# Study of the impact of radiation treatment upon biochemical properties of lyophilized dairy products

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The effect of freeze-drying and radiation treatment on the protein profile and fatty acid composition of lyophilized cow's milk and curd was studied. The results of SDS-PAGE showed that lyophilization did not alter the qualitative composition of the main proteins in milk and curd. A significant decrease in casein and whey proteins was observed after irradiation with 10 kGy. These changes in protein components are associated with a decrease in allergenicity after radiation treatment. Changes in fatty acid composition are reflected by an increase in saturated fatty acid content and a decrease in monounsaturated and polyunsaturated fatty acids. In connection with the reported experimental data it is recommended that milk and curd should be totally skimmed before radiation treatment for potential decrease of the allergenicity.

**Keywords:** dairy products, fatty acids profile, irradiation, lyophilization, protein profile.

## INTRODUCTION

The irradiation of food is a perspective technology for prevention of food spoilage by reducing the number of microorganisms, increasing the safety and extending the shelf life with minimal impact over the functional, nutritional and sensorial properties [1-3].

In addition, some results indicate that treating with ionizing radiation could cause decrease of the allergenicity by destruction of IgE-binding epitopes in nutritional allergens [4-6].

The appearances of allergic diseases, especially in children, are becoming more frequent during the last several decades. Food allergy is a serious health problem that is affecting 4-6% of the children and 1-3% of the adults [7]. Cow milk allergy is probably the most common food allergy in infancy and it is a clinical manifestation of an abnormal immunological response to one or more milk proteins [8]. The main allergens in cow milk are caseins, β-lactoglobulin, α-lactalbumin, bovine serum albumin and bovine serum IgG [9]. The β-lactoglobulin is considered as the most important allergen of them, because it is not present in the human milk and gives the highest percentage of positive reactions in oral administration (66%), although the casein shows the highest reactivity degree in skin test (68%) [10, 11].

Different technological approaches are tested to solve the problem with allergenicity of cow's milk. The decrease of the allergenicity of cow's milk by enzyme hydrolysis with proteolytic enzymes is the most common method [12, 13].

These hypoallergenic formulas have unsatisfactory taste, due to the presence of bitter peptides and amino acids [8]. Also it is reported about cases of allergy to some milk hydrolysates [14].

Other strategies for elimination or modification of allergens in milk are heat treatment, glycation reactions, high pressure processing and lactic acid bacteria fermentation. A lot of research for the structural modification of food allergens by gamma irradiation is done over the last few years.

The ionizing radiation could decrease allergenicity of the milk proteins by eliminating of the IgE-binding epitopes [4, 15]. Lee *et al.* investigated the changes in the allergenicity and antigenicity of irradiated milk proteins by competitive indirect enzyme-linked immunosorbent assay. The authors reported that epitopes in milk allergens are structurally changed by the gamma-irradiation and concluded that allergenicity of the milk proteins could be decreased by this method [16]. For better understanding of the possible decrease of the allergenicity by irradiation technology, it is important that the effect upon the specific milk proteins is clarified. Besides this, the information for the changes in fatty acids compounds of milk fat after irradiation of milk or dairy products is limited.

The purpose of the present study is to perform a comparative analysis of the protein profile and fatty acids content of the lyophilized milk and curd, and to evaluate the impact of radiation treatment for potential decrease of the allergenicity to these indicators.

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## EXPERIMENTAL

*Material*: Skimmed cow milk (3.1% protein, 0.5% fat) and whey curd from cow milk (13.2 % protein, 0.5 % fat) were bought from a local supermarket in Sofia, Bulgaria. The curd was prepared by coagulation of the proteins in the whey after producing of the white brine cheese. All the chemicals used were of analytical grade.

The samples from homogenized curd and milk were distributed in metal trays (Food-Grade Stainless Steel) and frozen under (-25°C).

*Lyophilization:* The freeze drying was carried out in a freeze dryer TG 16.50 (Hochvacuum–Germany), at the following parameters: primary drying temperature – (-40)$^0$C, temperature of the desublimator – (-65°C), maximal working vacuum – $2.10^1$ Pa; secondary drying temperature + 30°C. Duration of the process – 24 ± 1 h.

The lyophilizates were crushed and distributed in vacuum-sealed packages of triple aluminium foil. All samples were kept at 10 ± 2°C until the analyses were done.

