STUDIES IN PHYSICAL CULTURE AND TOURISM
Vol. 13, No. 2, 2006

ANNA SKARPAŃSKA-STEJNBORN[1], PIOTR BASTA[1], ŁUCJA PILACZYŃSKA-SZCZEŚNIAK[2]
[1] Department of Water Sports, Branch Faculty of Physical Culture in Gorzów Wlkp.
[2] Department of Hygiene, University School of Physical Education, Poznań

THE INFLUENCE OF SUPPLEMENTATION WITH THE BLACK CURRANT *(Ribes nigrum)* EXTRACT ON SELECTED PROOXIDATIVE-ANTIOXIDATIVE BALANCE PARAMETERS IN ROWERS

Key words: antioxidant enzymes, TBA-reactive products, black currant, exhaustive exercise.

ABSTRACT

The extent of oxidative damage after intensive physical exercise depends not only on the level of free radicals but also on the efficiency of antioxidative defense. One of the factors contributing to the increase of the antioxidative potential is supply of natural plant antioxidants. The aim of this study was to investigate the influence of black currant extract (*Ribes nigrum)* on prooxidative-antioxidative balance parameters in rowers. The research was conducted on 19 subjects, members of the Polish national rowing team. The subjects were divided into two groups. The subjects from the supplemented group (n=10) received 1 gelatin capsule containing 250 mg of ground black currant fruits, 3 times a day, during 6 weeks. The control group (n=9) received a placebo. Before and after the supplementation the competitors performed a 2000 m test on the rowing ergometer with the maximum velocity and power. A 5-minute warm-up preceded the exercise test. During the test, the minute ventilation equivalent (VE), oxygen uptake (VO_2) and carbon dioxide elimination (VCO_2) were continuously monitored. From the obtained data, the maximum oxygen uptake (VO_2max) was calculated. Before the exercise test, one minute after its completion and after 24 hours, the blood was taken from the antecubital vein. In the haemolysate of red blood cells the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and the level of thiobarbituric acid reactive lipid peroxidation products (TBARS) were determined. In the plasma, the total antioxidative capacity (TAC) and – in the capillary blood – the lactic acid concentration were measured. The supply of ground black currant preparations contributed to the increase of the total antioxidative defense, thus improving the protection against oxidative processes in the body that were expressed by the decrease of the antioxidative enzymes activity and restriction of erythrocyte lipids impairments.

INTRODUCTION

The increased oxygen consumption during physical exercise is a cause of changes in the prooxidative-antioxidative balance. However, the extent of oxidative impairments during physical exercise depends on such factors as intensity of the exercise, its duration, degree of the body's adaptation to the effort, and efficiency of endogenous protective mechanisms [1, 5, 10, 24].

Studies of Karolkiewicz and Szcześniak [11], Di Massimo et al., [4] and Oztasan et al., [20] have

Correspondence should be addressed to: Anna Skarpańska-Stejnborn, Department of Water Sports, Branch Faculty of Physical Culture in Gorzów Wlkp., University School of Physical Education, 66-400 Gorzów Wlkp., Estkowskiego 13, tel. +48 95 7271208, e-mail: ankass@poczta.onet.pl

revealed that physical training reinforces the endogenous antioxidative system, this way defending the competitor's body from the oxidative stress. However, the stable redox balance concerns mainly exercises of moderate intensity and duration. The increase of the exercise intensity induces generation of free radicals, which contributes to the decrease of the endogenous antioxidative defense and the development of free radical impairments [13, 22, 23].

The necessity of counteracting generation of free radicals during intensive physical exercise requires introduction to athlete's diet supplements that may effectively protect him or her from these unwanted processes. Until recently, a great significance has been attributed to phenol compounds from plants due to their strong antioxidative properties. Antioxidative actions of plant polyphenols are manifested by their direct reactions with free radicals, their scavenging, chelating of metal ion that catalyze oxidative processes, and activating other antioxidants, e.g. lipid soluble vitamins [7, 6, 17].

Black currants are among plant products that are rich in flavonoids. They are not only a source of vitamin C, but also contain antocyans, catechins, and querticin [25]. For these reasons, the purpose of our study was to investigate whether and to what degree the supply of the black currant extract contributes to the increase of the enzymatic antioxidative potential of erythrocytes and to the total antioxidative capacity of the blood plasma in athletes performing exercises of the maximal intensity.

