POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES

www.pan.olsztyn.pl/journal/
e-mail: joan@pan.olsztyn.pl

Pol. J. Food Nutr. Sci.
2008, Vol. 58, No. 2, pp. 273-278

COMPARISON OF THE EFFECTS OF CHOKEBERRY FRUIT EXTRACT, CHICORY FLOUR AND THEIR DIETARY COMBINATION ON BLOOD PARAMETERS AND ANTIOXIDANT STATUS OF HEALTHY AND DIABETIC RATS

Adam Jurgoński, Jerzy Juśkiewicz, Zenon Zduńczyk

Department of Biological Analysis of Food, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn

Key words: diabetes, antioxidant status, chokeberry polyphenols, chicory fructans, glycaemia

In this paper we compare the effects of a polyphenol-rich extract from black chokeberry fruit, chicory flour containing non-digestible fructans and their dietary combination on blood parameters and antioxidant status of healthy and diabetic rats. The rat model of diabetes was induced by streptozotocin (STZ) and characterised by severe hyperglycaemia, hypercholesterolaemia, hypertriglyceridaemia and some disturbances in the antioxidant status, connected with body weight gain (BWG) depression, as well as liver and kidneys enlargement. The dietary treatments had no influence on glycaemia and BWG of the diabetic animals, but significantly alleviated liver and kidneys hypertrophy. The chicory preparation to a lesser extent, but the chokeberry extract – alone or in combination – effectively reduced total cholesterol level in the serum of diabetic rats. Moreover, the dietary combination of both examined preparations diminished triglycerides concentration to the control level observed in healthy animals. The STZ administration substantially decreased integral antioxidant capacity of hydrophilic substances in plasma (ACW), as well as increased the concentration of a lipid peroxidation indicator (TBARS) in the kidney tissue. Both tested dietary additives did not increase ACW, while the extract decreased TBARS concentration in the diabetic rats. Although the preparations used in our experiment did not fully protect against serious diabetic dysfunctions, positive changes observed in the blood parameters (total cholesterol and triglycerides level), as well as attenuated kidneys' hypertrophy and lipid peroxidation, indicate that the chokeberry fruit extract and/or chicory flour constituents may provide a useful supplementary therapeutic option in the treatment of diabetes and its complications.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterised by persistent high blood glucose level, caused by insulin resistance and/or impaired insulin secretion. Well known risk factors of the most frequent type 2 diabetes are obesity and metabolic syndrome [Kopelman & Albon, 1997]. Beside the disturbances in carbohydrate metabolism, all these abnormalities are frequently connected with dyslipidaemia and can be considered as diet-related; they are associated with increased energy consumption derived mainly from the products with saturated fat and refined sugar high-contents, connected with a decreased intake of unprocessed plant raw materials and fibre [Mann, 2002; Biesalski, 2004]. An often result of these nutritional faults, except body weight increase, is a decreased intake of vitamins and other low-molecular antioxidative compounds, as well as microelements which are parts of antioxidative enzymes in the body. Moreover, the undiagnosed or uncontrolled diabetes leads to persistent hyperglycaemia which *i.a.* can disturb oxidative/reductive balance in an organism, and is an important factor of the development and progression of diabetic complications [King & Loeken, 2004; Rolo & Palmeira, 2006]. All the foregoing factors can induce the oxidative stress which seems to be the underlie of diabetes development and progression.

Due to high amount of polyphenols, recently, many experiments have been performed on plant-derived products with antioxidative properties, in order to estimate the influence of native substances on organism with diabetes. The rat's streptozotocin-induced diabetes was recognized as a very useful model where, besides severe hyperglycaemia and dyslipidaemia, disturbed antioxidant status was also revealed [Garg *et al*., 1996]. It is well known that black chokeberry (*Aronia melanocarpa*) offers a strong antiradical activity resulting from high amount of natural antioxidants, especially polyphenols [Benvenuti *et al*., 2004]. Few evidences about a positive impact of the chokeberry derivatives on animal model of diabetes have also been presented in literature. Interestingly, Maslov *et al*. [2002] pointed at the extract from chokeberry leaves as a substance of hypoglycaemic actions, whereas the last report of Valcheva-Kuzmanova *et al*. [2007] indicates hypolipidaemic and hypoglycaemic activity of chokeberry fruit juice.

