

EVALUATION OF THE EFFECTIVENESS OF THE ANTIOXIDANT PROPERTIES OF FUNCTIONAL BAKERY PRODUCTS

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Abstract: *The objective of our experimental studies was to evaluate the effectiveness of the antioxidant properties of functional bakery products. In the course of the experiment, we evaluated the effectiveness of antioxidant properties of functional bakery products containing antioxidants (selexen, α -tocopherol) and a number of B vitamins. Spectrophotometric methods (FRAP and DPPH) have established that the antioxidant capacity of the loaves studied is preconditioned by the presence of vitamin E. Field studies have revealed that the combined effect of selenium, which is an ingredient of selexen, and vitamins on the activation of antioxidant protection of the human body when consuming functional bakery products gives a statistically significant reduction in indicators of lipid peroxidation (LPO) in the blood of the examined volunteers within 30 days.*

Key words: *functional nutrition products, antioxidants.*

1. Introduction

One of the top-priority public projects of the Russian Federation is to improve the quality of life of its citizens by preserving health and working capacity. This is especially important for industrial regions of the country characterized by the anthropogenic impact of xenobiotics of various origins on the human body alongside imbalances of essential nutrients and a deficiency of vital micronutrients. Among the latter group, antioxidants (vitamins A, E, C, and K;

carotenoids; microelements selenium and zinc; polyphenols, flavonoids, etc.) occupy the key positions in the processes of detoxification and correction of metabolic disturbances, as they are capable of leveling out the influence of harmful environmental factors by supporting peroxide homeostasis and increasing resistance to oxidative stress and antioxidant protection of the human body [5].

The goal of designing food products enriched with antioxidants and furnishing evidence of the advantages of said new products is to effectively promote

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functional food systems to the consumer market. At the same time, the information on a functional food product should be justified and confirmed by approbation in representative groups of people, demonstrating not only its total safety and acceptable taste, but also the ability to significantly improve health indicators.

As for the health benefits of functional food products, in 1999, the International Life Sciences Institute proposed the "oxidative stress protection" category as a classifying objective function of the organism to be corrected by such products [13].

The objective of our experimental studies was to evaluate the effectiveness of the antioxidant properties of functional bakery products.

2. Materials and Methods

Individual antioxidants and bakery products enriched with them were used as a research material in the first stage of the experiment:

- Selexen, a selenium-containing additive (manufacturer Medbiofarm, Obninsk, Russia) is a synthetic heterocyclic organic selenium compound (containing not less than 95% of selenopyran); the content of selenium in the product is 23-24%;
- Vitamin E ($\geq 97\%$) manufactured by SIGMA-ALDRICH (USA);
- Functional food products, namely, Selenium-containing Gorodskaya loaf and Selenium-containing Gorodskaya loaf enriched with vitamins (manufactured by "First Bakery", Chelyabinsk, Russia), the micronutrient composition of which is presented in Table 1; the quality and nutritional value of the loaves are confirmed by the test reports from the Center for

Hygiene and Epidemiology in the Chelyabinsk Region (Chelyabinsk, Russia). The daily intake of selenium and vitamins for an adult was taken from the current standards [15].

In addition to the standard ingredients (premium quality wheat flour, drinking water, sugar, cream margarine, baking yeast, salt), both loaves contain selexen, which produces a high content of selenium microelement in the products. Premix 986 (manufacturer DSM Nutritional Products Europe Ltd, Switzerland) is also added to the Selenium-containing Gorodskaya loaf enriched with vitamins.

At the second stage of the experiment, biosubstrate (blood serum) was used to study the antioxidant activity of the human body.

The antioxidant activity of selexen, vitamin E, and functional products measured *in vitro* was determined using FRAP and DPPH spectrophotometric methods [10].