METHODS

The subjects were 19 male members of the Polish National Rowing Team, who participated in a six-week training camp between the preparatory and competitive periods. The subjects' characteristics are presented in Table 1. The competitors were randomly assigned to groups receiving the black currant preparation (the supplemented group, n = 10), or the placebo (the control group, n = 9). The rowers of the supplemented group were given one black currant gelatin capsule (produced by Herbapol, Poland), three times a day, for six weeks. One capsule of 326 mg contained 250 mg of ground black currant fruits. The concentration of polyphenol compounds was calculated as the content of gallic acid which amounted to 8.5 mg in one capsule. At the same time and with the same dosage regime, the subjects from the control group received dyed gelatin capsules containing Poznańska flour (produced by Polskie Zakłady Zbożowe, Kraków).

Every day during the week the subjects filled in their food intake questionnaires, which allowed us to calculate the energy equivalent of the food rations and the content of antioxidant vitamins. Data concerning the daily energy and antioxidant vitamin intake in the supplemented and control groups are given in Table 2, according to Tables of Composition and Nutritional Value of Foodstuffs [15].

On the first day (before supplementation) and at the end of the training camp (after supplementation), the athletes performed a controlled 2000-meter rowing exercise test.

Aerobic capacity was estimated from the maximum oxygen uptake (VO_2max) obtained during the ergometer test. Each subject had to cover 2000m distance in the shortest time on rowing ergometer (Concept II – USA). Before the main test competitors performed 5 minutes individual warm-up. During the test, minute ventilation equivalent (VE), oxygen uptake (VO_2) and carbon dioxide elimination (VCO_2) were continuously monitored using Oxycon Mobile ergospirometer (VIASYS Healthcare GmbH. – Germany). Heart rate (HR) was recorded using a sport tester (Polar PE 3000, Finland). On the basis of the obtained gasometric values the oxygen uptake was calculated for each subject. The VO_2max was also expressed in relative values (ml/kg/min).

Blood samples for redox parameters were drawn from the antecubital vein, with K2EDTA (dipotassium ethylenediamine tetraacetic acid) as the anticoagulant before each incremental exercise test (in the morning, after an overnight fast), 1 minute after the test completion, and following the 24-hour recovery period. Samples were centrifuged immediately to separate red blood cells from plasma. Packed erythrocytes were washed three times with saline and lysed with ice-cold, redistilled water. Plasma and lysed erythrocytes were frozen immediately and stored at –28°C until use (up to one week). Additionally, capillary blood samples were drawn from the fingertip before and after each exercise test to assess the lactate levels (LA).

The Total Antioxidant Capacity (TAC), used as the overall measure of plasma antioxidant capacity, was assessed using commercial kits (Randox-TAS, Cat No. NX 2332, UK). According

to Miller et al. [16], this assay is based on the interaction between a chromogen (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonatel] $ABTS^{\cdot}$) and ferryl-myoglobin, a free radical formed by the reaction of metmyoglobin and hydrogen peroxide. Antioxidants in added serum scavenge $ABTS^{\cdot}$ prevent absorbance to a degree related to the overall serum antioxidant capacity. This radical had a stable green color, measured at 600 nm. The TAC levels in the sample caused suppression of the color development proportional to the antioxidant concentration.

The superoxide dismutase (SOD) activity was measured in washed erythrocytes after their lysis, by means of commercial kits (Randox-Ransod, Cat No. SD 125, UK). SOD catalyzes the dismutation of superoxide anion ($O_2^{-\cdot}$), leading to the formation of oxygen and hydrogen peroxide. The determination of the SOD activity was based on the production of O_2 by the xanthine and xanthine oxidase system. Superoxide anions reacted with the 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The units of the SOD activity were calculated on the basis of changes in the absorbance over 3 min, at 505 nm and 37°C, and from data in the standard curve generated with known amounts of purified SOD, which was obtained from the manufacturer. The superoxide dismutase activity was expressed in U/gHb.

