Спортивное питание

DOI: 10.14529/hsm190114

PROSPECTS FOR THE APPLICATION OF TAXIFOLIN BASED NANOEMULSIONS AS A PART OF SPORT NUTRITION PRODUCTS

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Aim. The article deals with the analysis of the effect of sport nutrition products enriched by taxifolin encapsulated into nanoemulsion on people involved in low-intensity sports for forecasting changes in homeostasis. **Materials and methods.** 3 groups of volunteers aged 25–35 (n = 30) and involved in sports on a regular basis (low-intensity training, 2 times per week) participated in a placebo-controlled study. The extract and nanoemulsion of taxifolin were used as antioxidants. To estimate the effect of enriched products on homeostasis, we performed a non-invasive study of hematological and biochemical indicators. **Results.** This paper demonstrates the results of the study of oil-water emulsions obtained with ultrasonic treatment for the encapsulation of biologically active substance – taxifolin – to improve and preserve its bioactive properties. We conducted an analysis of metabolism after the inclusion of taxifolin-enriched products in a diet. We established that the inclusion of taxifolin-enriched products does not result in worsening of lipid, carbohydrate, and protein metabolism. **Conclusion.** The paper demonstrates the reason for the inclusion of products enriched with taxifolin encapsulated into nanoemulsion in the diet of people involved in low-intensity sports for minimizing the risk of oxidative stress and providing homeostasis regulation.

Keywords: *nanoemulsion, taxifolin, encapsulation, sports nutrition.*

Introduction. It was established that intense loads in sports result in disturbing and, in most cases, stress effects [6]. Such manifestations are a substantial threat to human homeostasis. Many scientists agree that physical strain as stress increases significantly the concentration of various hormones in blood plasma not just under load. For example, the concentration of various hormones and metabolism products changes spontaneously in the first 4–10 minutes of submaximal anaerobic exercise. This period provokes a certain disbalance of regulatory factors [1, 19].

Muscular activity improves the performance of the respiratory system in accordance with the increase of gas exchange. Lung ventilation also increases significantly. It may increase both with the respiratory rate or the depth of respiration movements.

Physical exercises provoke increased oxygen consumption *invivo*, which leads to the formation of active oxygen forms (AOF). Free radicals are produced during aerobic cell metabolism and play a key role as regulatory mediators in signal transmission. Disbalance between AOF formation and adequate antioxidant protection triggers the process of oxidative stress [12, 20].

Therefore, the support of endogenic protection by exogenic antioxidants can be regarded as an effective non-invasive tool for preventing or reducing oxidative stress during training.

One of the main classes of natural antioxidants is plant polyphenols such as quercetin, curcumin, resveratrol, taxifolin, luteolin, catechin, and others that possess proven immunomodulatory, antioxidant, anti-inflammatory, cardioprotective, anticarcinogenic, and mitochondrial stimulating activity [2, 10, 15, 18, 21].

Several experimental studies prove the potential benefit of polyphenol consumption by athletes. They describe such effects as preserving

higher oxygen consumption, reducing oxidation of fatty acids and glucose, increasing endurance, and reducing inflammatory indicators in athletes consuming polyphenols.

Studies demonstrate that the application of Taxifolin during 2 or 3 weeks contributes to the increase of muscular aerobic capacity in highly skilled athletes from cyclic sports and improves the speed of recovery after intense physical loads. Based on the data obtained, it is recommended to use Taxifolin as an effective, natural, and safe antioxidant, capillary protector, and immunomodulator in sports connected with local and global muscular endurance during intense training aimed at improving muscular aerobic capacities [8, 9, 22, 23].

Studies prove that the use of Taxifolin belonging to P vitamins is recommended both for qualified athletes and people with an active lifestyle practicing fitness, diving, alpinism, bodybuilding, etc.

To create sport nutrition products, it is necessary to take into account several factors including the features of physical and chemical properties of antioxidant, its activity, and bioavailability.

Numerous studies indicate the feasibility of using nanoemulsions for encapsulating, protecting, and effective delivering of biologically active substances of lipophilic nature.

Emulsions were used for the encapsulation of numerous biologically active substances including ω-3 fatty acids, carotenoids, curcumin, resveratrol, coenzyme Q10. Several studies demonstrated that the encapsulation of hydrophobic BAA in emulsions and nanoemulsions improves their bioavailability and/or biological activity. *Invivo* studies showed that the encapsulation of curcumin in nanoemulsions improved its bioavailability in comparison with free curcumin. *Invivo* studies also revealed that per oral availability of β-carotene was approximately 11.5 times higher when delivered as nanoemulsion instead of a simple dispergation by oil [4, 5, 11, 19].

