Artículo de investigación

Assessment of blood lipids peroxidation after the introduction of spirulina-containing melted cheese product (experimental study)

Оценка Процессов Перекисного Окисления Липидов Крови при Включении В Рацион Спирулиносодержащего Плавленого Сырного Продукта (Экспериментальное Исследование)

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Abstract

The article presents the results of activating the free-radical lipids oxidation in the erythrocytes and blood plasma of white rats that received spirulina-containing melted cheese product along with the basic diet in the amount of up to 10 % of the body weight of animals. A more pronounced cytotoxic effect was detected after the introduction of Chinese arthrospira into the composition of the cheese product; on the contrary, activation of lipid peroxidation of the blood plasma was more pronounced in animals after the introduction of melted cheese containing the arthrospira from the Solenoye Lake near Omsk into the diet.

Keywords: Lipid peroxidation, free-radical oxidation, antioxidants, cyanobacteria, spirulina, innovative technology, melted cheese product.

Аннотация

В статье приведены результаты активации свободнорадикального окисления липидов эритроцитов и плазмы крови белых крыс, получавшие к основному рациону 10% к массе тела животных спирулиносодержащего плавленого сырного продукта. Определен более выраженный цитотоксический эффект, при включении в состав сырного продукта артроспиры Китайского происхождения, напротив, активация липидпероксидации плазмы крови более выражена у животных, при включении в их рацион плавленного сыра, содержащего артроспиру озера Соленое г. Омска.

Ключевые слова: перекисное окисление липидов, липидпероксидация, свободнорадикальное окисление, антиоксиданты, цианобактерия, спирулина, инновационная технология, плавленый сырный продукт.

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Introduction

Cyanobacterium Arthrospira (Spirulina) platensis (family Oscillatoriaceae; Department Blue-green algae – Cyanophyta) has microscopic autotrophic structure and grows in an alkaline solution of inorganic minerals. Due to its unique chemical composition, arthrospira (spirulina) is a popular object of biotechnology. A number of scientists recommend using it as a source of protein, β-carotene, vitamins, mineral substances for improving organism resistivity, and as a source of natural antioxidants. The positive effect of arthrospira on the useful intestinal microflora (lactobacilli and bifidobacteria) by reducing the level of opportunistic microorganisms has been proven.

Currently, a wide range of arthrospira-based products is present in the market of biologically active additives. The main suppliers of spirulina in the world are Mexico, the USA, Japan, India, China, and Thailand, where the production of spirulina exceeds 100 tons per year. In Russia, spirulina is quite popular but is mostly imported from other countries. The domestic production of spirulina in located in Moscow (Spirulina MSU), Sochi (Sochi Spirulina), Samara (live spirulina in the form of a frozen paste), and in Sevastopol. On the territory of Omsk, during the study of shallow lake Solyenoye, mass vegetation of cyanobacteria of another species – Arthrospira fusiformis – was discovered. The Omsk scientists (Bazhenova, Konovalova, 2012) in their works noted the high nutritional value of arthrospira and its positive influence on the organism of experimental animals after its introduction into the diet in the form of phytomass (Bazhenova, Isirgepova, Konovalova, 2012; Vodolaga, et al., 2017).

Despite the fact that the overall effect of spirulina on the human and animal organisms has been fairly well studied, the literature contains no data about the state of the antioxidant system in the organism after introducing spirulina into the composition of products.

The work was aimed at studying the intensity of erythrocytes and blood plasma lipid peroxidation in white rats after the introduction of melted cheese product with spirulina into their diet.

Materials and methods

Experiments were performed with male white outbred rats with the body weight of 180 – 200 g, which were divided by principle of analogues into four groups, five animals in each: the first – the reference biological group that received every day only the standard main diet of the vivarium (group I); the second – the experimental group that received the main diet of the vivarium along with 2,0 g sliced melted cheese product made according to the traditional methods (group II); the third – the experimental group that received every day the main diet of the vivarium with 2,0 g melted cheese product containing 10 % of Arthrospira fusiformis from the Solyenoe Lake in the Omsk region (group III); the fourth – the experimental group that received every day the main diet of the vivarium with 2,0 g melted cheese product containing 10 % of biologically active additives based on Arthrospira platensis made in the People's Republic of China (group IV).

