DE GRUYTER OPEN

Research Article **Open Access**

Erik Krošlák, Tibor Maliar, Mária Maliarová, Peter Nemeček, Peter Hozlár, Miroslav Ondrejovič, Michaela Havrlentová, Ján Kraic*

Antioxidant and protease-inhibitory potential of extracts from grains of oat

DOI 10.1515/chem-2016-0035
received June 29, 2016; accepted December 6, 2016.

Abstract: The most of important crops cultivated for production of foods and feeds could be considered as plants possessing nutraceutical or medically interesting compounds , especially if can be eaten without processing. Chemical and biological parameters that were evaluated in 100 oat (*Avena sativa* L.) genotypes were others than those that are important in food and feed production. Contents of polyphenols and flavonoids, radical scavenging activity (DPPH), and inhibitory activities against five proteases (trypsin, thrombin, urokinase, elastase, cathepsin B) were analyzed in extracts from mature grains. The antioxidant activity (DPPH) correlated to the content of total polyphenols. Only a minority (15 from 100) of analyzed genotypes created separate subgroup with a high content of polyphenols, flavonoids, and high antioxidant activity. The best in these parameters were genotypes CDC-SOL-FI, Saul, and Avesta, respectively. Fifteen other genotypes assembled another minority subgroup (also 15 from 100) on the basis of their high inhibitory activities against tested proteases. The highest trypsin–, urokinase–, and elastase–inhibitory activities were in genotype Racoon, the best in thrombin-, and cathepsin B-inhibitory activities were genotypes Expression and SW Kerstin, respectively. Three oats genotypes – Rhea, AC Percy, and Detvan appeared in both subgroups.

***Corresponding author: Ján Kraic:** Department of Biotechnology, Faculty of Natural Sciences, University of Ss. Cyril and Methodius, Námestie J. Herdu 2, 91701 Trnava, Slovakia, E-mail: jan.kraic@ucm.sk
Erik Krošlák, Tibor Maliar, Miroslav Ondrejovič, Michaela Havrlentová, Ján Kraic: Department of Biotechnology, Faculty of Natural Sciences, University of Ss. Cyril and Methodius, Námestie J. Herdu 2, 91701 Trnava, Slovakia
Mária Maliarová, Peter Nemeček: Department of Chemistry, Faculty of Natural Sciences, University of Ss. Cyril and Methodius, 91701 Trnava, Slovakia
Peter Hozlár, Michaela Havrlentová, Ján Kraic: Research Institute of Plant Production, National Agricultural and Food Centre, 92168 Piešťany, Slovakia

Keywords: *Avena sativa* L., mature grains, biological activity

1 Introduction

Plant secondary metabolites play essential roles in different physiological processes during growth, reproduction, and defensive reactions against pathogens and pests. Secondary metabolites have potential to affect specific biochemical and physiological pathways also in organism after consumption of foods or feeds containing plants and plant seeds. They may act as antioxidants, anti–infective, antibacterial, antifungal, metabolic, lipid–lowering agents, as well as substances with multiple protective effects against cardiovascular, gastrointestinal, neuroprotective, and degenerative diseases, aging, and carcinogenesis [1,2]. Many plant secondary metabolites were identified as inhibitors of human, animal, and viral proteases, e.g. human Hageman factor fragment, kallikreins, plasmin, thrombin, bovine Factor X_a, trypsin, chymotrypsin, metaloproteinases [3-6]. The most (above 43%) of all discovered proteases have been found in plants and about one quarter of them store plants in seeds [7]. Generally, proteases regulate and control different processes and often featured in the position of triggers of many steps of cascade mechanisms. Inhibitors of proteases, particularly those located in plant seeds and tubers, participate in the mechanisms of response to attacks of insects and microorganisms [8] by inhibition of invader's proteases. This basic characteristic of inhibitors of plant proteases is attractive enough to be expressed in transgenic plants as their protective agents against pathogens [9,10].

From the medicinal point of view, there are important relationships between proteases and the pathophysiological processes in the body. Trypsin (EC 3.4.21.4) acts as potential pathophysiological agent for both the acute and chronic types of pancreatitis [11]. Thrombin (EC 3.4.21.5) plays a role in the coagulation disorder diseases, inflammation, and

metastasis progression [12,13]. Urokinase (urokinase type plasminogen activator, uPA) (EC 3.4.21.75) is an important component of the extracellular protease system specifically converting plasminogen to plasmin, participating in a number of pathophysiological processes, including tumor progression and metastasis [14]. Neutrophile elastase (EC 3.4.21.37) is described as the promoter of onco-transformed cell spreading by extracellular matrix lysis, promoter of atherosclerosis, coagulation, and inflammation diseases [15,16]. Cathepsin B (EC 3.4.22.1) plays a critical role in the protein degradation process coming into the lysosome space during phagocytosis or endocytosis [17] and was found to be promoter of various inflammations and extracellular-matrix proteins degrading diseases [18,19].

Some common and daily consumed grains are known for their positive effects on consumer health and fitness. Grains of oat (*Avena sativa* L.) are known as a valuable source of biologically active compounds. Similarly to barley, oat grains possess high content of the β-D-glucan [20] utilized in the development and production of functional foods [21]. The health benefit of oat grains and the oat "young grass" for consumers relates to composition of phytochemicals with antioxidant and other biological activities [22-26]. The main sources of antioxidants found in oats are phenolic compounds such as tocopherols and tocotrienols (vitamin E), hydroxycinnamic acids (caffeic, *p*-coumaric, ferulic, and sinapic acid), avenanthramides, and to lesser extent flavonoids [22]. Valuable low-molecular compounds responsible for antioxidant activity are partially free and partially linked to husk skeletal structures and could be released by enzymatic treatment [27,28]. Besides of the well-known polyphenolic acids as antioxidants are interesting also avenanthramides with anti-inflammatory, antioxidant, anti-itch, anti-irritant, and antiatherogenic activities [29-31]. Nevertheless, protease inhibitory activities in extracts of oat grains and young leaves have been reported very rarely [32-35].

Natural phytochemicals are indispensable in discovery of new biologically active agents known as new "hit to lead" in early drug discovery and development of natural and semi-synthetic medicinal products for human and veterinary medicine. The aim of this study was to analyse and evaluate variation in content of total polyphenols, total flavonoids, antioxidant activity, as well as in inhibitory activities against five proteases in extracts from mature grains of diverse oat genotypes.

2 Experimental Procedure

2.1 Plant Material and Chemicals

One hundred oat (*Avena sativa* L.) genotypes (Table 1) were obtained from the collection of genetic resources maintained in the Genebank of the Slovak Republic (Research Institute of Plant Production, Piešťany, Slovakia). Twenty-eight of them were of the Slovak origin, others originated from different countries. Oat genotypes differed in morphological, agronomical, qualitative, phytopathological, as well as other traits and characteristics. All oats were grown in the same year (2012), in the same location (Vígľaš-Pstruša, Slovakia, altitude of 370 m, average annual temperatute 7.6 °C, average annual rainfall 600 mm), and were cultivated by the same growing technology (sown on March 22, after red clover), fertilization (54 kg/ha of ammonium nitrate), and chemical treatments (0.8 L/ha of Mustang Forte). Temperature during the vegetation period from April to July was above of long-term average, rainfall in April was close to normal, in May, June and July were above the long-term average rainfall.