*Radiation treatment:* Part of the lyophilized samples were irradiated in the National Centre of Radiobiology and Radiation Protection (NCRRP), Sofia, Bulgaria, on a gamma-irradiating installation - “NIGU-7”, with cobalt-60 as a source of gamma-rays and dose rate - 2 $kGy.h^{-1}$. The applied dose was 10 kGy. The measurements of the dose were done by alanine dosimetry system with film alanine dosimeters (BioMax Kodak) and ESR spectrometer E-scan Bruker.

### *Physicochemical analysis*

Determination of residual moisture after lyophilization - the moisture content of the lyophilized samples was measured with a Sartorius Thermo Control YTC 01L balance; total protein content – by the Kjeldahl method [17]; total fats - by extraction with hexane in a “Soxtec 2005” apparatus; total ash – by sample mineralization in a muffle oven [18].

### *SDS – Polyacrylamide gel electrophoresis*

SDS–PAGE was performed by the method of Laemmli [19] at concentrations of stacking gel – 6% and running gel – 12%, using an OmniPAGE WAVE Electrophoresis System (Cleaver Scientifics). Samples were rehydrated with distilled water and diluted with sample buffer (0.2 M Tris-HCl, pH 6.80, containing 2% SDS, 16% glycerol and 10 mM DDT). Protein Test Mixture for SDS PAGE (SERVA Electrophoresis), α–lactalbumin, β-lactoglobulin, α-casein and β-casein (Sigma-Aldrich) were used as standard proteins. Gel was stained using 0.1% Coomassie Brilliant Blue (30-40 min).

### *Fatty acids analysis*

The extraction of total lipids was done by the method of Roese-Gottlieb [20] using diethyl and petroleum ether. The transesterification of milk fat was done using sodium methylate (Merck) followed by drying with $NaHSO_4.H_2O$.

The fatty acids methyl esters (FAME) were analyzed using a gas chromatograph Shimadzu-2010 (Kyoto, Japan) with a flame ionization detector and an automated injection system (AOC-2010i). The analysis was done on a capillary column CP7420 (100 m × 0.25 mm i.d., 0.2 μm film, Varian Inc., Palo Alto, CA). Hydrogen was used as a carrier gas, and nitrogen as a make-up gas.

### *Statistical analysis*

All experimental measurements were carried out in triplicate. Differences in mean values for fatty acids before and after irradiation were calculated by t-test. The statistical processing of data was performed by using Microsoft Excel 2013. The molecular weights of the main protein fractions in cow’s milk and curd samples were calculated using the protein standards. The applied software was GelAnalyzer 19.0.

## RESULTS AND DISCUSSION

### *Physicochemical analysis of lyophilized milk and curd*

The obtained lyophilized products are with significant differences in quantity of the total protein – from 33.58% in milk to 43.58% in curd (Table 1). The lipid content in both types of samples is about 5 %. All samples are with low residual moisture: 3.14% and 2.96%, respectively. The low water content guarantees the long-term storage at room temperature without any changes in the main components.

**Table 1**. Composition of samples of lyophilized cow's milk and curd. Data represent means ± SD (n = 3)

| Parameter | Lyophilized milk | Lyophilized curd |
|---|---|---|
| Residual moisture (%) | 3.14±0.32 | 2.96±0.24 |
| Fat (%) | 4.80 ± 0.15 | 5.20±0.56 |
| Protein (%) | 33.58± 0.78 | 43.58±0.89 |
| Ash (%) | 1.95±0.38 | 2.82±0.21 |