The successive group of native substances which may also beneficially affect diabetes are linear type fructans, the non-digestible oligo- and polysaccharides obtained on an industrial scale from the chicory root (*Cichorium intybus*). The fructans, also well known as inulin, oligofructose or fruc-

Author's address for correspondence: Adam Jurgoński, Ph.D. student; Institute of Animal Reproduction and Food Research, Tuwima 10, 10-747 Olsztyn, Poland; e-mail: adam@pan.olsztyn.pl

tooligosaccharides (depends on the degree of polymerization, DP), are categorised as parts of dietary fibre with prebiotic properties [Roberfroid, 2005]. Despite this, authors suggest that fructans also attenuate blood lipid and insulin level [Kok *et al*., 1998; Jackson *et al.*, 1999], as well as improve glucose tolerance [Kok *et al*., 1998; Cani *et al*., 2006], however, contrary results have been described as well [Alles *et al*., 1999; Luo *et al.*, 2000].

Although polyphenols and fructans are recognized as potential phytochemicals which may favourably affect diabetic state, the reports have been scarce and unclear thus far. Moreover, Larrauri *et al.* [1996] have proposed both glucose retardation index and antioxidant capacity as the major health-promoting properties associated to dietary fibre. Therefore, the purpose of this study was to compare the effects of a polyphenol-rich extract from chokeberry fruit, chicory flour containing non-digestible fructans and their combination on blood parameters and antioxidant status of healthy and diabetic rats.

MATERIAL AND METHODS

Preparations

The chicory flour was obtained by drying comminuted roots at a temperature not exceeding 70°C. Dried roots (*ca.* 95% d.m.) were disintegrated to particles with the size smaller than 0.4 mm. The final preparation contained 3% of mono- and disaccharides, 15% of fructooligosaccharides (DP 2-7) and 60% of inulin (DP >10). The content of oligo-, di- and monosaccharides in the flour was measured with the HPLC method using Knauer (Berlin, Germany) chromatograph with RI detector, Animex HPX 87C (300 x 7.8 mm) column (Bio-Rad, Hercules, CA, USA) and water as a mobile phase (flow rate of 0.5 mL/min, temp. of 85°C). A commercial extract from black chokeberry fruit (*Aronia melanocarpa*) was a source of polyphenolic compounds purchased from Agropharm Co. (Łódź, Poland). The extract was characterised in details by Frejnagel [2007].

Animals and diets

The animal protocol used in this study was approved by the University of Warmia and Mazury Institutional Animal Care and Use Committee. The experiment was conducted on 64 grownup male Wistar rats divided into 8 groups of 8 animals each. All experimental treatments were applied in 2 ways using healthy and diabetic animals. Diabetes was induced by a single intraperitoneal injection of 65 mg/kg body weight of streptozotocin (STZ, Sigma Chemical Co., St. Louis, USA) freshly dissolved in 0.05 mol/L sodium citrate buffer (pH 4.5). Control rats received an injection of citrate buffer. Blood glucose level was measured 48 h after STZ injection. Rats with glycaemia ≥300 mg/dL were considered diabetic and used for the study. To prevent hypoglycaemia, rats were kept on a 5% glucose solution for 24 h. All treatments of the rats were started at 9 a.m. and the sacrifice was also carried out at 9 a.m. Three days after the administration of STZ, diabetic rats were randomly assigned to the experimental groups. Then the healthy and diabetic rats were fed over 4 weeks with four experimental diets: the control casein diet containing 5% of cellulose as a source of dietary fibre (C group), a diet supplemented with 6.7% of chicory flour (F group) added at the expense of cellulose and a part of maize starch, which provided 5% of dietary fructans; a diet in which 0.2% of chokeberry fruit extract was added at the expense of maize starch (P group), as well as a group fed with a diet containing both the fructans (6.5% chicory flour) and polyphenolic (0.2% chokeberry fruit extract) preparation (FP group). The detailed composition of the diets is given in Table 1. All animals were housed individually in standard conditions with free access to water and diets.