Common laboratory chemicals including the Folin-Ciocalteu reagent were supplied by the Mikrochem Ltd. (Pezinok, Slovakia). The gallic acid (Sigma product no. G7384), 2,2-diphenyl-1-picrylhydrazil (DPPH, Aldrich product no. D9132), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, Aldrich 238813), 5-(3-carboxy-4-nitrophenyl)disulfanyl-2-nitrobenzoic acid (DTNB, 5,5'-dithiobis(2-nitrobenzoic acid, Sigma D8130), phosphate buffered saline tablets (Sigma P4417), Tris(hydroxymethyl)-aminomethane hydrochloride (Trizma® hydrochloride, Sigma T3253), Z-L-Lys-SBzl hydrochloride (Sigma C3647), and all enzymes subjected to the tests, i.e. trypsin from bovine pancreas (Sigma T8003), thrombin from bovine plasma (Sigma T7513), urokinase from human urine (Sigma U0633), elastase from porcine pancreas (Sigma E0258), and cathepsin B from bovine spleen (Sigma C6286) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA)

Extracts were prepared from mature grains by extraction in 5 mL of 100% methanol, in the ratio 1:5 (solid:liquid, w/v) for 24 h in the dark at 4 °C. Extracts were evaporated at 40 °C and the dry residue were dissolved in 1 mL of methanol and stored at 4 °C before analysis.

Table 1: The list and origin of analyzed genotypes of oats (*Avena sativa* L.).

Genotype (country of origin)	Genotype (country of origin)	Genotype (country of origin)	Genotype (country of origin)
Racoon (Great Britain)	PS-181 (Slovakia)	Flämingsfprofi (Germany)	Dakar (France)
Rhea (France)	PS-167 (Slovakia)	SW Betania (Sweden)	Expression (Great Britain)
Ac Percy (Canada)	Adler (Germany)	Dagny (Sweden)	CDC-Dancer (Canada)
Zuton (Great Britain)	Freddy (Germany)	PS-122 (Slovakia)	CDC-Mistrel (Canada)
Lenon (Great Britain)	Ogle (USA)	Hetman (Poland)	CD-SO-I (Canada)
100 260 CN(Great Britain)	Krezus (Poland)	Rajtar (Poland)	CDC-SOL-FI (Canada)
Saul (Czech republic)	Čína 4 (China)	Markant (Denmark)	CDC-Weaver (Canada)
Tatran (Slovakia)	Atego (Czech republic)	Nelson (Germany)	Hronec (Slovakia)
Izák (Czech republic)	Vendelin (Slovakia)	Husky (Germany)	Važec (Slovakia)
Avenuda (Czech republic)	Valentin (Slovakia)	Carron (Germany)	Vaclav (Slovakia)
Detvan (Slovakia)	Viliam (Slovakia)	Berdysz (Poland)	Vojtech (Slovakia)
Akt (Poland)	Prokop (Slovakia)	Zuch (Poland)	PS-186 (Slovakia)
Bandicoot (Australia)	Zvolen (Slovakia)	Bohun (Poland)	PS-190 (Slovakia)
Abel (Czech republic)	Vok (Czech republic)	Breton (Poland)	Dunajec (Slovakia)
Bullion (Great Britain)	Monarch (Austria)	Barra (Sweden)	PS-194 (Slovakia)
SV-5 (Slovakia)	Aragon (Germany)	SW Kerstin (Sweden)	PS-195 (Slovakia)
Avenuda x Atego (Slovakia)	Kanton (Germany)	SW Ingeborg (Sweden)	PS-196 (Slovakia)
OT 258 (Canada)	Neklan (Czech republic)	Fussion (Great Britain)	PS-197 (Slovakia)
PS-165 (Slovakia)	Typhon (Germany)	Nusso (Germany)	PS-198 (Slovakia)
Raven (Czech republic)	Jumbo (Germany)	Amaris (USA)	PS-199 (Slovakia)
Avesta (France)	Dalimil (Czech republic)	GEM (Canada)	PS-200 (Slovakia)
Paddock (France)	Lutz (Germany)	Zorro (France)	Taiko (Australia)
Kentucky (USA)	Flämingsgold (Germany)	Duffy (France)	AC Lotta (Canada)
Nem 1125 (Germany)	BE 201 700 (Germany)	Corneil (France)	Black (France)
Auteuil (France)	Triton (Germany)	Ranch (France)	Sirene (France)

2.2 Radical scavenging activity

Free radical scavenging activities were measured by 2,2-diphenyl-1-picrylhydrazil radical (DPPH•) using the method [36] modified for microplate assay system. Decreasing in absorbance indicated higher free radical scavenging activity. Grain extract (25 µL) was mixed with 100 µL of DPPH solution (120 mg L^{-1} dissolved in methanol). Absorbance at 490 nm (Microplate Reader Opsys MR™, Dynex, Chantilly, USA) was measured after 10 min of incubation in the dark. The DPPH radical scavenging activity of the extracts was expressed as Trolox equivalent antioxidant capacity (TEAC).

2.3 Total polyphenols and flavonoids

The total polyphenol content in extracts was determined by the method [37] modified for microplate assay system. The reaction mixture contained 20 µL of the grain extract, 20 µL of Folin-Ciocalteu reagent, and after 5 min was added 200 µL of 10% (w/v) water solution of sodium carbonate. Absorbance at 690 nm was measured (Microplate Reader Opsys MR™, Dynex, Chantilly, USA) after 30 min of incubation in the dark. The total polyphenols content was determined as a milligram of gallic acid equivalent per 1 kg of grain sample.

The total flavonoid content of oat samples was determined according to method of [38]. The reaction mixture contained 50 µL of the grain extract and 50 µL of a 2% (w/v) methanol solution of aluminium chloride. Absorbance at 405 nm was measured after 10 min of incubation. Total flavonoid content was expressed as a milligram of quercetin equivalent per 1 kg of grain sample.

2.4 Enzyme inhibitory assays

The Z-Lys-SBzl.HCl with DTNB [39] was used for determination of protease inhibitory activities as the chromogenic substrate. The substrate was cleaved with

trypsin, thrombin, urokinase, elastase, and cathepsin B and released DTNB-S-Bz was detected at 405 nm. Each well contained buffer solution with 0.6 mmol substrate, 1% DMSO (v/v), and grain extract. Reactions started by addition of enzyme solution containing 10 mg/l of trypsin, 4 mg L^{-1} of thrombin, 4 mg L^{-1} of urokinase, 2 mg L^{-1} of elastase, or 2 mg L^{-1} of cathepsin B, respectively. Reaction temperature was 37 °C. The inhibitory activities (IA) were calculated as: % IA = [(1-(ΔOD sample/ΔOD control)] × 100), where ΔOD is difference between the optical density measured in the 61st minute and 1st minute in sample and control, respectively (Microplate Reader Opsys MR™, Dynex, Chantilly, USA). The control sample was methanol itself.