For an *in vivo* evaluation of the effectiveness of antioxidant properties of functional bakery products, we carried out an experimental study (before and after) of the blood serum of 64 adult volunteers aged 25 years out of the local population chosen according to the inclusion-exclusion criteria (informed consent to participate in the study, absence of acute diseases and chronic diseases at the stage of exacerbation or decompensation). The control group is not used in the so-called "uncontrolled" "before-after" field study. Such an experimental study is based on the assumption that any gain in health in the course of the research is due precisely to the applied means [18], in our case, functional bakery products.

Provision of the physiological need (PhN) for micronutrients
when consuming an average daily portion of loaves

Table 1

Micronutrients	PhN [mg/day]	Micronutrient content [mg/150 g]			
		Selenium-containing Gorodskaya loaf		Selenium-containing Gorodskaya loaf enriched with vitamins	
		content	% of PhN	content	% of PhN
Selenium	0.07	0.031 ± 0.002	45.0	0.031 ± 0.001	45.0
Vitamin B ₅	5.0	0.79 ± 0.01	15.9	1.98 ± 0.01	39.6
Vitamin B ₁₂	0.003	not found	–	0.00109 ± 0.00002	36.3
Vitamin E	15.0	2.40 ± 0.01	16.0	5.34 ± 0.02	35.6
Vitamin PP	20.0	1.39 ± 0.01	6.9	6.85 ± 0.01	34.2
Vitamin H	0.05	not found	–	0.016 ± 0.002	32.0
Vitamin B ₁	1.5	0.12 ± 0.02	8.0	0.46 ± 0.01	31.0
Vitamin B ₂	1.8	0.052 ± 0.001	2.9	0.49 ± 0.01	27.2

All volunteers were divided into two groups (32 individuals each): group 1 consumed 150 g (average daily portion) of Selenium-containing Gorodskaya loaf daily, group 2 consumed the same amount of Selenium-containing Gorodskaya loaf enriched with vitamins over 30 days. Nutrition was also monitored to avoid intervening factors.

Medical personnel monitored the health status of the volunteers during the experimental trial.

To study the indicators of the human antioxidant system in the blood serum of the volunteers, we determined the content of: primary and secondary products of lipid peroxidation (LPO) using the method of Volchegorsky et al. [17]; TBC-active products - using the method of Stalnaya et al. [14]; and Schiff bases - using the method of Lvovskaya et al. [7]. Catalase activity was determined using the methods of Korolyuk et al. [6], and superoxide dismutase (SOD) activity - using the method of Chevari et al. [2].

All the studies were carried out according to the ethical principles of

medical research of the Helsinki Declaration of the World Medical Association in cooperation with the specialists of the clinic and the biochemical department of the Central Scientific Research Laboratory of the South Ural State Medical University (Chelyabinsk, Russia).

The studies of selexen, vitamin E, and enriched loaves were carried out five times. Statistical error did not exceed 5% (with a 95% confidence level). Statistical processing of the research results was carried out using Microsoft Excel XP, Maple 14, Statistica 8.0 suites, and biomedical statistics.

3. Results and Discussion

3.1. Study of the Antioxidant Activity of Micronutrients and Enriched Loaves

According to the manufacturer Mediobiopharm, the high electron-donating ability of selexen is not only confirmed by experimental data on study of the antioxidant activity in fats and in

the organism of agricultural and laboratory animals, but also by direct voltammetric studies of oxidation of pure selexen products in acetonitrile and methylene chloride [8]. In light of this, it was of interest in the first stage of research to study the antioxidant activity of selexen and vitamin-antioxidant (vitamin E) in a comparative aspect in order to establish the role of each in the

formation of the overall antioxidant activity of the enriched loaves. Antioxidant activity was determined using the FRAP and DPPH spectrophotometric methods as the most widespread in the practice of studying biological activity [9, 10]. The results of the light absorption of antioxidant solutions are presented in Table 2.

