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ANTIOXIDANT AND ANTIPROTEINASE EFFECTS OF BUCKWHEAT HULL EXTRACTS

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ABSTRACT

Buckwheat is known not only due to its appropriate nutritional composition but the content of prophylactic compounds, too. These are responsible for buckwheat beneficial impact on human health. Most of them are concentrated in outer layers of buckwheat grain. The subject of this work was to screen hulls of nine common and one tartary buckwheat cultivar for the content of flavonoids and its antioxidant and antiproteinase effects. The highest content of total flavonoids was determined for tartary buckwheat cultivar *Madawaska* (0.6% of hulls weight). Among common buckwheat cultivars the best values reached samples *Bamby* (0.23%) and *KASHO-2* (0.11%). Antioxidant activity as detected via binding radical ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and monitoring reducing power was the most effective for samples with highest flavonoid content. Buckwheat hulls effectively inhibited pathophysiological proteases thrombin and urokinase, whereas only little effects were seen to trypsin and elastase. In this testing there were again the best samples with highest flavonoid content. Only tartary buckwheat *Madawaska* effectively inhibited elastase at tested concentrations. No significant correlation was determined between flavonoid content and measured antioxidant or protease inhibitory action. Obtained results allow us to commend tartary buckwheat cultivar *Madawaska* as well as common buckwheat cultivars *Bamby* and *KASHO-2* for further experiments.

Keywords: antioxidant activity; buckwheat hulls; enzyme inhibition; flavonoids; serine proteases

INTRODUCTION

Food is not only a source of energy and nutrition for maintenance and growth of the body but is also a source of bioactive compounds that have beneficial effects on humans. Common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*) are traditional foodstuffs available worldwide. Buckwheat is a traditional crop in Central and Eastern Europe and Asia. It is not a cereal, but buckwheat seed has chemical, structural and utilisation characteristics similar to those of cereal grains and thus is usually classified as a pseudocereal **(Yildiz, Bilgiçli, 2012)**.

Buckwheat represents raw material interesting in term of its health beneficial properties. It contains many prophylactic compounds that can in the positive way influence genesis and development of many diseases. Dietary fibre is contained mainly in coating layers. It is usefull in preventing gastrointestinal disorders. Phytosterols present in endosperm lower blood cholesterol. Buckwheat bran is rich in B group vitamins. Due to binding proteins they are more bioavailable than from other sources. In comparison with other cereals and pseudocereals buckwheat is better source of magnesium, potassium, phosphorus, zinc, manganese and copper. They are located in peripheral layers and in embryo **(Danihelová, Šturdík, 2012)**.

Buckwheat is known as one of the richest sources of polyphenols and flavonoids. These are concentrated mainly in outer layers of buckwheat grain **(Sedej et al., 2012)**. Among them the most abundant is rutin with its content from 0.02% to 2% **(Jiang et al., 2007)**. In buckwheat we can found also other polyphenols – sinapic, ferulic, syringic or protocatechuic acid **(Sedej et al., 2012)** and flavonoids such as quercetin, catechin, epicatechin, quercitrin, orientin or luteolin **(Verardo et al., 2010)**.

In vitro, *ex vivo* and some *in vivo* experiments have shown that buckwheat possess many positive effects. Plant parts, seeds and even hulls displayed antioxidant properties **(Sun, Chi-Tang, 2005)**, the ability to inhibit cancer cell proliferation **(Kim et al., 2007)**, have anti-allergic **(Kim et al., 2003)**, anti-obesity and anti-inflammatory action **(Wieslander et al., 2011)**. There were investigated inhibitory effects mainly to pathophysiological proteases trypsin and chymotrypsin. In most cases molecules of protein origin were detected as inhibitors **(Tsybina et al., 2004)**.

This paper links to the previous one, that was aimed at screening of buckwheat cultivars for their cytotoxic and antioxidant activity **(Danihelová, Jantová, Šturdík, 2013)**. The subject of this work was to screen nine common buckwheat cultivars and one tartary buckwheat cultivar for total flavonoid content. We have tested buckwheat hull methanolic extracts. Samples were examined for antioxidant activity as detected via binding radical ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and via measuring reducing power

(FRAP). Inhibitory activity to serine proteases trypsin, thrombin, urokinase and elastase was also determined.