The Spirulina platensis-containing melted cheese product was made based on the accumulated experience of scientists and professionals, and according to the following legislative and normative documents: SanPiN 2.3.2.1293-03, SanPiN 2.3.2.1078-01, SanPiN 2.3.2.2804-10, CU TR 029 2012, 52349-05 GOST R, GOST R 5406-10, GOST R 55577-2013, Guidelines MK MR 2.3.1.1915-04, as well as the technology of making spirulina-containing melted cheese product developed at the Department of Food and Nutritional Biotechnology of the Omsk State Agrarian University. The percentage of introducing the spirulina-containing component did not contradict the basic requirements for enriching food products with functional ingredients. The following factors (Gordeev, et al., 2016; Moliboga, Gavrilova, 2014) were the basis for using spirulina combined with the dairy base:

- Increasing the food and biological value of the products;
- Forming functional properties of the product;
- Improving or modifying the organoleptic parameters: appearance, taste, texture;
- Stimulating the growth of probiotic microorganisms, i.e., the prebiotic effect;
- Saving the main raw milk by reducing milk content in the product; and
- Improving the quality, safety and prolonging the shelf life of the product, which did not contradict the assortment policy of healthy food products (Gordeev, et al., 2016; Moliboga,

Gavrilova, 2014; Khoroshavina, Moliboga, Boiko, 2017).

The animals were observed within 40 days by assessing the palatability of the cheese product, the appearance of the experimental animals, and the state of fecal boluses. At the end of the observation period, the animals under sedation were removed from the experiment by exsanguination. Whole blood was centrifuged and separated into plasma and erythrocytes.

Parameters of lipid peroxidation were studied in heptane-isopropanol extracts of erythrocytes and blood plasma in the modification of I. A. Volchegorsky et al. (1989). The necessity of using two phases is due to the nature of extraction; for instance, heptane mainly extracts neutral lipids, and isopropanol extracts phospholipids, which are the main substrates of lipid peroxidation. The content of molecular products of lipid peroxidation (LPO) was determined in each extracted phase of erythrocytes and blood plasma by spectrophotometry at 220, 232, 278, and 400 nm: diene conjugates (DC), ketodienes and conjugate of trienes (KD/CT), and final LPO products – Schiff's bases (SB). The results were expressed in oxidative index units (o.i.u.), which was calculated as the ratio of E232/220, E278/220, and E400/220. The obtained results were mathematically processed using the standard methods of variational statistics using the Statistica 6.0 software. The differences were considered statistically significant at $p \leq 0.05$ from the level of the reference group (Rebrova, 2006).

Results

Throughout the period of observation, no death and behavioral disorders were observed in the experimental animals. On the contrary, the animals with high activity ate the cheese product and equally preferred it to the basic diet. The state of the hair coat did not visibly change; fecal boluses in the rats from the experimental groups had lighter color than in the reference.

It is known that cyanobacteria Arthrospira platensis have rich mineral composition, contain calcium, phosphorus, magnesium, copper, iron, potassium, sodium, chlorine, and some other substances. Ions of variable valency metals – iron and copper – in the composition of spirulina are in organically bound form, so they are better absorbed. However, it has been noted that most natural antioxidants, depending on the conditions of the redox reactions and their concentrations (ascorbic acid), in the presence of ions of metals Fe3+ or Cu2+ induce decomposition of peroxides, and, like flavonoids, can act as pro-oxidants.

In initiating the chain of LPO, particular importance is attached to H2O2 and the hydroxyl radical (Figure 1). Due to its relative lipophilicity, H2O2 can freely diffuse through the phospholipid bilayer of the cell membranes. Taking an electron from Fe+2, hydrogen peroxide restores to a hydroxyl radical, which, in turn, interacts with polyunsaturated fatty acelas with the formation of alkyl radicals.