The glutathione peroxidase (GPx) activity in the hemolysate samples was measured using commercial kits (Randox-Ransel, Cat No. RS 506, UK). According to the method of Paglia and Valentine [21], GPx catalyzes the oxidation of reduced glutathione in the presence of cumene hydroperoxide. The rate of glutathione oxidation was measured by monitoring the disappearance of $NADPH+H^+$ in the reaction medium, since $NADPH+H^+$ is consumed for the reduction of oxidized glutathione by glutathione reductase. The decrease in absorbance was measured at 340 nm and 37°C. Glutathione peroxidase activity was expressed in U/gHb.

The concentrations of the thiobarbituric acid reactive substances (TBARS) in the hemolysate samples were assessed as a measure of oxidative damage to red blood cells. TBARS concentrations were evaluated with the method described by Buege and Aust [2] involving the acidic breakdown of lipid peroxides into malonaldehyde molecules. Malonaldehyde subsequently reacted with the thiobarbituric acid (TBA), producing substances suitable for spectrophotometric detection. The tested samples contained 0.025 mL of hemolysate, 0.5 mL of TBA solution (0.375g/100 mL in 0.25 mol/L hydrochloric acid), 0.5 mL of trichloroacetic acid solution (15g/100 mL trichloroacetic acid in 0.25 mol/L hydrochloric acid) and 0.475 mL of water. The blank samples contained 0.025 mL of hemolysate, 0.5 mL of trichloroacetic acid solution and 0.975 mL of water. All samples were mixed vigorously and heated for 15 min in boiling water. Next, they were cooled down in ice-cold water and centrifuged at 2500 *g* for 15 min. The absorbance of the supernatant was determined at 535 nm and 37°C. The absorbance of the blank sample was subtracted from the absorbance of the tested sample and the concentrations of TBARS were determined from a standard curve generated with known amounts of tetramethoxypropane. The concentrations of TBARS (malondialdehyde equivalents) were expressed in μmol/gHb.

The concentration of hemoglobin was assessed using the cyanmethemoglobin method with the Drabkin's reagent and maximal absorbance at 540 nm. The results were expressed in g/100 mL.

The lactate levels in the capillary blood were determined immediately after the collection of the samples using a diagnostic kit (Dr Lange, Cat No. LKM 140, Germany). The lactate concentration was expressed in mmol/L.

Statistical analyses were performed with STATISTICA v. 6.0 software package. The normally distributed data (TAC, TBARS) were compared using a two-way analysis of variance (ANOVA). When significant changes were observed in ANOVA tests, these data were also analyzed by paired and unpaired Student's *t*-tests, with Scheffe's post-hoc test for multiple comparisons. The data without normal distribution (SOD, GPx) were analysed with nonparametric tests. The Mann-Whitney test was used to compare mean values between the two groups, and the data within each group were analyzed with Wilcoxon's test. All values were reported as mean ± standard deviations (SD). Statistical significance was set at $p < 0.05$.

RESULTS

The results are combined in Tables 1-4. Table 1 contains basic anthropometric characteristics and training experience of the competitors. Table 2 presents the mean daily intake of energy and antioxidative vitamins. No statistically significant differences were found in the diet of the rowers

Table 1. Basic characteristics of the studied groups (means ± SD)

Parameters	Supplemented group (n=10)	Control group (n= 9)
Age (years)	20.1 ± 0.8	20.9 ± 1.7
Body mass (kg)	87.5 ± 6.2	83.2 ± 8.1
Body height (cm)	193.0 ± 5.9	187.0 ± 6.6
Years of training (years)	6.4 ± 1.8	5.6 ± 1.4
Sport class (number of subjects):		
– Country Master Class	8	4
– Class I	2	5

Table 2. Daily energy and antioxidant vitamin intake in the supplemented and the control groups (means ± SD)

	Supplemented group	Control group	Student's *t*-test
Kcal	5416.3 ± 1311.0	4398.6 ± 1672.0	NS
Beta-carotene (IU)	7493.6 ± 6067.1	7415.3 ± 6426.1	NS
Vitamin C (mg)	226.5 ± 205.2	260.4 ± 201.1	NS
Vitamin E (mg)	19.6 ± 6.9	16.4 ± 7.1	NS

