural component of milk fat [6]. Its content is highest in the control samples – 1.49 g/100 g fat and lowest after irradiation with 4 kGy – 1.23 g/100 g fat. From nutritional point of view of importance are only the configurations cis 9, trans11- and trans 9, cis 11 which represent a functional nutritive component for prevention of intestine and stomach cancer.

The unsaturated long-chain fatty acids hold an important place in human nutrition, in the treatment of coronary and cardio-vascular diseases /omega-3 and omega-6 fatty acids/. The presented generalized results for the fatty acid composition of buffalo yoghurt, analyzed in three groups – non irradiated, irradiated – 2 kGy and 4 kGy, show a comparatively low content of anticarcinogenic substances (CLA). Buffalo yoghurt features a very good proportion ω -6 : ω -3, which is in the range from 2,14 to 2,73. Raw materials and natural foods for which this proportion is < 5 are with a low risk factor for human health. The content of ω -3 decreases from 1.01 g/100 g fat for the control group to 0.85 g/100 g fat after irradiation with 2 kGy and to 0.74 g/100 g fat

Table 3. Polyunsaturated fatty acids, g/100 g fat.

Fatty acid	Control	2 kGy	4 kGy
C-18:2c9,12/19:0	1.70±0.04	1.67±0.01	1.58±0.00
gC-18:3n6	0.06±0.01	0.06±0.00	0.05±0.00
aC-18:3n3	0.85±0.01	0.73±0.01	0.64±0.00
CLA9c,11t	1.49±0.02	1.43±0.01	1.23±0.01
C-18:4n3	0.01±0.00	0.01±0.01	0.00±0.00
CLA9c,11c	0.10±0.00	0.12±0.00	0.11±0.00
CLA9t,11t	0.04±0.01	0.06±0.02	0.04±0.02
C-20:2n6	0.08±0.00	0.08±0.00	0.07±0.00
C-20:3n6	0.05±0.00	0.05±0.00	0.04±0.00
C-20:4n6	0.09±0.00	0.08±0.00	0.06±0.00
C-20:3n3	0.01±0.00	0.00±0.00	0.01±0.00
C-20:5n3	0.06±0.00	0.04±0.00	0.04±0.00
C-22:2n6	0.05±0.00	0.07±0.00	0.06±0.00
C-22:5n3	0.09±0.00	0.07±0.00	0.05±0.00

Table 4. Branched fatty acids, g/100 g fat.

Fatty acid	Control	2 kGy	4 kGy
C-13iso	0.30±0.00	0.04±0.00	0.04±0.00
C-13aiso	0.01±0.00	0.01±0.00	0.01±0.00
C-14iso	0.16±0.03	0.13±0.00	0.15±0.00
C-15iso	0.30±0.00	0.26±0.00	0.26±0.00
C-15aiso	0.59±0.01	0.57±0.00	0.59±0.00
C:16iso	0.31±0.01	0.27±0.00	0.28±0.00
C-17iso	0.33±0.00	0.34±0.00	0.35±0.00
C-17aiso	0.41±0.00	0.41±0.00	0.41±0.00
C-18iso	0.06±0.00	0.07±0.00	0.06±0.00

Table 5. Groups fatty acids, g/100 g fat.

Fatty acid	Control	2 kGy	4 kGy
Σ CLA	1.64±0.01	1.61±0.01	1.38±0.02
Σ C-18:1Trans-FA	5.79±0.05	5.97±0.04	5.81±0.01
Σ C-18:1Cis-FA	20.87±0.21	21.22±0.15	20.76±0.10
SFA	65.49±0.73	66.27±0.47	66.96±0.43
MUFA	29.42±0.27	29.73±0.18	29.19±0.13
PUFA	4.70±0.03	4.47±0.02	4.00±0.02
Σ n-3	1.01±0.01	0.85±0.01	0.74±0.01
Σ n-6	2.17±0.04	2.15±0.01	2.02±0.00
Σ n-6 / Σ n-3	2.14±0.07	2.53±0.04	2.72±0.03
Branched FA	2.47±0.01	2.08±0.01	2.15±0.01
CLA	1.49±0.02	1.43±0.01	1.23±0.01

after irradiation with 4 kGy. In contrast to ω -6 the content of which decreases insignificantly from 2.17 g/100 g fat for the control group to 2.02 g/100 g fat after irradiation with 4 kGy.

The data for the branched fatty acids (Table 4) show that depending on the degree of irradiation their quantity varies in very narrow ranges and their total content is from 2.47 g/100 g fat for the control group to 2.15 g/100 g fat for the irradiated with 4 kGy lyophilized buffalo yoghurt (Table 5). The branched fatty acids represent position isomers with concentration which does not exceed 3 % of the fatty acid spectrum of milk and dairy products.

CONCLUSIONS

The assessment of the fatty acid profile of lyophilized buffalo yoghurt, treated with 2 different doses of gamma rays, for prolongation of their shelf-life, has shown that significant changes in the content of the saturated fatty acids are not observed.

The content of the short-chain fatty acids (C4:0, C6:0, C8:0 and C10:0) increases insignificantly during the process of irradiation.

The level of the useful for healthy nutrition oleic and vaccenic fatty acids is comparatively constant as a result of treatment of the buffalo lyophilized yoghurt with gamma-radiation.

With the increasing of the radiation value, the CLA concentration decreases from 1.49 g/100 g fat for the non irradiated group to 1.43 g/100 g fat for the yoghurt irradiated with 2 kGy and to 1.23 g/100 g fat at 4 kGy.

The lyophilized buffalo yoghurt is characterized by a very good proportion of ω -6 : ω -3 which varies from 2,14 to 2,73, i.e. the obtained irradiated yoghurts are with a low risk factor for human health status.

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