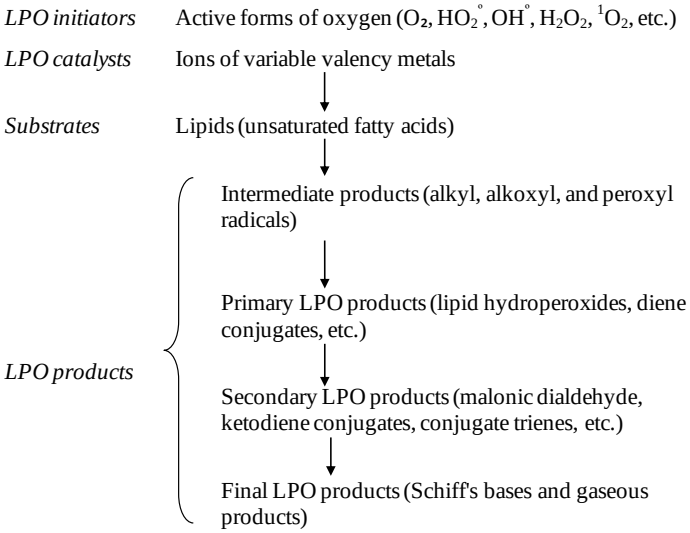

Figure 1. Scheme of lipid peroxidation chain

The next stage is the interaction of the alkyl radical with molecular oxygen with the formation of the hydroperoxide radical. Further LPO stages are associated with oxidation of new lipid molecules with the formation of lipid hydroperoxides, which are unstable compounds. Spontaneous decomposition of hydroperoxides with the emergence of alkoxyl radicals characterizes the free-radical oxidation (FRO) as an expressed excessive chain multistage process with additional branches.

In this paper, the intensity of LPO processes was assessed with separate identification of LPO products in heptane and isopropanol phases of the lipid extract of erythrocytes and blood plasma. The effect of Arthrospira platensis and Arthrospira fusiformis in the rats' diet on the content of blood plasma LPO products is shown in Tables 1 and 2.

Table 1. The relative content of LPO products in the heptane phase of blood plasma lipid extract, (M ± m)

Indicator (*o.i.u.)*	Groups of animals			
	Group I	Group II	Group III	Group IV
Diene conjugates	0.8166 ± 0.0070	0.7959 ± 0.0083	0.7584 ± 0.0102^{x}	0.8014 ± 0.0081
Ketodienes and conjugated trienes	0.0978 ± 0.0022	0.0883 ± 0.0031^{x}	0.1168 ± 0.0050^{x}	0.1040 ± 0.0051
Schiff's bases	0.0198 ± 0.0024	0.0086 ± 0.0021^{x}	0.0287 ± 0.0037^{x}	0.0280 ± 0.0036^{x}

Note: ^{x} *– p < 0.05*

The content of diene conjugates in all studied groups was equal, and in group III of animals, a decrease of 7.1 % was noted, compared to the reference. The level of ketodienes and conjugated trienes that characterize the content of secondary LPO products, in the heptane phase of the blood plasma lipid extract decreased by 9.7 % in group II of the rats that received melted cheese product, but increased in experimental groups III and IV of animals whose diet contained additional component, namely, spirulina. The increase in the content of secondary LPO products in group III, compared to the reference, was 19.4 %. Similar changes were also detected in the content of Schiff's bases – the final products of lipid oxidation.

Identification of LPO products in the isopropanol phase allows assessing the intensity of free radical oxidation of polar lipids. The content of primary products of LPO polar lipids in the blood plasma of male white rats increased in group II by 12.3 %, compared to the reference (Table 2). The greatest increase in the content of diene conjugates was observed in the animals that received melted cheese product with spirulina; in group III, this indicator increased by 38.5 %, and in group IV – by 25.9 %.

Table 2. The relative content of LPO products in the isopropanol phase of blood plasma lipid extract, (M ± m)

Indicator (*o.i.u.)*	Groups of animals			
	Group I	Group II	Group III	Group IV
Diene conjugates	0.2823 ± 0.009	0.3169 ± 0.010^x	0.3909 ± 0.013^x	0.3553 ± 0.008^x
Ketodienes and conjugated trienes	0.1157 ± 0.002	0.1047 ± 0.003	0.1359 ± 0.005^x	0.2668 ± 0.005^x
Schiff's bases	0.0064 ± 0.002	0.0098 ± 0.002^x	0.0374 ± 0.003^x	0.0324 ± 0.003^x

Note: ^{x} – $p < 0.05$

The introduction of melted cheese product into the diet of white rats did not change the level of secondary (KD, CT) and final products of peroxidation (FPO) of the blood plasma lipids, while their content in experimental groups III and IV of the animals that received melted cheese product with Arthrospira fusiformis from the Solyenoye Lake near Omsk and Arthrospira platensis made in the People's Republic of China increased, compared to the reference: secondary products – by 17.5 % and 130.6 %, final products – 5.8 and 5.1 times, respectively.

The content of LPO products in the blood of the male white rats in all experimental groups is shown in Tables 3 and 4.

In the second experimental group of animals, diet of which included only melted cheese product, a decrease of primary (DC) and secondary products (KD, CT) of LPO by 5.1% and 14.5%, respectively, was detected in the heptane phase of erythrocytes extract, compared to the values in the reference group of animals.