NS – difference non-significant ($p < 0.05$)

Table 3. Exercise characteristics (means ± SD)

Parameters	Supplemented group (n=10)			Control group (n= 9)		
	Before supplementation x ± SD	After supplementation x ± SD	Student's *t*-test	Before supplementation x ± SD	After supplementation x ± SD	Student's *t*-test
HR (ud./min)	194.2 ± 5.9	191.9 ± 7.06	NS	187.2 ± 9.4	185.0 ± 13.56	NS
Power (watt)	432.1 ± 20.59	434.8 ± 19.97	NS	369.4 ± 25.43	390.1 ± 14.93	NS
V'E (L/min)	190.0 ± 18.5	197.3 ± 27.12	NS	180.6 ± 18.2	188.9 ± 18.2	NS
V'O2 (L/min)	5.7 ± 0.63	6.2 ± 0.45	*	5.3 ± 0.35	5.7 ± 0.23	*
VO2/kg (ml/kg/min)	65.3 ± 5.48	67.2 ± 6.26	NS	68.0 ± 8.92	71.0 ± 6.89	NS
LA_{max} (mmol/L)	15.6 ± 2.83	15.3 ± 2.09	NS	15.4 ± 2.80	14.8 ± 2.05	NS
Time (s)	372.1 ± 5.22	368.7 ± 5.52	NS	393.2 ± 9.43	385.1 ± 4.79	NS

NS – difference non-significant; * – difference significant at $p < 0.05$

Table 4. Balance between oxidants and antioxidants before and after supplementation with the black current extract – comparisons within the supplemented and the control groups (means ± SD)

Parameter	Supplemented group			Control group		
	Before supplementation x ± SD	After supplementation x ± SD	Test	Before supplementation x ± SD	After supplementation x ± SD	Test
SOD (U/gHb)						
At rest	1237.6 ± 98.35	1196.1 ± 65.11	*	1088.2 ± 381.28	1207.4 ± 50.83	NS
After ET	1661.5 ± 39.17	1626.3 ± 73.77 †	NS	1619.8 ± 39.17	1665.8 ± 43.63 †	NS
24 hrs after ET	1738.2 ± 56.6	1632.0 ± 86.22 †	*	1749.8 ± 26.37	1651.6 ± 111.46 †	NS
GPx (U/gHb)						
At rest	57.8 ± 12.65	62.0 ± 10.16	NS	58.0 ± 8.22	58.0 ± 14.16	NS
After ET	62.4 ± 11.98	54.4 ± 7.77 †	NS	61.5 ± 11.98	57.7 ± 12.72	NS
24 hrs after ET	69.6 ± 18.85	50.5 ± 12.00 †	*	63.6 ± 15.93	54.3 ± 9.52	NS
TAC (mmol/L)						
At rest	1.21 ± 0.09	1.25 ± 0.07	NS	1.17 ± 0.13	1.23 ± 0.07	NS
After ET	1.36 ± 0.09 †	1.35 ± 0.08 †	NS	1.25 ± 0.12 †	1.28 ± 0.04	NS
24 hrs after ET	1.29 ± 0.20	1.45 ± 0.15 †	*	1.29 ± 0.13 †	1.28 ± 0.07#	NS
TBARS(µmol/gHb)						
At rest	1.5 ± 0.29	1.3 ± 0.61	NS	1.6 ± 0.28	1.9 ± 0.82	NS
After ET	3.1 ± 1.48 †	3.1 ± 1.0 †	NS	3.1 ± 1.15 †	3.5 ± 0.87 †	NS
24 hrs after ET	3.3 ± 1.66 †	1.8 ± 0.96	*	3.2 ± 1.16 †	3.1 ± 1.36 † #	NS

The compared values were collected at rest (At rest), 1 minute after the exercise test (After ET), and 24 hours after the exercise test (24 hrs after ET).

GPx – glutathione peroxidase; SOD – superoxide dismutase; TAC – Total Antioxidant Capacity; TBARS – thiobarbituric acid reactive substances; N.S. – difference non-significant; * – difference significant at $p < 0.05$; † – significantly different from the resting value ($p < 0.05$); # – significant difference between the supplemented and control groups ($p < 0.05$)

under study and the values were in agreement with those recommended for physically active men [28].