2.5 Data processing

The first step of the evaluation of each sample was the calculation of primary parameters – standard equivalent for all composite variables, TEAC variable for antioxidant activity by DPPH method, and percentage expression of inhibitory activity for all enzyme inhibitory assays. The second step was the principal component analysis (PCA) and the cluster analysis (CA) for all parameters as well as for two groups (Field 1 and Field 2) separately. The next step was construction of histograms depicting the frequency distribution of primary extract collection for each studied variable, as well as for subsets of genotypes from CA. All were calculated using the software Microsoft Excel 2010. All histograms were fitted and replaced by the Gauss curve by the Oakdale engineering software DataFit version 9.0.59. PCA and CA analyses were performed using the JMP 9 software. The quality of the fitting by Gauss function was evaluated by coefficients of determination (r^2) for each curve.

3 Results and Discussion

3.1 Differences within oats

Two variables – total polyphenols (TPP), total flavonoids (TFL), six activity variables – protease inhibitory activities to trypsin (IA_TY), thrombin (IA_TR), urokinase (IA_UR), elastase (IA_ELA), cathepsin B (IA_CATB), and radical scavenging ability (DPPH) were analyzed in set of oats containing geographically and morphologically diverse genotypes. The principal component analysis (PCA) revealed that the minority of oats was significantly different from the majority whose members were concentrated around the zero point of intersection (Fig. 1). Two groups of oats located either in the Field 1 or in the Field 2 were significantly different mutually (Fig. 1). Vectors within the Field 1, parallel with the second principal component, included oats with the highest content of TPP and TFL, both significantly correlated with antioxidant activity (DPPH). Such correlation is known to be a common phenomenon of plant extracts from grains also in oat [40,41]. Besides of free phenolic compounds present in cereal grains, the phenolic acids bonded to the cell walls significantly contribute to the antioxidant activity of seed extracts [27,28]. From the nutritional as well as medicinal points of view might be interesting that the amount of antioxidants released from the cereal matrix into the human intestine *in vivo* could be higher than expected by measurements in vitro [42].

Oats placed inside and close to the Field 2 (Fig. 1) possessed the highest inhibitory activities to five tested proteases – trypsin, thrombine, urokinase, elastase, and cathepsin B. Vectors directed to the Field 1 (TPP, TFL, DPPH) not declared correlation with vectors directed to the Field 2 (protease inhibitory activities). Subsequently, samples located in both fields (Field 1 and Field 2) were evaluated separately to improve the percentage of explained variance as well as better insight into oat genotypes from both points of view – protease inhibitory and antioxidant activities, respectively.

3.2 Polyphenols, flavonoids, antioxidant activity

According to values of three interrelated variables TPP, TFL, and DPPH, oats were grouped by the PCA and CA into two major and one minor subset (Fig. 2, Fig. 3). The first major group (diamond symbols in Fig. 2 and Fig. 3) were relatively uniform in their low contents of TPP, TFL, and antioxidant activity (DPPH). This subset included predominantly yellow hulled oats. The second major subset (cross symbols in Fig. 2 and Fig. 3) represented genotypes with median values of all three variables. Fifteen oats of the minor subset (ring symbols in Fig. 2 and Fig. 3) were genotypes of different geographical origin, but they contained significantly higher values of DPPH, TPP, and TFL than others. The best of them are named in the Fig. 2. Thirteen of them were hulless, remaining two had black hulls. The antioxidant activity (DPPH) correlated with the content of total polyphenols (TPP) and also total flavonoids (TFL) (Fig. 1). Positive correlation between antioxidant activity and content of soluble

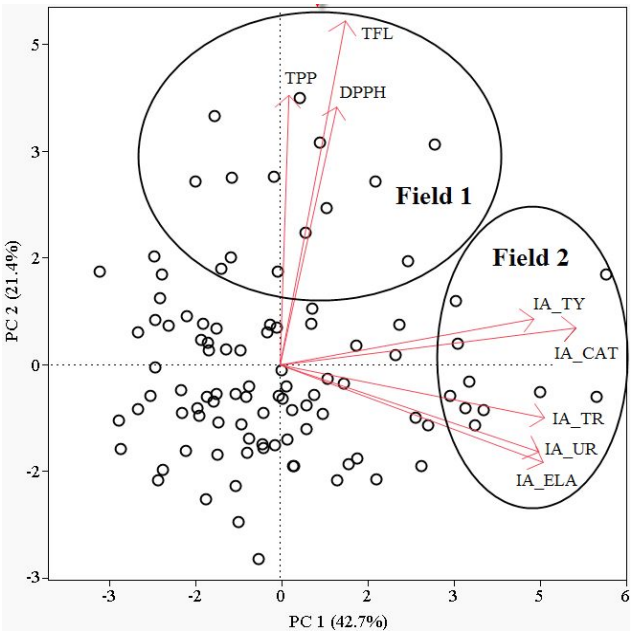

Figure 1: Principal component analysis of composite variables total polyphenols, total flavonoids (TPP, TFL), and activity variables radical scavenging ability (DPPH), protease inhibitory activities to trypsin (IA_TY), thrombin (IA_TR), urokinase (IA_UR), elastase (IA_ELA), and cathepsin B (IA_CATB) detected in seed extracts prepared from one hundred oat genotypes.

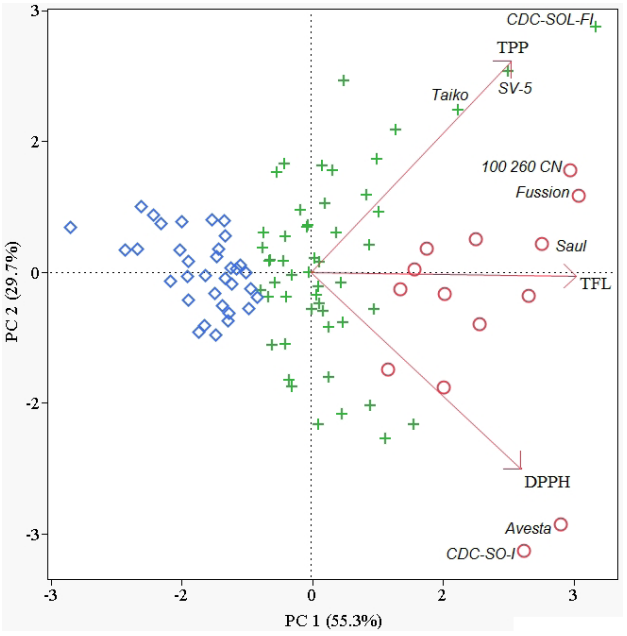

Figure 2: The principal component analysis of total polyphenols (TPP), total flavonoids (TFL), and radical scavenging activity (DPPH) (shape and color of individual points representing oat genotypes is the same as in dendrogram in Fig. 3).