MATERIAL AND METHODOLOGY

MATERIAL

Trypsin from porcine pancreas (EC 3.4.21.4, 2000 BAEE U/mg), thrombin from bovine plasma (EC 3.4.21.5, 2000 NIH U/mg), elastase from porcine pancreas (EC 3.4.21.36, 4 U/mg), Nα-benzoyl-D,L-arginine-paranitroanilide hydrochloride, N-glycine-arginine-paranitroanilide dihydrochloride, Nα-benzoyl-L-phenylalanyl-L-valyl-L-arginine-paranitroanilide hydrochloride, N-succinyl-L-alanyl-L-alanyl-L-alanine-paranitroanilide, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium persulfate and 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ) were purchased from Sigma-Aldrich. Urokinase 500 000 HS from human urine (EC 3.4.21.73, 500 000 IU/mg) was from Medac GmbH. All solvents and other reagents were supplied from local companies and were of analytical or HPLC grade.

PLANT MATERIAL TREATMENT

Nine common buckwheat cultivars and one tartary buckwheat cultivar were kindly provided from Plant production research center in Piešťany (SR). Overview of tested cultivars is outlined in Table 1. Buckwheat grains were mechanically dehulled. Obtained hulls were extracted using methanol (p. a.) for 24 hours at room temperature (diluent : weighing material = 10 : 1), filtered and used for flavonoid content determination and antioxidant activity testing. For purposes of enzyme inhibition evaluation extracts were evaporated and dissolved in dimethyl sulfoxide.

Table 1. Overview of tested buckwheat cultivars.

Buckwheat cultivars	Buckwheat variety	Crop year
Pyra	Common buckwheat	2011
Špačinská 1	Common buckwheat	2011
Siva	Common buckwheat	2011
Emka	Common buckwheat	2011
Bamby	Common buckwheat	2011
Aiva	Common buckwheat	2011
Madawaska	Tartary buckwheat	2011
KASHO-2	Common buckwheat	2011
JANA C1	Common buckwheat	2011
Hrusowska	Common buckwheat	2011

TOTAL FLAVONOID CONTENT

The content of flavonoids was determined spectrophotometrically according **Kreft et al. (2002)**. The 200 µl of 5% $AlCl_3$ methanolic solution was added to 2 ml of sample. After 30 min flavonoid-aluminium complex was detected via measuring absorbance at 420 nm. Samples were measured in three replicates. Standard curve of rutin was prepared using the similar procedure. Results were expressed in rutin equivalents (mg RE/g dry sample). Data were presented as means of the percentage of control ± SD (standard deviation).

FREE RADICAL SCAVENGING ACTIVITY (ABTS)

The ability to scavenge free radicals was observed using spectrophotometric method according **Re et al. (1999)**. Cationradical $ABTS^{\cdot+}$ was prepared by reaction between 7 mM ABTS in phosphate buffered saline (0,1 M, pH 7.4) and 2.45 mM potassium persulfate in phosphate buffered saline (0.1 M, pH 7.4) in the rate of 1:1. This mixture stayed at room temperature in the dark for 12 hours. Solution of cationradical $ABTS^{\cdot+}$ was diluted with methanol (1.5 ml of $ABTS^{\cdot+}$ was pipetted into 60 ml of methanol) to get an absorbance of 0.700 at 734 nm. Then 1.95 ml of diluted $ABTS^{\cdot+}$ was added to 0.05 ml of sample. Reaction mixture was incubated 7 min at room temperature in the dark. Thereafter the absorbance was measured at 734 nm. Samples were measured in three replicates. Trolox served as standard antioxidant control. Results were expressed in trolox equivalents (µM TE/g dry sample). Data were presented as means of the percentage of control ± SD (standard deviation).

FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

Antioxidant reducing power of tested samples was performed using FRAP method according **Benzie and Strain (1996)**. This spectrophotometric procedure measures the ability to reduce ferric complex to ferrous. Working FRAP reagent was prepared by mixing 10 ml of acetate buffer (0.1 M, pH = 3.6), 2.5 ml of 10 mM TPTZ (2,4,6-tris(2-pyridyl)-S-triazine) in 40 mM HCl and 2.5 ml of 20 mM $FeCl_3 \cdot 6\ H_2O$. 25 µl of sample and 175 µl of FRAP reagent were pipetted into microplate well. The reaction lasted for 10 min at 37 °C. At the end, absorbance changes were measured spectrophotometrically at 593 nm. Samples were measured in five replicates. Trolox served as standard antioxidant control. Results were expressed in trolox equivalents (µM TE/g dry sample). Data were presented as means of the percentage of control ± SD (standard deviation).

ASSESSMENT OF ENZYME INHIBITION

For the purpose of enzyme inhibition determination we used spectrophotometric method that was reported previously. We adapted methodological modifications from **Jedinák et al. (2006)**. Suitable chromogenic substrates were applied for particular enzymes, concrete Nα-benzoyl-D,L-arginine-paranitroanilide hydrochloride for trypsin, N-glycine-arginine-paranitroanilide dihydrochloride for urokinase, Nα-benzoyl-L-phenylalanyl-L-valyl-L-arginine-paranitroanilide hydrochloride for thrombin and N-succinyl-L-alanyl-L-alanyl-L-alanine-paranitroanilide for elastase.

Hydrolysis of substrates released free nitroaniline, that was measured at 410 nm using microplate screening system. Hydrolytic reactions of substrates (0.03 M) and trypsin (30 BAEE U/ml), urokinase (62 500 IU/ml), thrombin (0.58 NIH U/ml) and elastase (0.02 U/ml) were carried out in phosphate buffered saline (0.01 M, pH = 7.6) at 37°C during 60 min.

All tested extracts were initially solubilized in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/ml and subsequently diluted in the reaction mixture to final concentrations 6.25 - 100 µg/ml. The highest concentration

of DMSO in the reaction mixture never exceeded 2 %. The absorbance was measured in the 1st and 61st minute after reaction started. Each experiment was performed in quintuplicate. Inhibitory activity was expressed as the concentration that is responsible for 50 % of substrate cleavage inhibition (IC_{50}). Data were presented as means of the percentage of control ± SD (standard deviation).

RESULTS AND DISCUSSION

TOTAL FLAVONOID CONTENT

Among natural plant sources rich in bioactive compounds we have choosed buckwheat due to its high content of rutin, tradition of cultivation in Slovakia as well as large scale of documented biological effects **(Krkošková, Mrázová, 2005)**. Buckwheat hulls represent waste material that has no important commercial utilization. But in comparison with other parts of buckwheat grain in hulls are concentrated present polyphenols and flavonoids **(Sedej et al., 2012)**. We therefore decided to use these in our experiments. From the collection of nine common buckwheat cultivars and one tartary buckwheat cultivar we prepared hull extracts in methanol using diluent to weighing material ratio 10:1. In samples we first determined total flavonoid content. Results are presented in Figure 1.

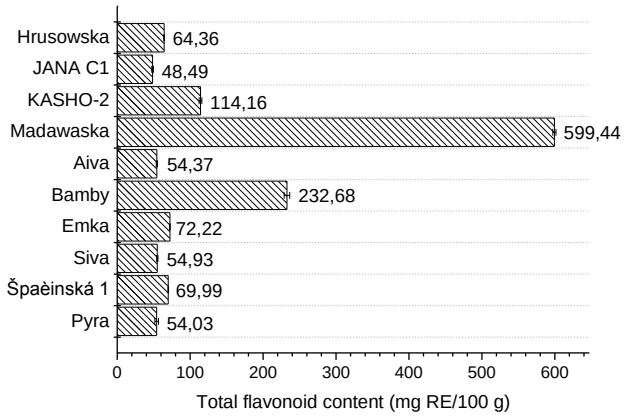

Figure 1. Total flavonoid content determined in buckwheat cultivars (RE = rutin equivalent).