Physiological characteristics of the exercise, together with concentrations of lactic acid have been collected in Table 3. The mean values of physiological parameters (HR, VE) obtained during the 2000 m test indicated that the maximum intensity of the exercise was reached, and values of the maximum oxygen uptake (VO_{2max}), ranged between 5.3 and 71.0 ml/kg/min, confirmed the subjects' high levels of physical capacity. Post-exercise concentration of the lactic acid, amounting to 15 mmol/L, indicated a significant contribution of anaerobic reactions to covering the energetic costs of exercises. A comparison of exercise parameters before and after the supplementation, showed a significant increase of oxygen uptake (VO_2) and no significant changes with respect to other parameters in both groups of rowers.

Table 4 contains a comparative analysis of mean values of the oxidative stress parameters in both terms of the study, for two groups of competitors under study. In the first term (before supplementation) the ergometric exercise test caused similar changes in both groups. In the second term, the lower SOD activity at rest and lower SOD and GPx activity after 24 hours of restitution were determined in the group supplemented with black currant.

In the supplemented group, the concentration of TBARS – indicator of impairments induced by free radicals, decreased after 24 hours ($p<0.05$) in comparison either to the results before supplementation or to the control group. On the other hand, the total antioxidant capacity of the plasma (TAC) appeared to be higher during the restitution period, in comparison either to the results before supplementation or to the control group receiving a placebo.

DISCUSSION

The 2000 m exercise test performed by rowers before and after six-week supplementation with the black current extract, has not induced any significant differences in the analyzed physiological parameters, except for a significantly higher oxygen uptake (Table 3). The maximum intensity of this exercise has been confirmed by the high concentration of the lactic acid (15 mmol/L) and high heart rate (about 190 bpm).

The analysis of prooxidative-antioxidative balance parameters has unequivocally confirmed that physical exercise with the maximum intensity performed by rowers shifts this balance towards oxidative processes. Despite the consistence of the daily vitamin intake in a diet (Table 2) with values recommended for highly physically active men [28], the system of the antioxidative defense in subjects from the control group has appeared to be less competent than in competitors from the supplemented group. This is proven by significantly lower TBARS concentrations in the control group immediately after the exercise and after 24 hours of restitution (Table 4). However, on the basis of comparison of results before and after black currant supplementation, and with the control group's, one may state that the preparation used in the study contributes to the decrease of this parameter during the restitution period.

The significantly lower activity of SOD in the restitution period for competitors supplemented with black currant points to lowered generation of the superoxide radical ($O^{-\cdot}_2$). The main, although not the only, source of $O^{-\cdot}_2$ during exercises of high intensity is the increase of purine nucleotides catabolism. This process is especially intensive following exercise, during the reperfusion period, when the increased level of hypoxanthin catalyzed by the xanthin oxydase to xanthin and the uric acid intensifies the monovalent reduction of oxygen to superoxide radical [9].

Vina et al. [26] have reported that allopurinol, the hypoxanthin analogue (blocking the synthesis of uric acid from hypoxanthin and xanthin), considerably decreases damages induced by free radicals during intensive physical exercise. However, studies of Lin et al. [14] show that certain flavonoids, such as polyphenols and antocyanidines [7], are more effective in inhibiting xanthin oxydase than allopurinol.

In vitro studies of Costantino et al. [3] show that black currants feature a high activity in suppressing chemically generated superoxide radicals and influence the inhibition of xanthin oxidase activity. This has been confirmed in the restitution period by the decreased dismutation of superoxide radical to hydrogen peroxide catalyzed by SOD and by a lower activity of GPx, responsible for its degradation (Table 4).

In the group receiving placebo, no significant differences in the activity of the above enzymes have been found between the two terms of the study. Thus, the 6-week training has not affected the activity of these antioxidative enzymes (Table 4).