Procedures

The whole blood glycaemia was determined in all rats in weekly intervals. At the termination of the experiment, the rats were anaesthetized with 20% urethane in physiological salt according to the recommendation for euthanasia of experimental animals [Close *et al.*, 1997]. After laparotomy blood samples were taken from tail vein, then liver, heart, kidneys, lungs and spleen were removed, weighed and subsequently stored at -70°C. The content of thiobarbituric acid-reactive substances (TBARS) in the internal organs tissue was determined after organs homogenization according to Uchiyama & Mihara [1978]. The concentrations of glucose (GLU), total cholesterol (CHOL) and triglycerides (TG) in the serum were determined with reagents from Alpha Diagnostics Ltd. (Cat. no. G6620, no. C6608, no. T6630, respectively). The activities of glutathione peroxidase (GPx) in heparinized blood and superoxide dismutase (SOD) in erythrocyte lysate were determined using the reagents from Randox Laboratories Ltd. (no. RS505 and no. SD125, respectively). Plasma ACW (integral antioxidant capacity of hydrophilic substances, kit Analytik Jena AG no. 400.801) and ACL (integral antioxidant capacity

TABLE 1. Composition of experimental diets fed to rats[1] (g/100 g).

	Diet			
	C	F	P	FP
Casein[2]	14.8	14.8	14.8	14.8
DL-methionine	0.2	0.2	0.2	0.2
Cellulose	5.0	-	5.0	-
Soybean oil	8.0	8.0	8.0	8.0
Cholesterol	0.5	0.5	0.5	0.5
Mineral mix[3]	3.5	3.5	3.5	3.5
Vitamin mix[3]	1.0	1.0	1.0	1.0
Chokeberry fruit extract[4]	-	-	0.2	0.2
Chicory flour[5]	-	6.7	-	6.5
Corn starch[6]	67.0	65.5	66.8	60.3

[1]All experimental treatments were applied in 2 ways: with a single intraperitoneal injection of 65 mg/kg body weight of streptozotocin (STZ) or with an injection of control citrate buffer. [2]Casein preparation (g/100 g): crude protein 89.7, crude fat 0.3, ash 2.0, water 8.0. [3]Recommended for AIN-93G diet [Reeves, 1997]. [4]Polyphenol concentration (mg/g): total 714, cyanidin glycosides 404.5, procyanidins 146.4, phenolic acids 105.2, quercetin and its glycosides 50.5, (-)epicatechin 7.6 [Frejnagel, 2007]. [5]Chicory flour preparation contained 3% of mono- and disaccharides, 15% of fructooligosaccharides (DP 2-7) and 60% of fructans with higher DP. [6]Corn starch preparation (g/100 g): total dietary fibre 0, crude protein 0.6, crude fat 0.9, ash 0.2, water 8.8.

of lipophilic substances, kit Analytik Jena AG no. 400.803) were determined with photochemiluminescence (PCL) detection method using a Photochem (Analytik Jena AG, Jena, Germany) according to the procedure of Popov & Lewin [1999]. In the PCL assay the generation of free radicals was partially eliminated by the reaction with antioxidants present in plasma samples, and the remaining radicals were quantified by luminescence generation. Ascorbic acid (AA) and Trolox (T) calibration curves were used in order to evaluate ACW and ACL, respectively, and the results were expressed as μmol of AA or T equivalent per mL plasma.

Data analysis

Pooled standard error of the mean (SEM) was calculated from the standard deviation from all rats divided by square root of rat number. Two-way repeated measures analysis of variance was performed with the type of diet (D) and health status (S) of the animals as factors, and their interaction (S×D). If the analysis revealed an overall significant effect of any factor ($p \leq 0.05$), the differences between individual groups were analysed with the Duncan's multiple range *post hoc* test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Body weight gain (BWG) and internal organ mass

All dietary treatments had no essential influence over the BWG of the healthy and diabetic animals (Table 2). A reduction of rats' weight was the result of a high dose of STZ, which probably led to thorough necrosis of pancreatic islet cells, and in turn, advanced hormonal disorders with its known consequences, *e.g* disturbances in carbohydrate, lipid and protein metabolism, impaired glucose uptake by tissues, osmotic diuresis, dehydration and disturbances of mineral metabolism, as well as enhanced production of reactive oxygen species [Chiasson *et al*., 2003; Rolo & Palmeira, 2006]. Except reduction of the weight, all the foregoing disorders connected with well known toxic effect of STZ [Bolzán & Bianchi, 2002] caused also the enlargement of liver and kidneys whose mass was definitely higher in the diabetic groups. Interestingly, the dietetic factor had an impact on the mass of liver ($p \leq 0.05$) and kidneys ($p \leq 0.01$), while the mass of spleen was not affected by both discussed factors (health status and diet). The highest liver mass was in the C group of the diabetic rats; the chokeberry fruit extract insignificantly, whereas chicory flour and the combination of both additives (FP group) significantly decreased liver mass of the diabetic rats. In the diabetic-C and diabetic-P group kidneys mass was slightly, while in the diabetic-FP significantly decreased, when compared to the F-diabetic group. Moreover, the two-way analysis of variance of liver and kidneys mass revealed interactions ($p \leq 0.05$ and $p \leq 0.01$, respectively) between the tested factors. However, these results indicate that the combination of both analysed additives had some positive influence over organs hypertrophy, nevertheless, the lowest mass of both liver and kidneys was recorded in the healthy rats.