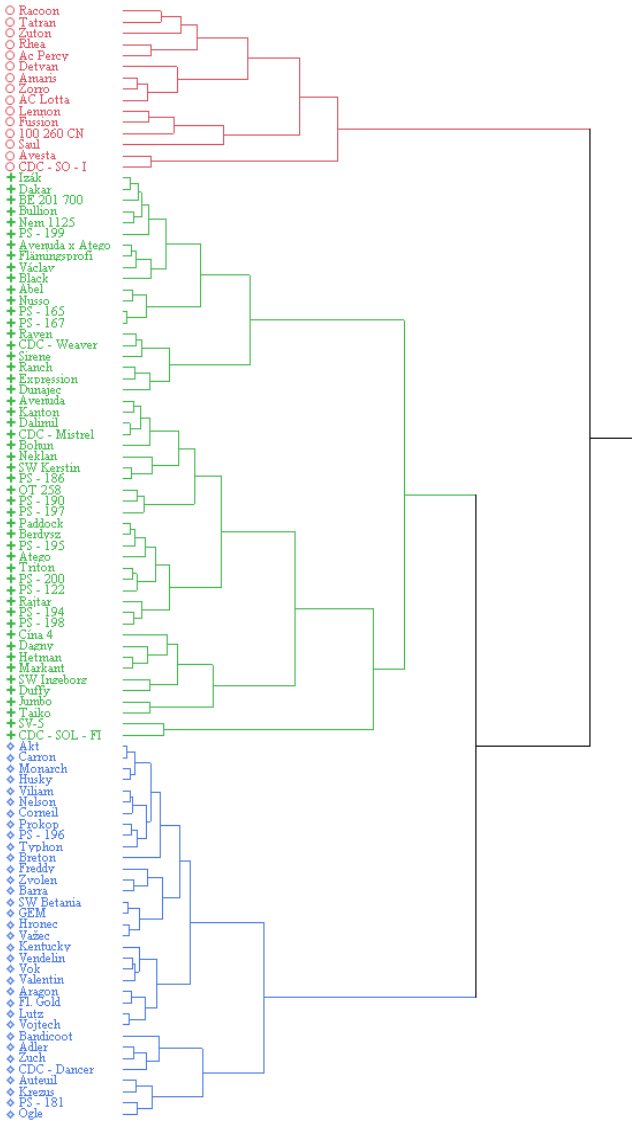

Figure 3: Cluster analysis of oats according to total polyphenols, total flavonoids, and radical scavenging activity.

phenolics in oats has previously been found [43,44]. Oat contains many compounds exhibiting antioxidant activity [22] that contribute together with the phenols to the total antioxidant capacity. Already known significant effect of growing location on content of TPP and antioxidant activity of oat grain extracts [47] was eliminated in our case.

The highest content of TPP was observed in the genotype CDC-SOL-FI (22.6 μg of gallic acid equivalent per 1 gram of grain). The highest content of TFL had genotype Saul (63.2 μg of quercetin equivalent per 1 gram of grain) located in the minor subset (ring symbols in Fig. 2 and Fig. 3). The most common flavonoids of oat grains are apigenin, luteolin, tricin, kaempferol, quercetin, and their glycoside derivatives [22].

Two oat genotypes possessing the highest antioxidant activity (DPPH) were also located in the minor subset (ring symbols in Fig. 2 and Fig. 3) – Avesta (50.8 μg of Trolox equivalent per gram of grains) and CD-SO-I (48.1 μg of Trolox equivalent per gram of grains). Avesta (black hulled oat) and CD-SO-I (white hulled) were the most different from all others (small subset within the minor cluster, ring symbols in Fig. 2). Higher recovery of polar compounds with antioxidant activity could be obtained from extracts of oat grains by methanol or alkali hydrolysis [45]. The comparable antioxidant activity of whole grain extracts exhibit also other cereals, e.g. barley, wheat, and rye [46]. Nevertheless, the presence of avenanthramides could significantly improve antioxidant activity in extracts from oat grains [31].

The common trait of living organisms is genetic diversity and variation in different traits. This was expected also in evaluated TPP, TFL, and DPPH within analyses set of one-hundred diverse oats. The frequency distribution within evaluated traits confirmed it. The shape of curves characterizing the frequency distribution of variable of grain extract samples within the complete set, two major subsets, as well as one minor subset confirmed the normal Gaussian distribution for all three parameters – TPP, TFP, and DPPH (Fig. 4-6). The central tendency of the frequency distribution in all three variables had the highest values in extracts of oats belonging to the minor red subset (Fig. 2, Fig. 3) and approximately two times higher in comparison with the central tendency of complete set of one-hundred oats.

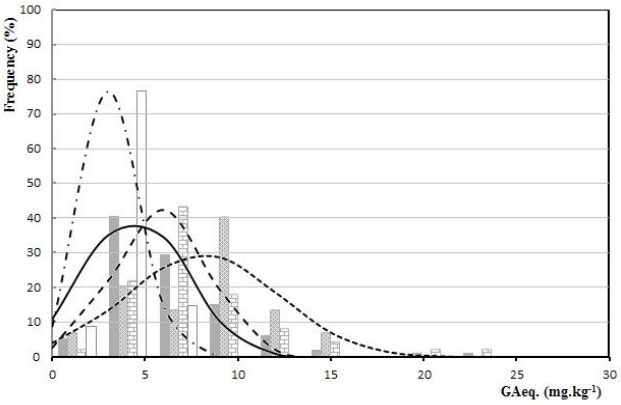

Figure 4: Distribution of total polyphenols expressed as galic acid equivalent in oats (solid column and line – complete set, $r^2 = 0.923$, bracket column and dash line – minor, red subset, $r^2 = 0{,}764$, dots column and dash-and-dot line – major, green subset, $r^2 = 0.967$, empty column and dots line – major, blue subset, $r^2 = 0.999$, the mentioned colors correspond to the colors in Fig. 2 and Fig. 3).

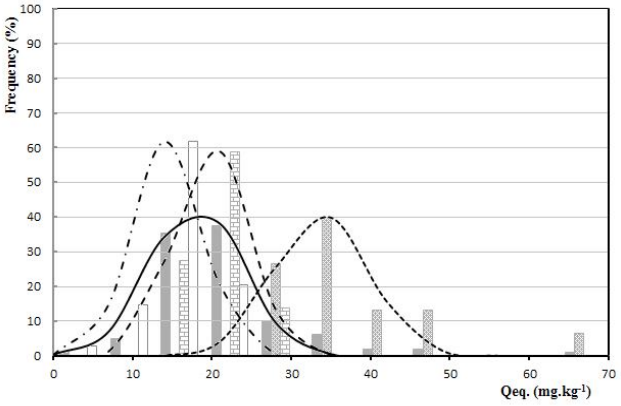

Figure 5: Distribution of total flavonoids in oats expressed as quercetin equivalent (solid column and line – complete set, $r^2 = 0.976$, bracket column and dash line – minor, red subset, $r^2 = 0.856$, dots column and dash-and-dot line – major, green subset, $r^2 = 0.999$, empty column and dots line – major, blue subset, $r^2 = 0.998$, the mentioned colors correspond to the colors in Fig. 2 and Fig. 3).

3.3 Proteinase inhibitory activity

The PCA analysis indicated that the IA_TY was relatively independent from other four proteinase inhibitory activities (Fig. 7). Significant correlation between IA_TR and IA_UR could be explained by the similarity in physiological role of both enzymes. The cluster analysis of variables of the Field 2 (Fig. 1) distinguished three subsets (Fig. 8, diamond, cross, and ring symbols) of oat genotypes. The minor subset included 15 oats separated from others according to relatively high values of IA_TR, IA_UR, IA_ELA, and IA_CATB (Fig. 7, Fig. 8, ring symbols). This subset contained mainly hulless oats, oats with black hull (PS-167, Kentucky, PS-165), white hulled (Dagny, SW Kerstin), as well as yellow hulled oats (Neklan). Relatively high values of the IA_TY possessed eight genotypes included in the minor (ring symbols) and major green (cross symbols) subsets. The highest values of IA_TY had genotypes Racoon (50.2%) and Kentucky (47.8%), (both located in subset with ring symbols in (Fig. 8).