Indicators of the antioxidant activity of micronutrients

Table 2

Name of antioxidants	Weight in 1 ml of methanol	Optical density	
		using the FRAP method	using the DPPH method
Selexen	0.1 mg	0.058 ± 0.018	0.034 ± 0.031
Vitamin E (α-tocopherol)		0.849 ± 0.015	1.836 ± 0.015

The order in which the tested substances increased antioxidant activity was the same according to the measurements of two different methods; Vitamin E manifests significantly greater antioxidant activity than selexen, regardless of the measurement method. The antioxidant activity of vitamin E is 15 times higher than that of selexen using the FRAP method and 54 times higher using the DPPH method. This a priori assumes the dominant role of vitamin E in the formation of the total antioxidant activity of the food products enriched with these antioxidants. It is important to understand that the antioxidant activity measured *in vitro* and *in vivo* does not always correlate [11, 12], because *in vitro* methods do not take into account metabolic transformations, tissue localization, and interaction with enzymes. Therefore, *in vitro* methods can be used as preliminarily in measuring antioxidant activity [12]. At the same time, the relatively low absolute optical density values established for selexen indicate the complex mechanism

of its action on a living organism, which differs markedly from the simple deactivation of active particles.

In the processes of ensuring the technological control of the quality of the food products containing antioxidants, it is necessary to evaluate the total content of analytes related in a structural or functional sense [1]. For this reason, we studied the total antioxidant activity of functional food products through calculating the theoretical value of the indicator using the quantitative content of antioxidants in the product and their previously established optical density, and through experiments obtaining the true value of the indicator using only the DPPH method, since the FRAP method is characterized by the non-additivity of the light absorption of mixtures (for example, the determination of vitamin C in a mixture with quercetin gives a negative deviation [16]).

The results of studying the total antioxidant activity of the enriched loaves in a comparative aspect are presented in

Table 3. Due to a low concentration of antioxidants in the food systems under study (compared to chemically pure analytes), the weight of the sample was increased and its concentration in 1 ml of methanol was 10.0 mg.

Table 3

Indicators of the total antioxidant activity of the loaves using the DPPH method

Name of products	Weight in 1 ml of methanol	Optical density	
		theoretical value	actual value
Selenium-containing Gorodskaya loaf	10.0 mg	0.0030	0.0031 ± 0.0007
Selenium-containing Gorodskaya loaf enriched with vitamins		0.0065	0.0066 ± 0.0015

It has been established that the antioxidant capacity of the enriched loaves is almost entirely preconditioned by the presence of vitamin E in their composition, which has relatively high rates of recovering oxidative (Fe^{3+}) and radical particles (DPPH). The vitaminized selenium-containing samples, despite having a similar content of selenium (as in the Selenium-containing Gorodskaya loaf), have a 2.3-times higher optical density against the background of a higher content of vitamin E (2.2 times higher).

3.2 Evaluation of the Effectiveness of Antioxidant Properties of the Enriched Loaves in Field Studies

Study of the LPO-AOP system (antioxidant protection) in the volunteers before consuming the functional products revealed an accumulation of intermediates of free radical reactions and a decrease in the total antioxidant activity. Free radicals in the cytoplasm are inactivated by SOD with the formation of H_2O_2 , which is decomposed by catalase. It is believed that H_2O_2 participates in the process of energy transformation in the cytochrome oxidase region of the respiratory chain, in the oxidation of sulfhydryl groups of proteins, peptides,

and amino acids, and in the initiation of LPO in biomembranes. Reduced control over the decomposition of H_2O_2 can have severe consequences [3]. During the study of the peculiar features of the state of the lipoperoxidic system of the volunteers' organism before and after the use of selenium-containing bakery products, we established the activity of the enzyme system of antioxidant protection of the organism, especially after consuming the Selenium-containing Gorodskaya loaf enriched with vitamins (Table 4) for 30 days.

Thus, the activity of catalase after consuming Selenium-containing Gorodskaya loaf increased by 15.9% ($p < 0.05$), when consuming Selenium-containing Gorodskaya loaf enriched with vitamins - by 23.6% ($p < 0.01$) compared to the analogous indicator before consuming the functional bakery products. This is not an accidental side effect, as it is known that the alimentary factors which increase catalase activity include a sufficient intake of B vitamins [4], which are contained in the vitaminized Selenium-containing Gorodskaya loaf. The activity of SOD in this case significantly decreased by 30.9% and by 35.0%, respectively.