TABLE 2. Body weight gain (BWG) and internal organ mass of healthy and diabetic rats fed the control and experimental diets.

		BWG (g)	Internal organ mass (g/100 g BW)		
			Liver	Kidneys	Spleen
Healthy rats	C	47.9^{a}	3.435^{c}	0.578^{c}	0.306
	F	49.4^{a}	3.376^{c}	0.528^{c}	0.287
	P	44.1^{a}	3.483^{c}	0.561^{c}	0.299
	FP	44.4^{a}	3.444^{c}	0.565^{c}	0.309
Diabetic rats	C	-12.5^{b}	5.211^{a}	1.014^{ab}	0.314
	F	-19.5^{b}	4.739^{b}	1.018^{a}	0.302
	P	-13.3^{b}	4.852^{ab}	0.962^{ab}	0.308
	FP	-20.1^{b}	4.573^{b}	0.907^{b}	0.307
Pooled SEM		0.63	0.105	0.029	0.005
P value	Health status (S)	≤0.001	≤0.001	≤0.001	NS
	Diet (D)	NS	≤0.05	≤0.01	NS
	Interaction (S×D)	NS	≤0.05	≤0.001	NS

Values not sharing the same superscript letters (a, b, c) within a column are different at $p \leq 0.05$; SEM – pooled standard error of the mean; NS – not significant; BW – body weight. C – control diet; F – 6.7% addition of chicory flour; P – 0.2% addition of chokeberry fruit extract; FP – 6.5% addition of chicory flour and 0.2% of chokeberry fruit extract.

Basic biochemical parameters

After each week of the experiment we observed strong differences in the blood GLU level between healthy and diabetic rats ($p \leq 0.001$, Table 3). The glycaemia was almost fivefold greater in the STZ-treated rats during the experiment than in the healthy ones. Contrary to expectations, both the chokeberry fruit extract and chicory flour, separately or in combination, had no influence on animals' glycaemia measured in whole blood throughout the study, and in the serum at the termination of the experiment. Reverse results can be found in the latest report of Valcheva-Kuzmanova *et al*. [2007], in which the application of 10 mL chokeberry fruit juice per kg body weight per day led to a 44% decrease of glycaemia in the diabetic rats, however without hypoglycaemic effect in the healthy ones. An explanation of these disagreements probably underlies in methodological differences. The dose of STZ used in our study equals 65 mg/kg body weight and results in the diabetic model with severe hyperglycaemia (above 500 mg/dL, measured in whole blood), while in the study of Valcheva-Kuzmanova *et al*. [2007] the dose of STZ was 15 mg/kg body weight lower, and glycaemia equalled to about 320 mg/dL (measured in plasma). Therefore, hypoglycaemic properties of the dietary factors applied in our experiment might be too subtle to overcome advanced pathological changes. Especially, it may refer to fructans from chicory flour which are characterised by rather local actions in the gastrointestinal tract, towards retardation of GLU absorption (similarly to the other dietary fibre fractions) [Jenkins *et al*., 1981] and stimulation of hormones secretion [Kok *et al*., 1998]. Furthermore, the polyphenol-reach extract used in our experiment might have lost some other important hypoglycaemic substances during processing, which might be present in chokeberry fruit juice. The hypoglycaemic effect of the chokeberry leaves observed by Maslov *et al*. [2002] may indicate that the hypothesis of other beneficial substances present in this plant should be considered.

TABLE 3. Basic biochemical parameters of healthy and diabetic rats fed the control and experimental diets.