The total antioxidant capacity of the plasma is an element of the non-enzymatic part of the system protecting the body against the effects of excessive amounts of ROS. The increased level of TAC in response to the increased oxidative stress induced by physical exercise, as revealed in this study, may be explained by increased concentrations of a few components of this system: the uric acid and possibly vitamin C, in which black currant is abundant. Especially quercitin and rutin display antioxidative functions in relation to vitamin C. *In vitro* studies have shown that these flavonoids delay the conversion of ascorbate to dehydroascorbate and protect from the activity of free radicals. In turn, the ascorbic acid inhibits the oxidative actions of flavonoids [19].

The uric acid, as the final product of purin metabolism, has been considered in *in vivo* studies [12] as an important plasma antioxidant – being also the key component of this system. Wayner et al. [27] have estimated the contribution of the uric acid to TAC to about 35-65%. Halliwell et al. [8] developed a succession of "usage" of individual antioxidants in decreasing effects of the oxidative stress. Ascorbate and thiol groups of proteins are used at the beginning, then bilirubin and uric acid, and then at the end α– tocopherol. Distribution of

particular antioxidants in the hydrophilic and hydrophobic phases of morphotic elements of the blood and plasma is also highly important. Namely, vitamin E reacts with superoxide radicals in the lipid phase (weakly reactive tocopherol radical – $TO^{\cdot}$, is formed this way), but does not participate in the water phase in which other antioxidants take part (ascorbic acid, uric acid, glutathione). Niki [18] has shown that hydrophilic antioxidants, except for the uric acid, may again reduce the tocopherol radical into tocopherol. Despite the high supply of vitamins C and E in the diet of the subjects (Table 2), the results indicate that supplementation with the black currant preparation contributes to the significant increase of TAC in the plasma during the restitution period (Table 4). This increase leads to strengthening of the endogenous antioxidative system and to limitation of erythrocyte lipid impairments.

Acknowledgment

The study was supported in part by the State Committee for Scientific Research funds (grant 4P05D 13626).

REFERENCES

[1] Aslan R., Sekeroglu M.R., Tarakcioglu M., Bayýroglu F., Meral I., Effect of acute and regular exercise on antioxidative enzymes, tissue damage markers and membrane lipid peroxidation of erythrocytes in sedentary students, Turkey Journal of Medical Sciences, 1998, 28: 411- 414.

[2] Buege J., Aust S.D., The tiobarbituric acid assay, (in:) Techniques in Free Radical Research. C.A. Evans, A.T. Diplock, M.C.R. Symones (eds), Elsevier Amsterdam London New York Tokyo, 1991: 147-148.

[3] Costantino L., Albasini A., Rastelli G., Benvenuti S., Activity of polyphenolic crude extracts as scavengers of superoxide radicals and inhibitors of xanthine oxidase, Planta Medica, 1992, 58 (4): 342-344.

[4] Di Massimo C., Scarpelli P., Penco M., Tozzi-Ciancarelli M.G., Possible involvement of plasma antioxidant defences in training-associated decrease of platelet responsiveness in humans, European Journal of Applied Physiology, 2004, 91 (4): 406-412.

[5] Finaud J., Scislowski V., Lac G., Durand D., Vidalin H., Robert A., Filaire E., Antioxidant status and oxidative stress in professional rugby players: evolution throughout a season, International Journal of Sports Medicine, 2006, 27 (2): 87-93.

[6] Frank J., Kamal-Eldin A., Lundh T., Maatta K., Torronen R., Vessby B., Effects of dietary anthocyanins on tocopherols and lipids in rats, Journal of Agricultural Food Chemistry, 2002, 50 (25): 7226-7230.

[7] Gabrielska J., Oszmianski J., Komorowska M., Langner M., Anthocyanin extracts with antioxidant and radical scavenging effect, Zeitschrift feur Naturforschung [C]. 1999, 54 (5-6): 319-324.

[8] Halliwell B., Gutteridge J.M., The antioxidants of human extracellular fluids, Archives of Biochemistry and Biophysics, 1990, 280 (1): 1-8.

[9] Hellsten Y., The role of xanthine oxidase in exercise, (in:) Exercise and Oxygen Toxicity. C.K. Sen, L. Packer, O. Hanninen (eds), Elsevier: 1994, 211-234.

[10] Ilhan N., Kamanli A., Ozmerdivenli R., Ilhan N., Variable effects of exercise intensity on reduced glutathione, thiobarbituric acid reactive substance levels, and glucose concentration, Archives of Medical Research, 2004, 35: 294-300.