The highest values of IA_TR expressed genotypes Expression (49.3%) and SW Kerstin (46.5%) both from the minor subset, Fig. 8, ring symbols). Several papers published the antithrombin effect of polyphenol-rich extracts from plants [48,49], nevertheless reports on extracts from cereal grains are not available.

The minor subsets (ring symbols) included also oats with the highest IA_UR – Racoon (33.0%) and Neklan (29.3%),both from the minor subset (ring symbols, Fig. 7, Fig. 8). The IA_UR was detected in extracts from many tropical plants [50], but our previous studies detected IA_UR in extracts from temperate medicinal plants

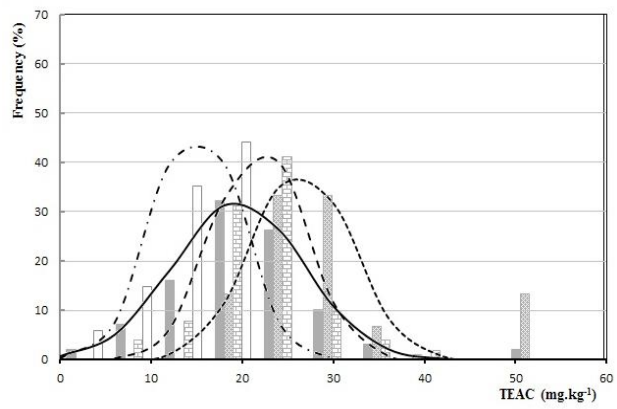

Figure 6: Distribution of antioxidant activity (ability to terminate DPPH radical) in oats expressed as Trolox equivalent antioxidant activity (solid column and solid line – complete set, $r^2 = 0.987$, bracket column and dash line – minor, red subset, $r^2 = 0.888$, dots column and dash-and-dot line – major, green subset, $r^2 = 0.982$, empty column and dots line – major, blue subset, $r^2 = 0.929$, the mentioned colors correspond to the colors in Fig. 2 and Fig. 3).

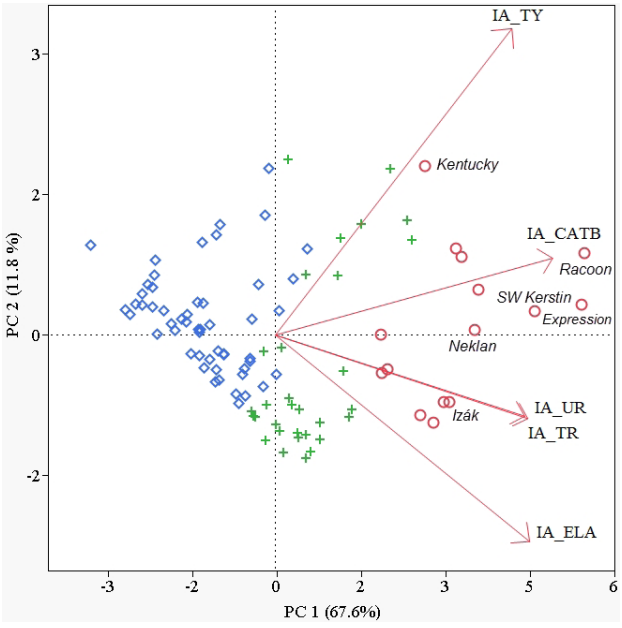

Figure 7: Distribution of oats according to protease inhibitory activities to trypsin (IA_TY), thrombin (IA_TR), urokinase (IA_UR), elastase (IA_ELA), and cathepsin B (IA_CATB) (Field 2, Fig. 1). Colors and symbols of samples are the same as in cluster analysis (Fig. 8).

Acer platanoides, *Rhus typhina* [51] as well as forage legume *Medicago sativa* L. [52]. Nevertheless, studies characterizing the IA_UR in grain extracts from oat are also not known.

The highest values of IA_ELA were detected in extracts from genotypes Racoon (47.9%), Izák (45.3%), and Neklan (44.3%). All of them are again from the minor subset

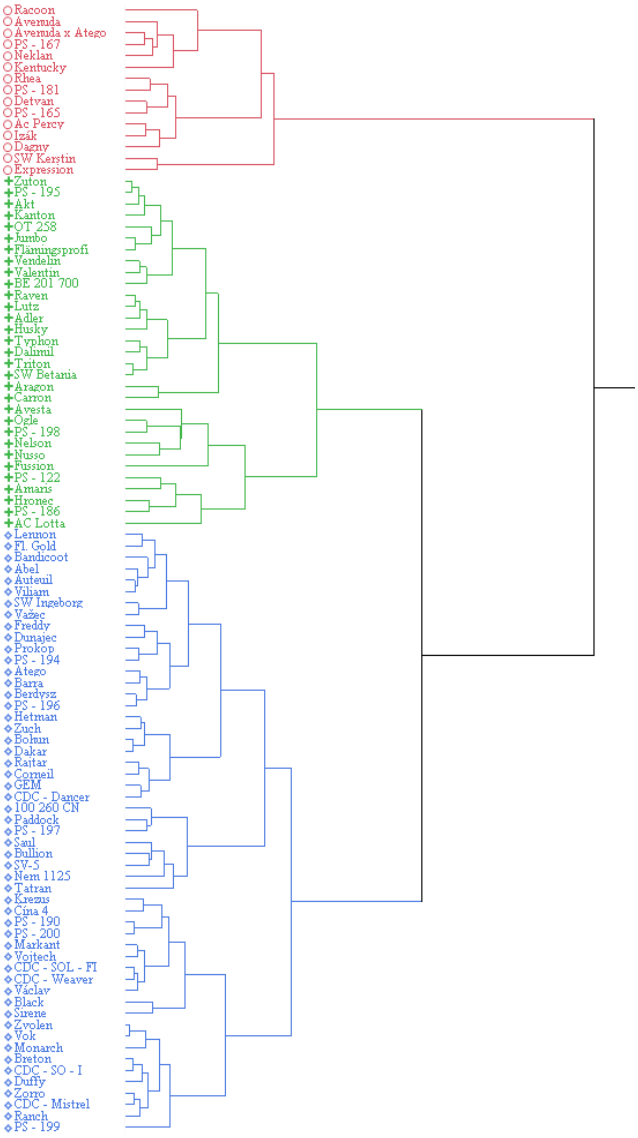

Figure 8: Cluster analysis of oats according to protease inhibitory activities to trypsin (IA_TY), thrombin (IA_TR), urokinase (IA_UR), elastase (IA_ELA), and cathepsin B (IA_CATB).

(Fig. 7 and Fig. 8, ring symbols). Studies describing inhibitory effects to elastase neither from oat nor from other cereals are not available. IA_ELA was detected in several aromatic [53], tannin rich plant extracts [54], and plant from the tropics [55]. Anti-elastase activity associated with radical scavenging activity should have considerable value in the future, for example in cosmetics [56].