In previous publications, we provided the characteristic of a plant antioxidant – Taxifolin – and described its physical and chemical properties limiting its biopharmaceutical features in case of its application in native form. We proved the possibility of compensating disadvantages by means of ultrasonic micronization [16, 17].

We made an attempt to improve the antioxidant properties of taxifolin by encapsulating it in the system of nanoemulsion based on vegetable oil as an effective source of polyunsaturated fatty acids.

The aim of this study is to develop a nanoemulsion bicomposite based on plant flavanone taxifolin and to study its application in the food matrix for people with an active lifestyle.

Materials and methods. For the purpose of this study, we prepared emulsions of a direct (water-oil) type based on linseed oil. These emulsions were used for bread enrichment.

The linseed oil used was produced by Altayskiy len company ТУ10.89.19-010-3089444-2016, certificate of state registration № RU.77.88.003.E.001946.04.16 dd. 29.04.2016.

Guar gum (E412) was used as a surface-active substance (SAS).

The extract obtained from the wood of the Dahurian Larch was used as an antioxidant. The extract contains a minimum 97.0 % taxifolin (certificate of state registration № RU 77.99.11.003.E.018404.05.11 dd. 06.05.2011).

Oil concentration in the emulsion was 5 %. Taxifolin was introduced in the amount of 0.1 % of total emulsion volume, guar gum – in the amount of 0.1 % for each percent of a fat fraction.

The control sample of the emulsion was prepared by 20-minute mixing at a speed of 10000 rpm and a temperature of 40 °C.

The sample (nanoemulsion) was obtained with Volna-L ultrasonic equipment, model УЗТА-0,63/22-ОЛ (frequency 22 ± 1.65 kHz). The performance mode used had the following characteristics: power – 315 W, duration – 15 min, temperature – 40 °C [3, 5, 11, 14].

The samples obtained were assessed using the following criteria: *disperse content (Nanotrac Ultra, Microtrac Inc., USA, ISO 13321); morphology (confocal microscopy, objective HCX PL APO lambdablue 63.0x1.40); total antioxidant activity (DPPH method).*

To assess the efficiency of the nanoemulsion bicomposite, the taxifolin emulsion obtained was lyophilized and introduced into the bread recipe.

At the premises of the laboratory of "Food and Biotechnology" Department (SUSU – NRU) we developed the following samples:

Sample 1 – Wheat Rye Bread without BAS (placebo);

Sample 2 – Wheat Rye Bread enriched with taxifolin dry extract (0.05 % of flour weight);

Sample 3 – Wheat Rye Bread enriched with taxifolin nanoemulsion (0.05 % taxifolin to flour weight).

The group of volunteers was formed to assess

the effect of products enriched with antioxidant on homeostasis. Young people aged 25–35 and involved in low-intensity sports (2 times per week) participated in the study. The total number of participants is 30 persons (3 groups, 10 persons each group). All participants received bread for 24 days on a daily basis.

We conducted a randomized double-blind placebo-controlled study from May to June 2018. The results of hematological and biochemical examinations were accepted as controlled parameters. The assessment of volunteers was performed before and after the intake of enriched products and took place at the premises of the Scientific and Research Center for Sports Science. The assessment was conducted with the help of a non-invasive blood analyzer (AMP, Ukraine, certificate № ФСЗ 2008/02305). This study was approved by the Ethical Committee of the South Ural State University and was based on following all the criteria required.

Results and discussion. On the first stage of the study, we assessed the quality of fresh emulsions. The results obtained allowed us to established a pronounced effect of ultrasound treatment on the controlled parameters (Table 1). The control sample of the emulsion was characterized by various size fractions from 5220 nm to 172 nm with the predominance of the particles of more than 1000 nm (42.3 %). Therefore, this control sample could not be treated as nanoemulsion. In the emulsion obtained with ultrasound, we revealed the fractions of the particles of less than 200 nm. This indicates the possibility of preparing nanoemulsions with ultrasound treatment. Micro photos, which characterize the morphology of the samples studied, go in line with the results obtained for their disperse content.

The results of the antioxidant activity assessment of the emulsions obtained revealed that ultrasonic emulsification allows increasing to some extent taxifolin bioavailability in comparison with a control sample (12–13 % on average) [16, 17].

The authors suppose that the application of nanoemulsions for enrichment should minimize the risks of oxidative stress under physical loads of various intensity. To prove this hypothesis, we conducted a randomized double-blind placebo-controlled study in a group of volunteers.