Table 3. The relative content of LPO products in the heptane phase of erythrocytes lipid extract, (M ± m)

Indicator (*o.i.u.)*	Groups of animals			
	Group I	Group II	Group III	Group IV
Diene conjugates	0.8084 ± 0.009	0.7673 ± 0.011^x	0.8034 ± 0.010	0.9148 ± 0.014^x
Ketodienes and conjugated trienes	0.0847 ± 0.002	0.0724 ± 0.004^x	0.0789 ± 0.005	0.0940 ± 0.003^x
Schiff's bases	0.0150 ± 0.002	0.0130 ± 0.002	0.0173 ± 0.003	0.0156 ± 0.003

Note: ^{x} – $p < 0.05$

The introduction of melted cheese product containing Arthrospira fusiformis from the Solyenoye Lake near Omsk into the diet of the rats (group III) did not affect the level of LPO products. However, biologically active additive Arthrospira platensis made in the People's Republic of China (group IV) increased the content of erythrocytes neutral LPO products. For instance, in the heptane phase of extraction, the contents of primary (DC) and secondary (KD, CT) products of erythrocytes LPO was higher, compared to the respective values in the reference group of animals, by 13.2 % and 11.0 %.

The intensity of the free radical damage to polar lipids of the erythrocytes of the animals in groups III and IV was higher than that in the reference, which indicated higher level of secondary (KD, CT) and final products (FPO), compared to the reference group (Table 4).

In the isopropanol phase of erythrocytes lipid extraction, more significant changes in the

content of conjugated trienes (KD, CT) (by 36.2 %) and those of Schiff's bases (by 64.7 %) of the erythrocytes in the animals that received melted cheese product with the addition of Arthrospira platensis made in the People's Republic of China (group IV) were observed.

Table 4. The relative content of LPO products in the isopropanol phase of erythrocytes lipid extract, (M ± m)

Indicator (*o.i.u.*)	Groups of animals			
	Group I	Group II	Group III	Group IV
Diene conjugates	0.3003 ± 0.00828	0.2793 ± 0.0096	0.3064 ± 0.0124	0.2927 ± 0.0085
Ketodienes and conjugated trienes	0.1120 ± 0.0021	0.1163 ± 0.0055	0.1445 ± 0.0062^{x}	0.1525 ± 0.0071^{x}
Schiff's bases	0.1078 ± 0.0027	0.1012 ± 0.0029	0.1507 ± 0.0031^{x}	0.1775 ± 0.0034^{x}

Note: ^{x} *– p < 0.05*

In the group of the rats whose diet included Arthrospira fusiformis from the Solyenoye Lake in the Omsk region (group III), these changes were less pronounced: the content of secondary (KD, CT) and final (FPO) products was higher, compared to the corresponding values in the reference group, by 29.0 % and 39.8 %. In the experimental group of the rats that received melted cheese product along with the basic diet (group II), the content of primary and secondary products of LPO did not differ from that in the reference group

Conclusion

Thus, as a result of the studies performed, an increase was detected in the content of primary, secondary and final products of oxidative degradation of heptane-soluble lipids in the blood plasma of the male white rats that within 40 days had received every day the basic diet along with melted cheese product containing a formulation of 10 % of Arthrospira fusiformis from the Solyenoye Lake in the Omsk region. The intensity of oxidative degradation of the neutral lipids of erythrocytes was more pronounced in the rats that received melted cheese product with 10 % content of Arthrospira platensis made in the People's Republic of China. In determining the degree of proneness of erythrocytes polar lipids and blood plasma to free radical oxidation in the groups of animals that had received melted cheese product with 10 % content of Arthrospira platensis and Arthrospira fusiformis, an increased intensity of LPO processes was detected, as evidenced by an increased level of products of secondary and final LPO in the isopropanol phase. However, the level of primary products (diene conjugates) of oxidative degradation of polar lipids increased only in the plasma of the rats that received melted cheese product with 10 % content of Arthrospira fusiformis. The carbonyl products of LPO, which include ketodienes and conjugated trienes, have greater toxicity, compared to diene conjugates.

The multidirectional nature of changes in the products of erythrocytes and blood plasma lipid damage upon the introduction into the diet of cheese product containing arthrospira of various origin and species is determined by the differences in its chemical composition. The cytotoxic effect is more pronounced upon the introduction of arthrospira of Chinese origin into the cheese product; on the contrary, free radical damage of the blood plasma lipids was more pronounced in the rats that received in their diet melted cheese containing arthrospira from the Solyenoye Lake near Omsk.

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