Genotypes with the highest IA_CATB – Expression (94.1%), SW Kerstin (93.5%), Racoon (77.1%), and Izák (62.8%) were also located in the minor subset of oats (Fig. 7 and Fig. 8, ring symbols). This type of inhibitory activity was detected in many plants [57] and in plant secondary metabolites like amentoflavone [58]. Studies

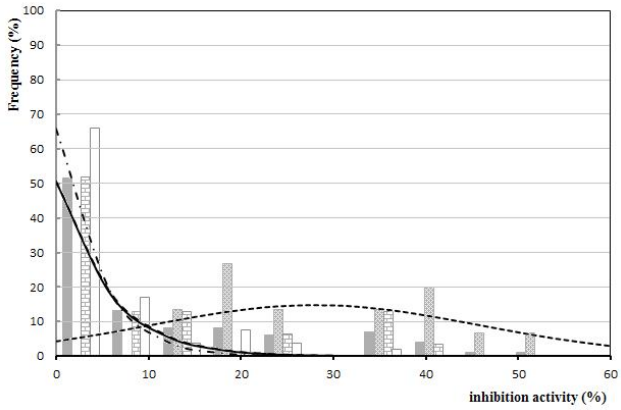

Figure 9: Distribution of inhibitory activity to trypsin of oat extracts expressed as % of inhibitory activity (solid column and solid line – complete set, $r^2 = 0.923$, bracket column and dash line – minor red subset, $r^2 = 0.286$, dots column and dash-and-dot line – major green subset, $r^2 = 0.875$, empty column and dots line – major blue subset, $r^2 = 0.984$, the mentioned colors correspond to the colors in Fig. 7 and Fig. 8).

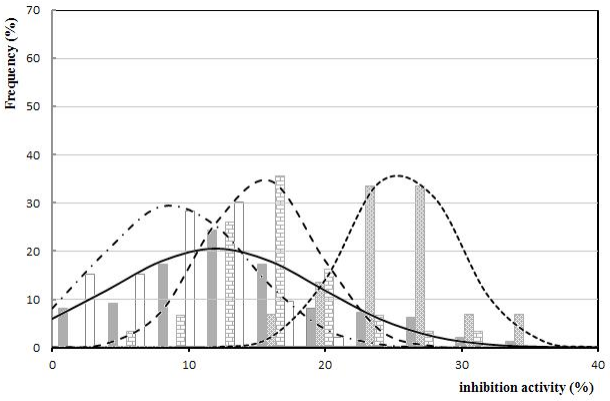

Figure 11: Distribution of inhibitory activity to urokinase in oat extracts expressed as % of inhibitory activity (solid column and solid line – complete set, $r^2 = 0.897$, bracket column and dash line – minor red subset, $r^2 = 0.957$, dots column and dash-and-dot line – major green subset, $r^2 = 0.973$, empty column and dots line – major blue subset, $r^2 = 0.915$, the mentioned colors correspond to the colors in Fig. 7 and Fig. 8).

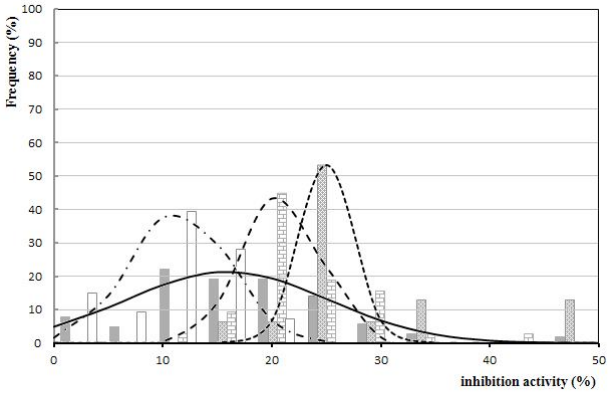

Figure 10: Distribution of thrombin inhibitory activity of oat extracts expressed as % of inhibitory activity (solid column and solid line – complete set, $r^2 = 0.886$, bracket column and dash line – minor red subset, $r^2 = 0.835$, dots column and dash-and-dot line – major green subset, $r^2 = 0.865$, empty column and dots line – major blue subset, $r^2 = 0.885$, the mentioned colors correspond to the colors in Fig. 7 and Fig. 8).

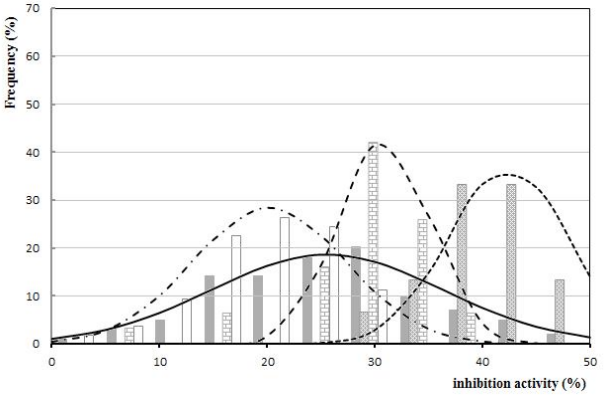

Figure 12: Distribution of inhibitory activity to elastase in oat extracts expressed as % of inhibitory activity (solid column and solid line – complete set, $r^2 = 0.927$, bracket column and dash line – minor red subset, $r^2 = 0.990$, dots column and dash-and-dot line – major green subset, $r^2 = 0.968$, empty column and dots line – major blue subset, $r^2 = 0.980$, the mentioned colors correspond to the colors in Fig. 7 and Fig. 8).

directly describing the inhibitory effect on cathepsin B in oat as well as in other cereal grain extracts have not been published yet.

According to obtained results were identified potentially valuable genotypes from the medical point of view within analyzed set of 100 oat genotypes. Especially the naked oat cultivar Racoon expressed very high inhibitory activity to all five tested proteases, moreover with high content of TFL. Other interesting oats were Avenuda, Expression, Dagny, Neklan, PS-165 expressed inhibitory activity to enzymes which hyperactivity is in relation with promotion of coagulation diseases, like thrombosis, haemorrhage, oncological diseases, etc. Genotypes Expression, Dagny, SW Kerstin, AC Percy, and Izák expressed inhibitory activity to proteases which hyperactivity is responsible for diseases related to connective tissue degradation like arthritis, rheumatism, and oncological diseases.

The frequency distribution of samples according to protease inhibitory activities represent Fig. 9-13. All curves relatively good fitted the Gaussian curve except the minor subset in IA_TY (Fig. 9, ring symbols) where only half wave of the curve was in the real quadrant and rest in the virtual

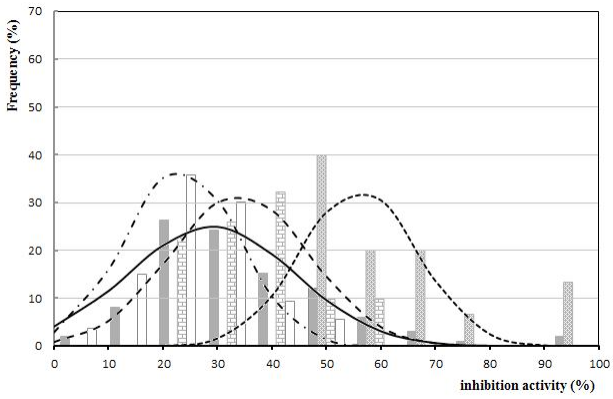

Figure 13: Distribution of inhibitory activity to catepsin B in oat extracts expressed as % of inhibitory activity (solid column and solid line – complete set, $r^2 = 0.901$, bracket column and dash line – minor red subset, $r^2 = 0.648$, dots column and dash-and-dot line – major green subset, $r^2 = 0.902$, empty column and dots line – major blue subset, $r^2 = 0.987$, the mentioned colors correspond to the colors in Fig. 7 and Fig. 8).