The analysis of blood indicators characterizing carbohydrate metabolism (Table 2) revealed

Table 1

Results for the study of taxifolin emulsion samples

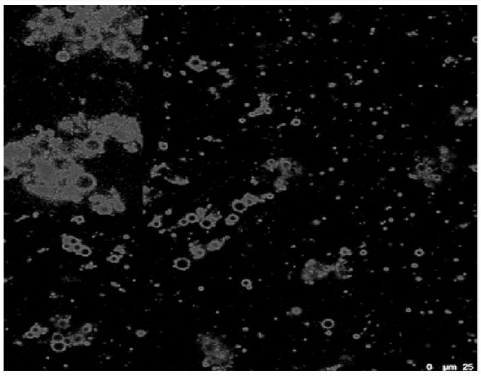
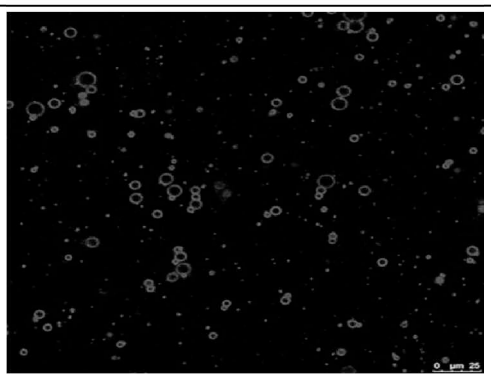
Samples	Micro photos (confocal microscope, objective HCX PL APO lambdablue 63.0x1.40)	Disperse content	AOA, % (DPPH)
Control sample		5220 nm – 30.5 % 1097 nm – 42.3 % 474.6 nm – 17.2 % 172 nm – 10.0 %	142.4 ± 2.4
Model sample (ultrasound)		174.8 nm – 74.3 % 82.6 nm – 25.7 %	155.0 ± 2.8

Table 2

Dynamics of blood indices in volunteers during the consumption of enriched products of various composition (averaged values)

Parameter, unit of measurement, mmol/l	Control group		Group 1		Group 2	
	Beginning	End	Beginning	End	Beginning	End
Lactic acid concentration **Reference values 0.99–1.38**	1,02 ± 0,08	1,22 ± 0,06	1,26 ± 0,04	1,14 ± 0,05	1,04 ± 0,08	0,99 ± 0,10
Glucose concentration **Reference values 3.9–6.2**	4,68 ± 0,18	4,02 ± 0,15	5,29 ± 0,16	5,22 ± 0,12	4,87 ± 0,12	4,81 ± 0,16
Total cholesterol concentration **Reference values 3.11–6.48**	4,93 ± 0,14	5,33 ± 0,20	4,30 ± 0,26	3,21 ± 0,25	4,56 ± 0,24	5,06 ± 0,20
Triglycerides concentration **Reference values 0.55–1.85**	1,46 ± 0,06	1,21 ± 0,08	1,75 ± 0,06	1,64 ± 0,05	1,64 ± 0,04	1,54 ± 0,08
Hemoglobin concentration **Reference values 120–160**	128,6 ± 0,21	126,2 ± 0,20	119,3 ± 0,18	120,6 ± 0,16	127,8 ± 0,14	128,6 ± 0,16

that in the control group at the end of the study glucose concentration tended to decrease to 4.02 ± 0.15 mmol/l in comparison with the beginning of the study.

In the beginning and at the end of the study glucose concentration in the first and second groups consuming bread enriched with taxifolin extract and nanoemulsion remained almost the same.

The change of blood glucose during muscle activity is individual and depends on the duration and capacity of exercises and physical fitness. Long-term physical loads usually led to a decrease in blood glucose. In untrained people, this decrease is usually more pronounced than in trained people. Decreased blood glucose is one of the factors contributing to fatigue. At the same time, physical performance decreases significantly. However, this parameter of carbohydrate metabolism is rarely used independently in sports diagnostics [6, 12].

The accumulation of lactic acid as the main metabolic intermediate is the result of obtaining energy during physical exercises. This process is similar to the decrease in blood glucose and determines fatigue appearance.

The analysis of the data obtained showed that, despite the fact that during the whole study the indicators of lactic acid concentration were within reference values for all participants, volunteers from the control group tended to accumulate lactic acid. Volunteers from the first group tended to the insignificant decrease of this indicator. The second group did not demonstrate any statistically significant changes.

It is proved that carbohydrate and lipid metabolism are closely connected under physical loads. During long-term physical loads the contribution of lipids to the total energy production increases; energy balance shifts from carbohydrate to lipid type.

The decrease of glucose concentration in blood inhibits the synthesis of triglycerides intensifying its breakdown. This tendency was registered in the control group, where the decrease of triglycerides concentration was 17.1 ± 0.06 % on average. In the first and the second groups, this parameter remained almost unchanged.

The results of various studies show that cholesterol decreases as a result of regular training. We registered the decrease of total cholesterol concentration only in the first group.

It is worth mentioning that all the parameters of lipid metabolism were within the reference values for this age group both in the beginning and at the end of the study.