According to the literature the highest flavonoid content was detected in tartary buckwheat (about 0.6% of hulls weight). The most of common buckwheat cultivars have shown approximately 10 times lower content of flavonoids as compared to tartary buckwheat. Among common buckwheat cultivars the most flavonoids contained cultivars *KASHO-2* (0.11%) and *Bamby* (0.23%).

Sedej et al. (2012) found significantly higher content of total flavonoids in buckwheat hull than in whole grain and groat. Also other authors discovered that flavonoids are more abundant in hulls than in the flour (45.6 mg/100 g DW for hulls and 9.8 mg/100 g DW for flour) **(Quettier-Deleu et al., 2000)**.

Obtained data from the literature about buckwheat flavonoid content lie in the wide range because they are dependent on varietal and growth conditions. Common buckwheat hulls contained total flavonoids from 36 mg/100 g to 1180 mg/100 g of hulls weight **(Watanabe, Ohshita, Tsushida, 1997; Quettier-Deleu et al., 2000; Sedej et al., 2008)**. Data stated for tartary buckwheat hulls are higher – 1100 mg/100 g to 3000 mg/100 g of hulls weight **(Yongyan et al., 2007; Xiong et al., 2009)**. Our results are comparable with these values, but flavonoid content for tartary buckwheat is lower.

ANTIOXIDANT ACTIVITY

Because flavonoids are known for their antioxidant properties and our previous investigations have shown, that buckwheat hull extracts possess antioxidant action, in the next step we have examined their antioxidant activity using other two different spectrophotometric methods. The first one follows the ability to bind cationradical $ABTS^{\cdot+}$. Antioxidants present in buckwheat caused radical binding and thereby its decolorization. The second one measures reducing power of samples (FRAP). Active samples could reduce ferric complex to ferrous, what resulted in color change. Activity was compared to standard antioxidant trolox (TE = trolox equivalent). Determined effects are presented in Table 2.

Table 2. Antioxidant activity of buckwheat hull samples as determined via binding radical 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and by measuring ferric reducing power (FRAP method).

Buckwheat cultivars	ABTS (μM TE/g DW)*	FRAP (μM TE/g DW)*
Pyra	691.19 ± 35.78	227.94 ± 4.77
Špačinská 1	822.87 ± 21.97	227.55 ± 9.08
Siva	765.85 ± 16.26	117.44 ± 3.74
Emka	781.91 ± 12.33	174.57 ± 2.09
Bamby	1083.48 ± 23.63	472.59 ± 12.27
Aiva	479.70 ± 11.15	96.64 ± 4.32
Madawaska	1603.14 ± 37.42	1103.61 ± 6.62
KASHO-2	1141.15 ± 25.11	581.98 ± 19.17
JANA C1	501.51 ± 5.28	84.25 ± 0.76
Hrusowska	698.39 ± 16.33	121.36 ± 3.14

*TE = trolox equivalent
DW = dry weight

Comparing obtained results of both measurements we came to the same conclusion. Antioxidant activity determined via binding radical ABTS as well as measuring reducing power (FRAP) was the highest in the case of tartary buckwheat *Madawaska*, which concurrent contained the highest amount of total flavonoids among tested samples. Among common buckwheat samples we observed best antioxidant properties for cultivars *Bamby* and *KASHO-2*, that was about one third lower than for tartary buckwheat. These two cultivars also contained relatively high amount of flavonoids.

Available literature documents, that tartary buckwheat because of higher polyphenol and flavonoid content exhibit higher antioxidant properties **(Tsai et al., 2012; Zhao et al., 2012)**. **Guo et al. (2011)** reported for tartary buckwheat antioxidant activity similar values with our determination. **Zielińska et al. (2010)** observed for common buckwheat hulls higher ability to bind free radicals than we have stated for our cultivars.