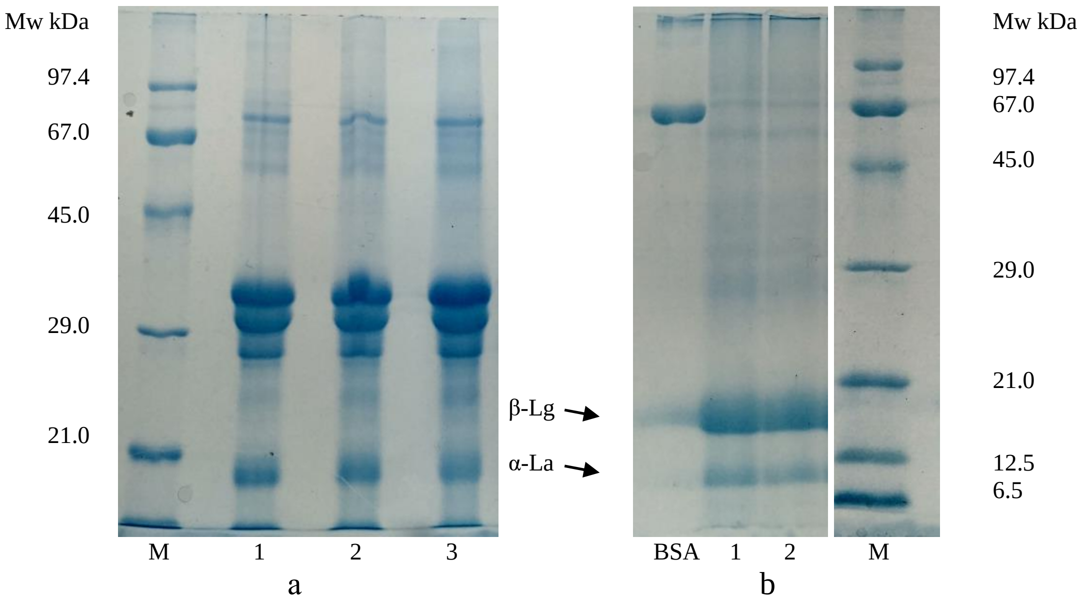


**Fig. 1**. SDS – PAGE of raw and lyophilized dairy products. a) milk: 1 and 2 – raw milk; 3- lyophilized milk; M-protein marker; b) BSA–bovine serum albumin,1-raw curd; 2-lyophilized curd; M-protein marker

*SDS – PAGE of the lyophilized and irradiated cow's milk and curd*

The results from electrophoretic analysis of milk samples show a similar picture of casein and whey fractions before and after lyophilization (Fig. 1a). In all samples the presence of intensive casein fractions (α-casein, β-casein and to a lesser extent κ-casein), β-lactoglobulin (β-Lg) and α–lactalbumin (α-La) is detected. A fraction with a molecular mass of 67 kDa, corresponding to serum albumin and a fraction with a molecular mass about 82-84 kDa, which respond to lactoferrin, are also observed.

The whey curd contains mainly β-lactoglobulin and α-lactalbumin while the casein fractions are minimal (Fig. 1b). In both curd samples (raw and lyophilized) an intensive band with Mw of 18 kDa (β-lactoglobulin) and a band with Mw – 12.4 kDa, (α-lactalbumin) are estimated. In the upper section of the gel, faint bands with molecular weight of 83 kDa (lactoferrin), 67 kDa (BSA) and 59 kDa are detected. Therefore, the lyophilization does not change the protein profile and the composition of the main protein components in milk and curd.

Figure 2 presents SDS – PAGE of lyophilized milk and curd, irradiated with a dose of 10.0 kGy. Distinguishable changes in the protein profile are observed in both types of irradiated samples, compared to unirradiated. In milk samples, irradiated with 10 kGy (2A), the fractions with higher molecular mass - 83 kDa, 67 kDa (BSA) and 59 kDa are missing. The intensity of the α-casein band is reduced by 20.0% and those of the β-casein - by 13.0%. For the whey proteins, β-lactoglobulin and α-lactalbumin, the decrease is with 11.0 and 19.8%, respectively. The main protein components of the curd are also changed after gamma-irradiation (2B). The decrease in the intensity of β-lactoglobulin and α-lactalbumin is by 15.5% and 32.1%, respectively.

A similar reduction in the content of the α-casein and β-casein in milk and Queso Blanco cheese after irradiation with 10 kGy was reported by Ham *et al.* [21]. Their results suppose that from the casein fractions, $\alpha_{S1}$-casein and $\beta_{A1}$-casein are more susceptible to gamma-irradiation and could be connected with the decrease of the allergenicity after radiation treatment. Significant changes in electrophoretic profile after gamma-irradiation of isolated β-lactoglobulin and α-casein in solution, are estimated, while the dose is increased [16].

These and other similar results from different authors refer mainly to the irradiation of milk and milk proteins in aqueous solutions. There is little data in the available literature about the changes in the proteins in lyophilized dairy products, after radiation treatment. According to Audette-Stuart *et al.* [22], the radiolysis induces mainly fragmentation and/or aggregation of the protein, and it matters whether it is in solution, or in solid form (frozen or lyophilisate). There is a significant reduction of structural damage in the lyophilized proteins and it is mainly represented by partial fragmentation.