Oxygen-carrying hemoglobin is the main source of protein for blood erythrocytes. Blood hemoglobin reflects the aerobic capacities of the body, the efficiency of aerobic training, and an athlete's health status.

Table 2 demonstrates the insignificant decrease of hemoglobin during the study (1.9 ± 0.20 % on average) in the control group. In people consuming bread enriched with taxifolin, we registered the slight increase of this parameter by 0.6 – 1.1 ± 0.17 %.

In general, in the majority of participants hemoglobin content was close to minimal reference values.

Conclusion. Our study revealed the possibility of applying ultrasound for obtaining oil-

water nanoemulsions. This approach can be regarded as efficient for the encapsulation of low-soluble biologically active substances and taxifolin in particular. The nanoemulsions obtained with ultrasound demonstrated the best results in terms of emulsion formation and the preservation of biologically active properties of taxifolin.

The study of lipid, carbohydrate, and protein metabolism in volunteers consuming bread with taxifolin nanoemulsions and extracts revealed that these products and their derivatives can be of interest for the correction of muscle fatigue and the main types of metabolisms in people practicing a sport. However, additional research is required with a larger number of the parameters studied.

The article was prepared with the support of the Government of the Russian Federation (Act No. 211 dd. 16.03.2013), Contract No. 02.A03.21.0011, and the financial support of state assignments No. 40.8095.2017/БЧ (2017123-ГЗ) and RFBR grant No. 18-53-45015.

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Received 5 December 2018

УДК 796.01:664 **DOI: 10.14529/hsm190114**

ПЕРСПЕКТИВЫ ИСПОЛЬЗОВАНИЯ НАНОЭМУЛЬСИЙ НА ОСНОВЕ ДИГИДРОКВЕРЦЕТИНА В СОСТАВЕ ПРОДУКТОВ ДЛЯ СПОРТИВНОГО ПИТАНИЯ

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Цель. Анализ воздействия на организм людей, занимающихся спортом малой интенсивности, продуктов питания, обогащенных инкапсулированным в наноэмульсию антиоксидантом дигидрокверцетином, для прогнозирования изменений процессов гомеостаза. **Материалы и методы.** Плацебо контролируемое исследование проводилось на 3 группах волонтеров в возрасте 25–35 лет (всего 30 человек), занимающихся спортом стабильно (тренировки малой интенсивности 2 раза в неделю). В качестве антиоксидантов применяли: экстракт и наноэмульсию дигидрокверцетина. Для оценки влияния включения обогащенных продуктов в рацион питания на процессы гомеостаза было проведено исследование гематологических и биохимических показателей с применением неинвазивного подхода. **Результаты.** Представлены результаты исследований эмульсий типа «масло-вода», полученных с применением ультразвукового воздействия, для инкапсуляции биологически активного вещества дигидрокверцетина с целью повышения и сохранения его биоактивных свойств. Проведен анализ характера метаболизма волонтеров при включении

в рационы питания продуктов, обогащенных антиоксидантом дигидрокверцетином. Установлено, что применение в рационе питания продуктов, обогащенных антиоксидантом, в целом не приводит к ухудшению показателей липидного, углеводного и белкового обменов. **Заключение.** Показана целесообразность включения в рационы питания людей, занимающихся спортом малой интенсивности, продуктов питания, обогащенных инкапсулированным в наноэмульсию дигидрокверцетином, для минимизации рисков оксидативного стресса и обеспечения регуляции процессов гомеостаза.

Ключевые слова: *наноэмульсии, дигидрокверцитин, инкапсуляция, спортивное питание.*

Статья выполнена при поддержке Правительства РФ (Постановление № 211 от 16.03.2013 г.), соглашение № 02.A03.21.0011, при финансовой поддержке государственного задания № 40.8095.2017/БЧ (2017123-ГЗ) и гранта РФФИ 18-53-45015.

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Поступила в редакцию 5 декабря 2018 г.

ОБРАЗЕЦ ЦИТИРОВАНИЯ

Prospects for the Application of Taxifolin Based Nanoemulsions as a Part of Sport Nutrition Products / I.V. Kalinina, I.Yu. Potoroko, A.V. Nenasheva et al. // Человек. Спорт. Медицина. – 2018. – Т. 18, № 4. – С. 100–107. DOI: 10.14529/hsm190114

FOR CITATION

Kalinina I.V., Potoroko I.Yu., Nenasheva A.V., Velyamov M.T., Bagale U. Prospects for the Application of Taxifolin Based Nanoemulsions as a Part of Sport Nutrition Products. *Human. Sport. Medicine*, 2018, vol. 18, no. 4, pp. 100–107. DOI: 10.14529/hsm190114