		GLU (mg/dL whole blood)			GLU (mg/dL)	CHOL (mg/dL)	TG (mg/dL)
		After 1st week	After 2nd week	After 3rd week	Measured in serum at the end of the experiment		
Healthy rats	C	105[b]	107[b]	104[b]	221[b]	89[d]	147[d]
	F	109[b]	105[b]	108[b]	176[b]	83[d]	131[d]
	P	101[b]	104[b]	105[b]	215[b]	84[d]	166[cd]
	FP	106[b]	107[b]	105[b]	222[b]	83[d]	161[cd]
Diabetic rats	C	521[a]	562[a]	562[a]	889[a]	595[a]	337[a]
	F	518[a]	565[a]	563[a]	894[a]	440[b]	322[b]
	P	527[a]	524[a]	520[a]	855[a]	329[c]	215[c]
	FP	526[a]	548[a]	544[a]	873[a]	313[c]	151[d]
Pooled SEM		27.8	28.5	28.4	50.0	28.6	14.1
P value	Health status (S)	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
	Diet (D)	NS	NS	NS	NS	≤0.001	≤0.001
	Interaction (S×D)	NS	NS	NS	NS	≤0.001	≤0.001

Values not sharing the same superscript letters (a, b, c, d) within a column are different at p≤0.05; SEM – pooled standard error of the mean; NS – not significant. C – control diet; F – 6.7% addition of chicory flour; P – 0.2% addition of chokeberry fruit extract; FP – 6.5% addition of chicory flour and 0.2% of chokeberry fruit extract. GLU – glucose; TG – triglycerides; CHOL – total cholesterol.

The diabetes induction led to a strong increase of both serum CHOL and TG levels. The chicory preparation to a lesser extent but the chokeberry extract, alone or in combination, effectively reduced CHOL level in the serum of diabetic rats. Separately, both tested additives were also observed to significantly decrease TG level, however, the extract had more hypotriglyceridaemic effect, whereas the dietary combination of both preparations diminished TG concentration to the control level observed in healthy animals. Similar influence on TG concentration was also reported by Valcheva-Kuzmanova *et al*. [2007] after chokeberry juice supplementation, however, the authors did not note any statistical influence of both STZ and juice on CHOL level as well as on its fractions. Furthermore, beside the confirmation of the hypolipidaemic actions of fructans presented in literature [Kok *et al*., 1998; Jackson *et* al., 1999], our results indicate combined effects of the chicory flour and chokeberry extract on lipaemia, as well as strong interactions between the analysed factors (health status and diet, p≤0.001).

TABLE 4. Antioxidant status of healthy and diabetic rats fed the control and experimental diets.

		Blood parameters				Tissue concentration of TBARS				
		ACW	ACL	SOD	GPx	Liver	Kidney	Lung	Heart	Spleen
Healthy rats	C	0.078[ab]	0.057	396[a]	64.3[b]	7.28	11.00[b]	9.88	7.00	12.61
	F	0.045[bc]	0.040	404[a]	69.7[ab]	7.45	10.74[b]	10.19	7.79	12.80
	P	0.069[ab]	0.053	411[a]	68.4[ab]	7.17	10.13[b]	10.82	8.25	12.17
	FP	0.084[a]	0.051	406[a]	72.1[ab]	7.40	10.47[b]	9.78	8.53	12.26
Diabetic rats	C	0.029[c]	0.044	331[b]	69.9[ab]	7.37	13.91[a]	10.06	8.90	13.25
	F	0.030[c]	0.053	328[b]	71.6[ab]	8.13	13.04[a]	9.50	9.05	13.59
	P	0.035[c]	0.050	383[ab]	77.6[a]	7.99	10.76[b]	9.92	7.12	13.69
	FP	0.030[c]	0.046	380[ab]	76.0[a]	7.92	13.85[a]	10.60	8.09	14.35
Pooled SEM		0.005	0.002	7.15	1.14	7.15	0.266	0.222	0.247	0.314
P value	Health status (S)	≤0.001	NS	≤0.001	≤0.05	NS	≤0.001	NS	NS	NS
	Diet (D)	NS	NS	NS	NS	NS	≤0.01	NS	NS	NS
	Interaction (S×D)	NS	≤0.05	NS	NS	NS	NS	NS	NS	NS

Values not sharing the same superscript letters (a, b, c) within a column are different at p≤0.05; SEM – pooled standard error of the mean; NS – not significant. C – control diet; F – 6.7% addition of chicory flour; P – 0.2% addition of chokeberry fruit extract; FP – 6.5% addition of chicory flour and 0.2% of chokeberry fruit extract. ACW – integral antioxidant capacity of hydrophilic substances (μmol AA/mL); ACL – integral antioxidant capacity of lipophilic substances (μmol T/mL); GPx – glutathione peroxidase (U/mL); SOD – superoxide dismutase (U/mL); TBARS – thiobarbituric acid-reactive substances (μmol/100 g).