### *Fatty acids composition of the milk fat in lyophilized milk and curd*

Milk fat is a mixture of short-, medium- and long-chain fatty acids, in different quantitative ratios depending on the nutrition, lactation and species specificity of the organism and technological treatment of producing dairy products. More detailed study of the fatty-acids profile of the samples of cow's milk and curd is presented in Tables 2 and 3. The lyophilized milk contains 70.91 g/100 g fat saturated fatty acids (SFA), while in the lyophilized curd the quantity of SFA is 61.38 g/100 g fat. The total amount of lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids in milk is 51.70 g/100 g fat, and in curd – 45.65 g/100 g fat. The content of stearic acid (C 18:0) in milk and curd is 12.31 g and 13.24 g/100 g fat, respectively. The data for the branched chain fatty acids (BFA) show that depending on the product, their content is different and varies from 3.10 g/100 g fat in lyophilized milk to 1.35 g/100 g fat in curd (Table 2).

Monounsaturated fatty acids (MUFA) are in concentrations of 24.37 g/100 g fat in lyophilized milk and 31.17g/100 g fat in lyophilized curd (Table 3). The main representatives of MUFA in the investigated dairy products are palmitoleic (C-16:1n7), vaccenic (C18:1trans11) and oleic (C18:1 cis9) acid. The total amount of trans fatty acids in lyophilized milk is 1.61 g/100 g fat, and in curd – 2.44 g/100 g fat.

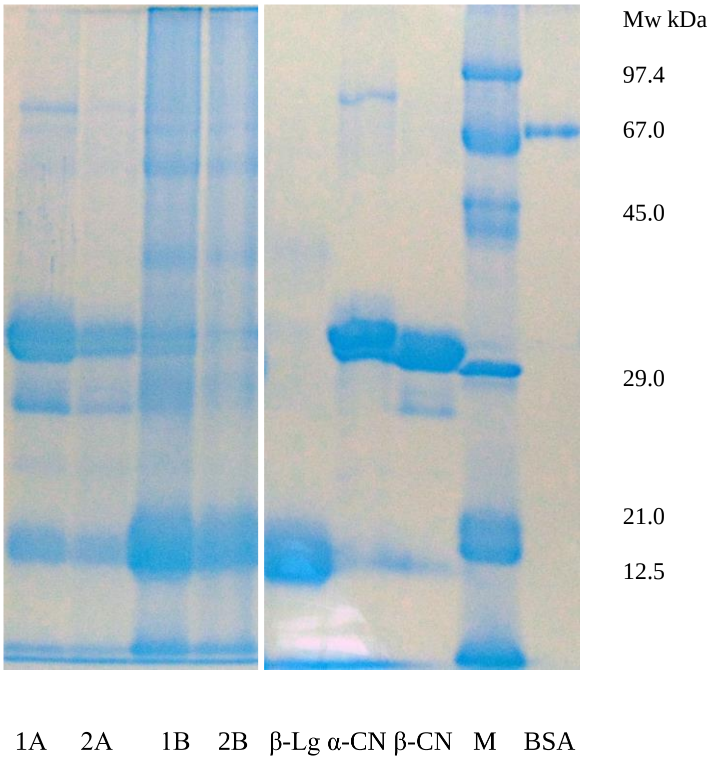


**Fig. 2**. SDS – PAGE of irradiated samples of lyophilized dairy products. 1A–lyophilized milk (0 kGy); 2A-lyophilized milk (10 kGy); 1B–lyophilized curd (0kGy); 2A- lyophilized curd (10 kGy); β-Lg – β-lactoglobulin; α-CN – α-casein; β-CN- β-casein; M – protein marker; BSA – bovine serum albumin.

**Table 2.** Saturated fatty acids and branched fatty acids (g /100 g fat) of lyophilized cow's milk and curd

| Saturated fatty acids | | | | | |
|---|---|---|---|---|---|
| SFA | Milk | Curd | SFA | Milk | Curd |
| C-4:0 | 2.37 | 0.48 | C-13:0 | 0.00 | 0.03 |
| C-6:0 | 1.11 | 0.23 | C-14:0 | 11.58 | 8.71 |
| C-7:0 | 0.06 | 0.02 | C-15:0 | 0.65 | 0.07 |
| C-8:0 | 0.56 | 0.16 | C-16:0 | 37.87 | 35.09 |
| C-9:0 | 0.03 | 0.02 | C-17:0 | 0.72 | 0.25 |
| C-10:0 | 1.27 | 1.03 | C-18:0 | 12.31 | 13.24 |
| C-11:0 | 0.02 | 0.01 | C-20:0 | 0.01 | 0.07 |
| C-12:0 | 2.25 | 1.85 | C-23:0 | 0.10 | 0.08 |
| Branched fatty acids | | | | | |
| BFA | Milk | Curd | BFA | Milk | Curd |
| C-13iso | 0.25 | 0.02 | C:16iso | 0.29 | 0.02 |
| C-13aiso | 0.03 | 0.04 | C-17iso | 0.42 | 0.24 |
| C-14iso | 0.09 | 0.00 | C-17aiso | 0.41 | 0.54 |
| C-15iso | 0.24 | 0.35 | C-18iso | 0.05 | 0.01 |
| C-15aiso | 1.33 | 0.14 | | | |