Most of authors detected positive correlation between flavonoid content and antioxidant activity in buckwheat samples **(Sedej et al., 2008; Markovic et al., 2009)**. But there were some that claimed no relationship in this case **(Oomah, Mazza, 1996)**. Our results show no significant

correlation between determined flavonoid content and measured antioxidant action.

SERINE PROTEASE INHIBITION

Data from the literature indicate potential inhibitory activity of buckwheat extracts to set of enzymes including serine proteases. This inhibitory activity authors ascribe mainly to various peptides present in buckwheat seed **(Tsybina et al., 2004)**.

Because flavonoids are known for their inhibitory action to various enzymes **(Jedinák et al., 2006)**, we decided to test buckwheat hull samples with proven flavonoid content for inhibition of serine proteases trypsin, thrombin, urokinase and elastase. Results were expressed in IC_{50} values, that represent extract concentration with 50% inhibitory activity in comparison with control (without an inhibitor). Determinations are listed in Table 3.

Table 3. Inhibitory effects of buckwheat hull extracts to serine proteases trypsin, thrombin, urokinase and elastase.

Buckwheat cultivars	IC_{50} (mg/ml)			
	Trypsin	Thrombin	Urokinase	Elastase
Pyra	> 0,5	0,352 ± 0,013	0,343 ± 0,012	> 0,5
Špačinská 1	> 0,5	0,350 ± 0,006	0,350 ± 0,006	> 0,5
Siva	> 0,5	0,371 ± 0,015	0,358 ± 0,015	> 0,5
Emka	> 0,5	0,332 ± 0,012	0,305 ± 0,016	> 0,5
Bamby	> 0,5	0,127 ± 0,006	0,156 ± 0,004	> 0,5
Aiva	> 0,5	0,272 ± 0,002	0,310 ± 0,010	> 0,5
Madawaska	> 0,5	0,134 ± 0,005	0,141 ± 0,002	0,353 ± 0,018
KASHO-2	> 0,5	0,113 ± 0,003	0,151 ± 0,005	> 0,5
JANA C1	> 0,5	0,386 ± 0,014	0,330 ± 0,012	> 0,5
Hrusowska	> 0,5	0,364 ± 0,011	0,338 ± 0,016	> 0,5

Among tested enzymes buckwheat hull extracts were the most potent inhibitors of thrombin and urokinase. Best inhibitory activities to both enzymes revealed common buckwheat cultivars *KASHO-2* and *Bamby* as well as tartary buckwheat cultivar *Madawaska*. These cultivars were about three times better than other tested samples. Buckwheat hull extracts have shown minimal inhibitory effects to trypsin and elastase. Only tartary buckwheat *Madawaska* inhibited effectively elastase at tested concentrations (IC_{50} = 0.353 mg/ml).

Tsybina et al. (2001) obtained low molecular weight protein inhibitors of serine proteinases from buckwheat seeds. These effectively inhibited trypsin, chymotrypsin and subtilisin. Other authors discovered inhibitory activity of peptide from buckweet seed to trypsin, chymotrypsin and cathepsin G **(Gladysheva et al., 1995)**. **Wang et al. (2006)** purified and characterized protease inhibitor from tartary buckwheat seeds with specific trypsin inhibitory activity. **Oparin et al. (2012)** obtained peptide trypsin inhibitor from buckwheat seeds.

As we can see, authors investigated protease inhibitory activity of buckwheat mainly to trypsin, chymotrypsin, subtilisin and cathepsin G. To our knowledge this is for the first time that was examined buckwheat extract inhibition of thrombin, urokinase and elastase. It seems that flavonoids are in this case effective components from buckwheat hull extracts.

CONCLUSION

Buckwheat belongs to traditional crops in Central and Eastern Europe and Asia. It is effective in management of many diseases, mainly cardiovascular and digestion disorders, cancer, diabetes and obesity. Effective prophylactic compounds are present mainly in outer layers of buckwheat grain.