(negative) quadrant. The common attribute of all analyzed parameters were their higher values in oats belonging to the minor subset (ring symbols). The central tendencies of a frequency distribution in all protease inhibitory activities were shifted in the direction of higher activities and were 2-2.5 times higher in comparison with the average values of complete set of 100 oats. Similarly to barley grain extracts [59] the oat grains could be interesting as source of natural compounds possessing different biological activities and their combinations.

4 Conclusions

Screening of selected compounds and biological activities was done within a wide range of oats – 100 different genotypes. Oats expressed different contents of polyphenols and flavonoids, radical scavenging activity (DPPH) tested *in vitro*, as well as inhibitory activities against five tested proteases (trypsin, thrombin, urokinase, elastase, cathepsin B). Extracts were prepared from edible parts of oat – mature grains, frequently used as food. Specifically, oats expressing the highest content of TPP (CDC-SOL-FI), TFL (Saul), antioxidant activity (Avesta) and the highest inhibitory activity against one of the five proteases (Racoon, Neklan, SW Kerstin, Izák, Expression), respectively, were clustered in a small minor subset (samples with ring symbols in Fig. 3, Fig. 8). The most interesting genotype was the cultivar Racoon, specifically expressed high inhibitory activities against fivethree tested proteases (IA_TY, IA_UR, and IA_ELA).

Acknowledgments: This study was supported by the Slovak Research and Development Agency (Grant no. APPV-0758-11) and the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and of Slovak Academy of Sciences (Grant no. VEGA 1/1188/12). Authors state no conflict of interest.

References

[1] Dillard C.J., German J.B., Phytochemicals: nutraceuticals and human health, J. Sci. Food Agric., 2000, 80, 1744-1755.
[2] Mena S., Ortega A., Estrela J.M., Oxidative stress in environmental-induced carcinogenesis, Mutat. Res.-Gen. Tox. En., 2009, 674, 36-44.
[3] Hojima Z., Pierce J.V., Pisano J.J., Plant inhibitors of serine proteinases: hageman factor fragment, kallikreins, plasmin, thrombin, factor Xa, trypsin, and chymotrypsin, Thromb. Res., 1980, 20, 163-171.
[4] Yamakawa S., Asai T., Uchida T., Matsukawa M., Akizawa T., Oku N., (–)-Epigallocatechin gallate inhibits membrane-type 1 matrix metalloproteinase, MT1-MMP, and tumor angiogenesis, Cancer Lett., 2004, 210, 47-55.
[5] Cheng X.W., Kuzuya M., Kanda S., Maeda K., Sasaki T., Wang Q.L., et al., Epigallocatechin-3-gallate binding to MMP-2 inhibits gelatinolytic activity without influencing the attachment to extracellular matrix proteins but enhances MMP-2 binding to TIMP-2, Arch. Biochem. Biophys., 2003, 415, 126-132.
[6] Roh C., Jo S., (–)-Epigallocatechin gallate inhibits hepatitis C virus (HCV) viral protein NS5B, Talanta, 2011, 85, 2639-2642.
[7] Kiyosaki T., Asakura T., Matsumoto I., Tamura T., Terauchi K., Funaki J., et al., Wheat cysteine proteases triticain α, β and γ exhibit mutually distinct responses to gibberellin in germinating seeds, J. Plant Physiol., 2009, 166, 101-106.
[8] Ryan C.A., Protease inhibitors in plants: Genes for improving defenses against insects and pathogens, Ann. Rev. Phytopathol., 1990, 28, 425-449.
[9] Altpeter I., Diaz H., McAuslane K., Gaddour P., Carbonero P., Vasil I.K., Increased insect resistance in transgenic wheat stably expressing trypsin inhibitor CMe, Mol. Breed., 1999, 5, 53-63.
[10] Gutierrez-Campos R., Torres-Acosta J.A., Saucedo-Aria L.J., Gomez Lim L.A., The use of cysteine proteinase inhibitors to engineer resistance against potyviruses in transgenic tobacco plants, Nat. Biotechnol., 1999, 17, 1223-1226.
[11] Whitcomb D.C. Molecular and genetic mechanisms of acute and chronic pancreatitis. Int. Congr. Ser., 2003, 1255, 49-60.
[12] Scatena M., Liaw L., Giachelli C.M., Osteopontin: a multifunctional molecule regulating chronic inflammation and vascular disease, Arterioscler. Thromb. Vasc. Biol., 2007, 27, 2302-2309.
[13] Bode W., Structure and interaction modes of thrombin, Blood. Cell. Mol. Dis., 2006, 36, 120-130.
[14] Ploug M., Structure-function relationships in the interaction between the urokinase-type plasminogen activator and its receptor, Curr. Pharm. Des., 2003, 9, 1499-1528.