**Table 3.** Monounsaturated and polyunsaturated fatty acids (g/100 g fat) of lyophilized cow's milk and curd

| Monounsaturated fatty acids | | | | | |
|---|---|---|---|---|---|
| MUFA | Milk | Curd | MUFA | Milk | Curd |
| C-10:1 | 1,10 | 0.02 | C-18:1c9/C-18:1t12/13/ | 18.07 | 26.02 |
| C-12:1n1 | 0.02 | 0.02 | C-18:1t15 | 0.17 | 0.25 |
| C-14:1n5 | 0.08 | 0.13 | C-18:1c11 | 0.04 | 0.03 |
| C-15:1n5 | 0.05 | 0.00 | C-18:1c12 | 0.03 | 0.03 |
| C-16:19tr | 0.49 | 0.03 | C-18:1c13 | 0.00 | 0.04 |
| C-16:1n7 | 2.33 | 1.77 | C-18:1t16 | 0.23 | 0.01 |
| C-17:1n7 | 0.05 | 0.02 | C-18:1c14 | 0.07 | 0.11 |
| C-16:3n4 | 0.02 | 0.00 | C-18:1c15 | 0.23 | 0.08 |
| C-18:1t4 | 0.03 | 0.21 | C-20:1n9 | 0.00 | 0.06 |
| C-18:1t5/6/7 | 0.13 | 0.38 | C-22:1n11 | 0.03 | 0.01 |
| C-18:1t9 | 0.24 | 0.65 | C-22:1n9 | 0.01 | 0.11 |
| C-18:1t10 | 0.37 | 0.53 | C-24:1n9 | 0.00 | 0.02 |
| C-18:1t11 | 0.58 | 0.63 | | | |
| Polyunsaturated fatty acids | | | | | |
| PUFA | Milk | Curd | PUFA | Milk | Curd |
| C-18:2t9,12 | 0.06 | 0.01 | CLA9t,11t | 0.01 | 0.04 |
| C-18:2c9,12/19:0 | 2.01 | 5.49 | C-20:2n6 | 0.08 | 0.08 |
| gC-18:3n6 | 0.29 | 0.26 | C-20:3n6 | 0.06 | 0.05 |
| aC-18:3n3 | 0.06 | 0.22 | C-20:4n6 | 0.40 | 0.04 |
| CLA9c,11t | 0.21 | 0.03 | C-20:3n3 | 0.16 | 0.01 |
| CLA10t,12c | 0.02 | 0.06 | C-20:5n3 | 0.03 | 0.20 |
| C-18:4n3 | 0.07 | 0.01 | C-22:2n6 | 0.06 | 0.05 |
| CLA9c,11c | 0.02 | 0.09 | | | |

MUFA, as a part of the human diet, do not cause cholesterol accumulation like the saturated acids, and do not oxidize easily like polyunsaturated ones. Also, the consumption of oils rich in monounsaturated fatty acids has a positive effect upon high-density lipoproteins (HDL) transporting the cholesterol from the walls of the blood vessels to the liver, where it is degraded. Meanwhile, monounsaturated fats lead to a decrease in the concentration of low-density lipoproteins (LDL) [23].

The content of the polyunsaturated fatty acids (PUFA) in lyophilized milk is 3.56 g/100 g, and in curd – 6.64 g/100g. The quantity of α- and γ-linolenic acid in milk and curd is 2.01 g/100 g and 5.49 g/100 g, and the total content of conjugated linoleic acids (CLA) is 0.26 g/100 g and 0.21 g/100 g, respectively.

The differences in the fatty-acids profile of the two lyophilized products are most significant in terms of the content of unsaturated fatty acids. The curd contains 27.90% more monounsaturated fatty acids and 86.50% more polyunsaturated fatty acids as compared to milk.