[11] Karolkiewicz J., Szcześniak Ł., Comperative analysis of reduced glutathione (GSH) concentration in red cells and lipid peroxidation products (TBARS) concentration in plasma in male rowers in four terms of investigation, Medicina Sportiva 2001, 5 (2): 1-9.

[12] Kaur H., Halliwell B., Action of biologically-relevant oxidizing species upon uric acid. Identification of uric acid oxidation products. Chemico-Biological Interactions, 1990, 73 (2-3): 235-347.

[13] Kayatekin B.M., Gonenc S., Acikgoz O., Uysal N., Dayi A., Effects of sprint exercise on oxidative stress in skeletal muscle and liver, European Journal of Applied Physiology, 2002, 87 (2): 141-144.

[14] Lin C.M., Chen C.T., Lee H.H., Lin J.K., Prevention of cellular ROS damage by isovitexin and related flavonoids, Planta Medica, 2002, 68 (4): 365-367.

[15] Łoś-Kuczera M., Composition and nutrition values of food products. PZWL: Warszawa, 1991.

[16] Miller N.J., Rice-Evans C., Davies M.J., Gopinathap V., Milner A., A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates, Clinical Science, 1993, 84: 407- 412

[17] Morel I., Lescoat G., Cillard P., Cillard J., Role of flavonoids and iron chelation in antioxidant action, Methods Enzymology, 1994, 234: 437-443.

[18] Niki E., Action of ascorbic acid as a scavenger of active and stable oxygen radicals, American Journal of Clinical Nutrition, 1991, 54 (Supplement 6): 1119S-1124S.

[19] Noroozi M., Angerson W.J., Lean M.E., Effects of flavonoids and vitamin C on oxidative DNA damage to human lymphocytes. American Journal of Clinical Nutrition, 1998, 67 (6): 1210-1218.
[20] Oztasan N., Taysi S., Gumustekin K., Altinkaynak K., Aktas O., Timur H., Siktar E., Keles S., Akar S., Akcay F., Dane S., Gul M., Endurance training attenuates exercise-induced oxidative stress in erythrocytes in rat, European Journal of Applied Physiology, 2004, 91 (5-6): 622-627.
[21] Paglia D.E., Valentine W.N., Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine, 1967, 70: 158-169.
[22] Pilaczyńska-Szcześniak Ł., Skarpańska-Stejborn A., Deskur E., Basta P., The influence of chokeberry juice supplementation on the reduction of oxidative stress resulting from an incremental rowing ergometer exercise, International Journal of Sport Nutrition and Exercise Metabolism, 2005, 14: 48-58.
[23] Sureda A., Tauler P., Aguilo A., Cases N., Fuentespina E., Cordova A., Tur J.A., Pons A., Relation between oxidative stress markers and antioxidant endogenous defences during exhaustive exercise, Free Radical Research, 2005, 39 (12): 1317-1324.
[24] Tauler P., Aguilo A., Guix P., Jimenez F., Villa G., Tur J.A., Cordova A., Pons A., Pre-exercise antioxidant enzyme activities determine the antioxidant enzyme erythrocyte response to exercise, Journal Sports Sciences, 2005, 23 (1): 5-13.
[25] Viljanen K., Kylli P., Hubbermann E.M., Schwarz K., Heinonen M., Anthocyanin antioxidant activity and partition behavior in whey protein emulsion, Journal of Agric Food Chemistry, 2005, 53 (6): 2022-2027.
[26] Vina J., Gomez-Cabrera M.C., Lloret A., Marquez R., Minana J.B., Pallardo F.V., Sastre J., Free radicals in exhaustive physical exercise: mechanism of production, and protection by antioxidants, International Union of Biochemistry and Molecular Biology Life, 2000, 50 (4-5): 271-277.
[27] Wayner D.D., Burton G.W., Ingold K.U., Barclay L.R., Locke S.J., The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxyl radical-trapping antioxidant activity of human blood plasma, Biochimica et Biophysica Acta, 1987, 924 (3): 408-419.
[28] Ziemlański Ś., Norm of man's nutrition, Warszawa: PZWL, 2001.