Antioxidant status

A high *in vitro* antiradical activity of chokeberries described in literature [Benvenuti *et al*., 2004] was not enough to overcome STZ-induced oxidative stress in our experiment. Neither the chicory flour nor the chokeberry extract were able to increase ACW affected by experimental diabetes ($p \leq 0.001$, Table 4). A lack of the effect of the extract on ACW was also confirmed between the healthy groups. Our results seem to be in agreement with the report of Bermudez-Soto *et al.* [2007] where chokeberry polyphenols were highly sensitive to mild alkaline *in vitro* conditions, similar to those presented in a small intestine. Thus, the intestinal environment might probably modulate the antioxidant activity of some polyphenols. Interestingly, ACL did not change statistically among all groups, yet an interaction was observed between the analysed factors. Moreover, the activities of both GPx and SOD became statistically dependent on the health status of rats ($p \leq 0.05$ and $p \leq 0.001$, respectively). The SOD activity in the C and F group lowered significantly after diabetes induction, whereas after the chokeberry extract inclusion, separately or in combination with chicory flour, it increased insignificantly. The activity of GPx was higher in P- and FP-diabetic groups when compared to the C-healthy one.

Only the kidneys' tissue concentration of the indicator of lipid peroxidation (TBARS) essentially changed after diabetes induction and was higher in diabetic groups, except P one where it was similar to those from all healthy groups. It is well known that kidneys and eyes are organs most sensitive to pathological changes (microangiopathies) during diabetes [Kopelman & Albon, 1997]. Therefore, our results confirm that dyslipidaemia connected with increased lipid peroxidation may by the key reason of microangiopathies development. Also Kowalczyk *et al.* [2004] observed a decreased TBARS concentration in mice with increased lipid peroxidation of internal organs tissue after administration of anthocyanins from black chokeberry. However, in our study completely unexpected was that the chokeberry extract in combination with chicory flour had no such a positive influence on kidneys' TBARS concentration as when applied separately.

CONCLUSIONS

Although the preparations used in our study did not fully protect against serious hyperglycaemia and oxidative stress induced by STZ in experimental rats, the positive changes observed in the blood parameters, *i.e.* decreased total cholesterol and triglyceride levels, as well as attenuated kidneys' hypertrophy and lipid peroxidation, indicate that the chokeberry fruit extract and/or its combination with chicory flour constituents may provide a useful supplementary therapeutic option in the prevention and treatment of diabetes and its complications.