The application of gamma-irradiation with 10 kGy in cow's milk and curd leads to a significant ($P \leq 0.05$ and $P \leq 0.01$) increase of saturated fatty acids. The content of SFA in lyophilized milk rises by 19.22%, and in curd - by 28.57%. The amount of short-chain fatty acids (C4:0, C6:0, C8:0 and C10:0) also rises during the irradiation, and so does the content of stearic acid (C 18:0) while the content of myristic (C14:0) and lauric (C12:0) acid remains almost constant in milk but grows in curd.

**Table 4**. Groups of fatty acids (g/100 g fat) of lyophilized cow's milk and curd, before and after treatment with 10 kGy. Data are expressed as means ± SD (n = 3)

| Group FA | Milk | | Curd | |
|---|---|---|---|---|
| | 0 kGy | 10 kGy | 0 kGy | 10 kGy |
| Σ CLA | 0.26±0.02*** | 0.04±0.01 | 0.21±0.03** | 0.27±0.05 |
| ΣC-18:1Trans-FA | 1.61±0.54* | 0.34±0.10 | 2.44±0.48** | 0.85±0.23 |
| Σ C-18:1Cis-FA | 18.40±0.60** | 12.88±1.12 | 26.28±1.81** | 17.51±1.43 |
| SFA | 70.91±4.83* | 84.54±6.07 | 61.38±2.40** | 78.62±3.87 |
| MUFA | 24.37±4.68* | 14.98±2.74 | 31.17±2.95** | 19.30±3.48 |
| PUFA | 3.56±1.17* | 0.23±0.05 | 6.64±1.99* | 1.85±0.38 |
| Σ n-3 | 0.33±0.01*** | 0.08±0.02 | 0.44±0.06 | 0.34±0.10 |
| Σ n-6 | 3.00±0.27*** | 0.10±0.02 | 6.02±0.78** | 1.31±0.17 |
| Σ n-6/Σ n-3 | 9.19±0.71*** | 1.28±0.09 | 13.60±2.46 | 3.84±0.37 |
| Branched FA | 3.10±0.32* | 2.59±0.31 | 1.35±0.15* | 0.85±0.20 |
| CLA | 0.21±0.02*** | 0.01±0.00 | 0.03±0.01** | 0.08±0.01 |

*P≤0.05, **P≤0.01, ***P≤0.001

The monounsaturated fatty acids in lyophilized milk are in a concentration of 24.37 g/100 g fat and decrease significantly ($P \leq 0.05$) to 14.98 g/100 g fat after irradiation with 10 kGy. The results of the studied curd are analogous – significant decrease from 31.17 g/100 g fat to 19.30 g/100 g fat ($P \leq 0.01$). The content of the polyunsaturated fatty acids significantly decreases ($P \leq 0.05$) in both milk and curd samples after gamma-irradiation. The same trend is observed in biologically active fatty acids from the group of omega-3, omega-6 and CLA.

The assumed data for the fatty-acids content of the lyophilized milk and curd samples, treated with 10 kGy are shown in Table 4.

## CONCLUSION

The results from the study show significant differences in the protein profile and fatty-acids content of the two investigated types of lyophilized products – skimmed cow's milk and curd produced by coagulation of the proteins of whey after the production of white brine cheese. The amount of total protein in the lyophilisates is much higher in curd, while the fat content in both products is about 5%. The results from SDS-PAGE show that the lyophilization does not change the content of the main protein component in cow's milk and curd. In the lyophilized milk, the main casein fractions, β-Lg, α-La, serum albumin and lactoferrin are established. The lyophilized whey curd contains mainly β-Lg and α-La and minimal amounts of casein fractions. As far as the fatty-acids content in the curd is concerned, a lower content of saturated fatty acids and higher content of mono- and polyunsaturated fatty acids was estimated in comparison to milk.

A change was observed in the electrophoretic image of casein fractions (mainly α-casein) and whey proteins, after irradiation of lyophilized milk with 10 kGy. There was also a significant decrease in the intensity of β-lactoglobulin and α-lactalbumin bands in irradiated curd. These changes in the protein components could be connected with the decrease in the allergenicity after radiation treatment. Changes in fatty acids profile after gamma irradiation in both types of lyophilized products were expressed in an increase in saturated fatty acids content and a decrease of monounsaturated and polyunsaturated fatty acids. In connection with the reported experimental data it is recommended that milk and curd should be totally skimmed before radiation treatment for potential decrease of the allergenicity.

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