A high degree of the antioxidant protection in the volunteers' organism

after consuming selenium-containing bakery products influenced the intensity of lipid peroxidation in the cells of organs and tissues (Tables 5).

Indicators of the antioxidant protection of the organism Table 4

Name of indicator	Research results			
	before consuming the enriched loaves		after consuming the enriched loaves	
	Group 1	Group 2	Group 1	Group 2
Serum catalase [mcb/l]	15.81 ± 0.50	15.53 ± 0.31	18.32 ± 0.11*	19.20 ± 0.21**
SOD [standards unit/l]	1.23 ± 0.05	1.20 ± 0.03	0.85 ± 0.03**	0.78 ± 0.04***
TBC-active products [nmol/ml]	4.73 ± 0.12	4.67 ± 0.14	3.89 ± 0.13**	3.69 ± 0.16**

Note: statistically significant at * p<0.05, ** p<0.01, *** p<0.001

Indicators of lipid peroxidation (in the heptane phase) in the volunteers' blood Table 5

Name of indicator	Research results			
	before consuming the enriched loaves		after consuming the enriched loaves	
	Group 1	Group 2	Group 1	Group 2
Total polyene [E ₂₂₀ /ml]	2.10 ± 0.11	2.01 ± 0.19	1.59 ± 0.21	1.27 ± 0.23*
Diene conjugates [E ₂₃₃ /ml]	1.76 ± 0.08	1.74 ± 0.05	1.19 ± 0.03**	1.07 ± 0.03***
Diene ketones [E ₂₇₈ /ml]	0.27 ± 0.03	0.29 ± 0.05	0.19 ± 0.02	0.15 ± 0.03*
Schiff bases [E ₄₀₀ /ml]	0.26 ± 0.04	0.29 ± 0.03	0.20 ± 0.03	0.13 ± 0.03*

Note: statistically significant at * p<0.05, ** p<0.01, *** p<0.001

When used as an extracting substance - heptane (extracts LPO products formed from neutral lipids), we obtained statistically-significant indicators on the mobilization of the antioxidant protection in examined volunteers. At the same time, a decrease in the blood concentrations of heptane-soluble LPO intermediates after consuming Selenium-containing Gorodskaya loaf enriched with vitamins turned out to be quite specific for this product, since a similar trend when consuming Selenium-containing Gorodskaya loaf proved to be statistically insignificant after the statistical processing of the obtained research results, except

for decreasing the blood concentration of diene conjugates (by 32.4% at p<0.01).

When consuming Selenium-containing Gorodskaya loaf enriched with vitamins, the content of primary LPO products in the blood changed as follows: total polyene decreased by 36.8% (at p<0.05), diene conjugates decreased by 38.5% (at p<0.001); secondary LPO products (dykeketones) decreased by 48.3% (at p<0.05), and final LPO products (Schiff bases) decreased by 55.2% (at p<0.05).

Despite the ambiguity of the situation, after consuming both types of bakery products, a decrease of one of the fractions of the secondary LPO products - TBA active substances, namely, malonic

dialdehyde (MDA), in the blood proved to be statistically significant; after consuming Selenium-containing Gorodskaya loaf, levels decreased by 17.8%, and after consuming Selenium-containing Gorodskaya loaf enriched with vitamins - by 21.0%.

4. Conclusions

The combined effect of selenium, which is part of Selexen, combined with vitamins, on the activation of the antioxidant protection of the human body when consuming enriched bakery products provides a more statistically-significant decrease in the lipid peroxidation in the blood of the examined volunteers. As a result, we scientifically justified and proved the higher effectiveness of consuming vitaminized selenium-containing bakery products over monocomponent enrichment of products with essential microelements as functional agents to reduce the intensity of oxidative processes in the human body.

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