[15] Garcia-Touchard A., Henry, T.D., Sangiorgi G., Spagnoli L.G., Mauriello A., Conover C., et al., Extracellular proteases in atherosclerosis and restenosis. Arterioscler. Thromb. Vasc. Biol., 2005, 25, 1119-1127.
[16] Lee W.L., Downey G.P., Leukocyte elastase. Physiological functions and role in acute lung injury, Am. J. Respir. Crit. Care Med., 2011, 164, 896-904.
[17] Bohley P., Seglen P., Proteases and proteolysis in the lysosome, Experientia, 1992, 48, 151-157.
[18] Hoegen T. Tremel N., Klein M., Angele B., Wagner H., Kirchning C., et al., The NLRP3 inflammasome contributes to brain injury in pneumococcal meningitis and is activated through ATP-dependent lysosomal cathepsin B release, J. Immunol., 2011, 187, 5440-5451.
[19] Buck M.R., Karustis D.G., Day N.A., Honn K.V., Sloane B.F., Degradation of extracellular-matrix proteins by human cathepsin B from normal and tumour tissues, Biochem. J., 1992, 282, 273-278.
[20] Havrlentová M., Kraic J., Content of β-D-glucan in cereal grains, J. Food Nutr. Res., 2006, 45, 97-103.
[21] Brennan C.S., Cleary L.J., The potential use of cereal (1→3,1→4)-β-d-glucans as functional food ingredients, J. Cereal Sci., 2005, 42, 1-13.
[22] Peterson D.M., Oat antioxidants, J. Cereal Sci., 2001, 33, 115-129.
[23] Gray D.A., Clarke M.J., Baux C., Bunting J.P., Salter A.M., Antioxidant activity of oat extracts added to human LDL particles and in free radical trapping assays, J. Cereal Sci., 2002, 36, 209-218.
[24] Randhir R., Kwon Y., Shetty K., Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain sprouts and seedlings, Innov. Food Sci. Emerg. Technol., 2008, 9, 355-364.
[25] Schellekens C., Perrinjaquet-Moccetti T., Wullschleger C., Heyne A., An extract from wild green oat improves rat behaviour, Phytother. Res., 2009, 23, 1371-1377.
[26] Chu Y., Wise M.L., Gulvady A.A., Chang T., Kendra D.F., van Klimken J.-W., et al., In vitro antioxidant capacity and anti-inflammatory activity of seven common oats, Food. Chem., 2013, 139, 426-431.
[27] Alrahmany R., Tsopmo A., Role of carbohydrases on the release of reducing sugar, total phenolics and on antioxidant properties of oat bran, Food Chem., 2012, 132, 413-418.
[28] Alrahmany R., Avis T.J., Tsopmo A., Treatment of oat bran with carbohydrases increases soluble phenolic acid content and influences antioxidant and antimicrobial activities, Food Res. Int., 2013, 52, 568-574.
[29] Fagerlund A., Sunnerheim K., Dimberg L.H., Radical-scavenging and antioxidant activity of avenanthramides, Food Chem., 2009, 113, 550-556.
[30] Koening R.T., Dickman J.R., Wise M.L., Ji L.L., Avenanthramides are bioavailable and accumulate in hepatic, cardiac, and skeletal muscle tissue following oral gavage in rats, J. Agric. Food Chem., 2011, 59, 6438-6443.
[31] Liu S., Yang N., Hou Z., Yao Y., Lü L., Zhou X., Ren G., Antioxidant effects of oats avenanthramides on human serum, Agric. Sci. China, 2011, 10, 1301-1305.
[32] Birk Y., Proteinase inhibitors from cereal grains, Methods Enzymol., 1976, 45, 723-728.
[33] Carson L., Doctor V.M., Mechanism of potentiation of antithrombin III and heparin cofactor II inhibition by sulfated xylans, Thromb. Res., 1990, 58, 367-381.
[34] Dace R., McBride E., Brooks K., Gander J., Buszko M., Doctor V.M., Comparison of the anticoagulant action of sulfated and phosphorylated polysaccharides, Thromb. Res., 1997, 87, 113-121.
[35] Mikola M., Mikkonen A., Occurrence and stabilities of oat trypsin and chymotrypsin inhibitors, J. Cereal Sci., 1999, 30, 227-235.
[36] Brand-Williams W., Cuvelier M.E., Berset C., Use of a free radical method to evaluate antioxidant activity, LWT-Food Sci. Technol., 1995, 28, 25-30.
[37] Slinkard K., Singleton V.L., Total phenol analysis: Automation and comparison with manual methods, Am. J. Enol. Viticult., 1997, 28, 49-55.
[38] Rakotoarison D., Greissier B., Trotin F., Brunet C., Dine T., Luyckx M., et al., Antioxidant activities of polyphenolic extracts from flowers, in vitro callus and cell suspension cultures of *Crataegus monogyna,* Pharmazie, 1997, 1, 60-64.
[39] Green G.D.J., Shaw E., Thiobenzyl benzyloxycarbonyl-l-lysinate, substrate for a sensitive colorimetric assay for trypsin-like enzymes, Anal. Biochem., 1979, 93, 223-226.
[40] Žilić S., Hadži-Tašković Šukalović V., Dodig D., Maksimović V., Maksimović M., Basić Z., Antioxidant activity of small grain cereals caused by phenolics and lipid soluble antioxidants, J. Cereal Sci., 2011, 54, 417-427.
[41] Angioloni A., Collar C., Polyphenol composition and "in vitro" antiradical activity of single and multigrain breads, J. Cereal Sci., 2011, 53, 90-96.
[42] Pérez-Jiménez J., Saura-Calixto F., Literature data may underestimate the actual antioxidant capacity of cereals, J. Agric. Food Chem., 2005, 53, 5036-5040.
[43] Serea C., Barna O., Phenolic content and antioxidant activity in oat. Annals Food Sci. Technol., 2001, 12, 164e168.
[44] Alfieri M., Redaelli R. Oat phenolic content and total antioxidant capacity during grain development, J. Cereal. Sci., 2015, 65, 39-42.
[45] Verardo V., Serea C., Segal R., Caboni M.F., Free and bound minor polar compounds in oats: Different extraction methods and analytical determinations, J. Cereal Sci., 2011, 54, 211-217.
[46] Zieliński H., Kozłowska H., Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions, J. Agric. Food Chem., 2011, 48, 2008-2016.
[47] Emmonds C.L., Peterson D.M., Antioxidant activity and phenolic content of oat as affected by cultivar and location, Crop Sci., 2000, 41, 1676-1681.
[48] Goun E.A., Petrichenko V.M., Solodnikov S.U., Suhinina T.V., Kline M.A., Cunningham G., et al., Anticancer and antithrombin activity of Russian plants, J. Ethnopharmacol., 2002, 81, 337-342.
[49] Bijak M., Saluk J., Ponczek M.B., Nowak P., Antithrombin effect of polyphenol-rich extracts from black chokeberry and grape seeds, Phytother. Res., 2013, 27, 71-76.
[50] Zha X., Diaz R., Franco J.J.R., Sanchez V. F., Fasoli E., Barletta G., et al., Inhibitors of urokinase type plasminogen activator and cytostatic activity from crude plants extracts, Molecules, 2013, 18, 8945-8958.

[51] Jedinak A., Valachová M., Maliar T., Šturdík E., Antiprotease activity of selected Slovak medicinal plants, Pharmazie, 2010, 65, 137-140.

[52] Maliar T., Drobná J., Kraic J., Maliarová M., Jurovatá J., Proteinase inhibition and antioxidant activity of selected forage crops, Biologia, 2011, 66, 96-103.

[53] Baylac S., Racine P., Inhibition of human leukocyte elastase by natural fragrant extracts of aromatic plants, Int. J. Aromather., 2004, 14, 179-182.

[54] Piwowarski J.P., Kiss A.K., Kozłowska-Wojciechowska M., Anti-hyaluronidase and anti-elastase activity screening of tannin-rich plant materials used in traditional Polish medicine for external treatment of diseases with inflammatory background, J. Ethnopharmacol., 2011, 137, 937-941.

[55] Bosisio E., Mascetti D., Cabalion P., Screening of plant from New Caledonia and Vanuatu for inhibitory activity of xanthine oxidase and elastase, Pharm. Biol., 2000, 38, 18-24.

[56] Sultana N., Lee N.H., Antielastase and free radical scavenging activities of compounds from the stems of *Cornus kousa*, Phytother. Res., 2013, 21, 1171-1176.

[57] Guo Y., Li Y., Xue L., Severino R.P., Gao S., Niu J., et al., *Salvia miltiorrhiza*: An ancient Chinese herbal medicine as a source for anti-osteoporotic drugs, J. Ethnopharmacol., 2004, 155, 1401-1416.

[58] Pan X., Tan N., Zeng G., Zhang Y., Jia R., Amentoflavone and its derivatives as novel natural inhibitors of human cathepsin B, Bioorg. Med. Chem., 2005, 13, 5819-5825.

[59] Maliar T., Slaba G., Nemeček P., Maliarová M., Benková M., Havrlentová M., et al., Antioxidants, enzyme inhibitors, and biogenic compounds in grain extracts of barleys, Chem. Biodiv., 2015, 12, 1678-1695.