REFERENCES

1. Alles M.S., de Roos N.M., Bakx J.C., van de Lisdonk E., Zock P.L., Hautvast J.G.A.J., Consumption of fructooligosaccharides does not favorably affect blood glucose and serum lipid concentrations in patients with type 2 diabetes. Am. J. Clin. Nutr., 1999, 69, 64–69.
2. Benvenuti S., Pellati F., Melegari M., Bertelli D., Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of rubus, ribes, and aronia. J. Food Sci., 2004, 69, 164–169.
3. Bermudez-Soto M.-J., Tomas-Barberan F.-A., Garcia-Conesa M.-T., Stability of polyphenols in chokeberry (*Aronia melanocarpa*) subjected to *in vitro* gastric and pancreatic digestion. Food Chem., 2007, 102, 865–874.
4. Biesalski H.K., Diabetes preventive components in the Mediterranean diet. Eur. J. Nutr., 2004, 43, 26–30.
5. Bolzán A.D., Bianchi M.S., Genotoxicity of streptozotocin. Mutat. Res., 2002, 512, 121–134.
6. Cani P.D., Knauf C., Iglesias M.A., Drucker D.J., Delzenne N.M., Burcelin R., Improvement of glucose tolerance and hepatic insulin sensitivity by oligofructose requires a functional glucagon-like peptide 1 receptor. Diabetes, 2006, 55, 1484–1490.
7. Close B., Banister K., Baumans V., Bernoth E-M., Bromage N., Bunyan J., Erhardt W., Flecknell P., Gregory N., Hackbarth H., Morton D., Warwick C., Recommendations for euthanasia of experimental animals: Part 2. Lab. Anim., 1997, 31, 1–32.
8. Chiasson J.L., Aris-Jilwan N., Bélanger R., Bertrand S., Beauregard H., Ekoé JM., Fournier H., Havrankova J., Diagnosis and treatment of diabetic ketoacidosis and the hyperglycemic hyperosmolar state. CMAJ, 2003, 168, 859–866.
9. Frejnagel S., Comparison of polyphenolic composition of extracts from honeysuckle, chokeberries and green tea - a short report. Pol. J. Food Nutr. Sci., 2007, 57, 83–86.
10. Garg M.C., Ojha S., Bansal D.D., Antioxidant status of streptozotocin diabetic rats. Indian. J. Exp. Biol., 1996, 34, 264–266.
11. Jackson K.G., Taylor G.R.J., Clohessy A.M., Williams C.M., The effect of the daily intake of inulin on fasting lipid, insulin and glucose concentrations in middle-aged men and women. Brit. J. Nutr., 1999, 82, 23–30.
12. Jenkins D.J.A., Wolever T.M.S., Taylor R.H., Barker H., Fielden H., Baldwin J.M., Bowling A.C., Newman H.C., Jenkins A.L., Goff D.V., Glycemic index of foods: a physiological basis for carbohydrate exchange. Am. J. Clin. Nutr., 1981, 34, 362–366.
13. King G.L., Loeken M.R., Hyperglycemia-induced oxidative stress in diabetic complications. Histochem. Cell Biol., 2004, 122, 333–338.
14. Kok N.N., Morgan L.M., Williams C.M., Roberfroid M.B., Thissen J.P., Delzenne N.M., Insulin, glucagon-like peptide 1, glucose dependent insulinotropic polypeptide and insulin-like growth factor 1 as putative mediators of the hypolipidemic effect of oligofructose in rats. J. Nutr., 1998,128, 1099–1103.
15. Kopelman PG, Albon L., Obesity, non-insulin-dependent diabetes mellitus and the metabolic syndrome. Brit. Med. Bull., 1997, 53, 322–340.
16. Kowalczyk E., Charyk K., Fijałkowski P., Niedworok J., Błaszczyk J., Kowalski J., Protective influence of natural anthocyanins of *Aronia melanocarpa* on selected parameters of antioxidative status in experimental intoxication with sulphide-2-chloroethyl-3--chloropropyl. Pol. J. Env. Stud., 2004, 13, 339–341.
17. Larrauri J.A., Goñi I., Martin-rarrón N., Ruperez P., Saura-Calixto F., Measurement of health-promoting properties in fruit dietary fibres: antioxidant capacity, fermentability and glucose retardation index. J. Sci. Food Agric., 1996, 71, 515–519.

18. Luo J., Van Yperselle M., Rizkalla S.W., Rossi F., Bornet F.R.J., Slama G., Chronic consumption of short-chain fructooligosaccharides does not affect basal hepatic glucose production or insulin resistance in type 2 diabetics. J. Nutr., 2000, 130, 1572–1577.
19. Mann J.I., Diet and risk of coronary heart disease and type 2 diabetes. The Lancet, 2002, 360, 783–789.
20. Maslov, D.L., Ipatova, O.M., Abakumova, O.Iu., Tsvetkova, T.A., Prozorovskii, V.N., Hypoglycemic effect of an extract from *Aronia melanocarpa* leaves. Vopr. Med. Khim., 2002, 48, 271–7 (in Russian; English abstract).
21. Popov I., Lewin G., Antioxidative homeostasis: characterization by means of chemiluminescent technique. Method. Enzymol., 1999, 300, 437–456.
22. Reeves P.G., Components of the AIN-93 diets as improvements in the AIN-76A diet. J. Nutr., 1997, 127, 838–841.
23. Roberfroid M.B., Introducing inulin-type fructans. Brit. J. Nutr., 2005, 1, 13–25.
24. Rolo A.P., Palmeira C.M., Diabetes and mitochondrial function: Role of hyperglycemia and oxidative stress. Toxicol. Appl. Pharmacol., 2006, 212, 167–178.
25. Uchiyama M., Mihara M., Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem., 1978, 86, 271–278.
26. Valcheva-Kuzmanova S., Kuzmanov K., Tancheva S., Belcheva A., Hypoglycemic and hypolipidemic effects of *Aronia melanocarpa* fruit juice in streptozotocin-induced diabetic rats. Methods Find. Exp. Clin. Pharmacol., 2007, 29, 1–5.

Received December 2007. Revision received January and accepted February 2008.