In this study there were screened hulls of ten buckwheat cultivars. We can conclude, that the highest total flavonoid content revealed tartary buckwheat *Madawaska*. Among common buckwheat best values achieved cultivars *Bamby* and *KASHO-2*. Samples with highest flavonoid content were the most effective in testing of their antioxidant and antiproteinase properties. In regard of achieved results we can commend tartary buckwheat *Madawaska* and common buckwheat cultivars *Bamby* and *KASHO-2* for further experiments.

LITERATURE

Benzie, I. F. F., Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of „antioxidant power“: the FRAP assay. *Analytical Biochemistry*, vol. 239, no. 1, p. 70-76. http://dx.doi.org/10.1006/abio.1996.0292, PMid:8660627

Danihelová, M., Šturdík, E. 2012. Nutritional and health benefits of buckwheat. *Potravinarstvo*, vol. 6, no. 3, p. 1-9. http://dx.doi.org/10.5219/206

Danihelová, M., Jantová, S., Šturdík, E. 2013. Cytotoxic and antioxidant activity of buckwheat hull extracts. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 2, special issue, p. 1314-1323.

Gladysheva, I. P., Dunaevskiĭ, I. a E., Belozerskiĭ, M. A., Gladyshev, D. P., Papisova, A. I., Larionova, N. I. 1995. Inhibition of exogenous serine proteinases by a trypsin inhibitor from the buckwheat IT-1 seeds. *Biokhimiia*, vol. 60, no. 9, p. 1530-1535. PMid:8562658

Guo, X.-D., Ma, Y.-J., Parry, J., Gao, J.-M., Yu, L.-L., Wang, M. 2011. Phenolics content and antioxidant activity of tartary buckwheat from different locations. *Molecules*, vol. 16, no. 12, p. 9850-9867. http://dx.doi.org/10.3390/molecules16129850 PMid:22117174

Jedinák, A., Maliar, T., Grančai, D., Nagy, M. 2006. Inhibition activities of natural products on serine proteases. *Phytotherapy Research*, vol. 20, no. 3, p. 214-217. http://dx.doi.org/10.1002/ptr.1836, PMid:16521112

Jiang, P., Burczynski, F., Campbell, C., Pierce, G., Austria, J. A., Briggs, C. J. 2007. Rutin and flavonoid contents in three buckwheat species *Fagopyrum esculentum*, *F. tataricum*, and *F. homotropicum* and their protective effects against lipid peroxidation. *Food Research International*, vol. 40, no. 3, p. 356-364. http://dx.doi.org/10.1016/j.foodres.2006.10.009,

Kim, C. D., Lee, W.-K., No, K.-O., Park, S.-K., Lee, M.-H., Lim, S. R., Roh, S.-S. 2003. Anti-allergic action of buckwheat (*Fagopyrum esculentum* Moench) grain extract. *International*

Immunopharmacology, vol. 3, no. 1, p. 129-136. http://dx.doi.org/10.1016/S1567-5769(02)00261-8,

Kim, S. H., Cui, C. B., Kang, I. J., Kim, S. Y., Ham, S. S. 2007. Cytotoxic effect of buckwheat (*Fagopyrum esculentum* Moench) hull against cancer cells. *Journal of Medicinal Food*, vol. 10, no. 2, p. 232-238. http://dx.doi.org/10.1089/jmf.2006.1089, PMid:17651057

Kreft, S., Štrukelj, B., Gaberščik, A., Kreft, I. 2002. Rutin in buckwheat herbs grown at different UV-B radiation levels: comparison of two UV spectrophotometric and an HPLC method. *Journal of Experimental Botany*, vol. 53, no. 375, p. 1801-1804. http://dx.doi.org/10.1093/jxb/erf032, PMid:12147730

Krkošková, B., Mrázová, Z. 2005. Prophylactic components of buckwheat. *Food Research International*, vol. 38, no. 5, p. 561-568. http://dx.doi.org/10.1016/j.foodres.2004.11.009

Markovic, G., Sedej, I., Mišan, A., Sakač, M., Tadić, V., Mandić, A., Pestorić, M. 2009. Phenolic compounds and antioxidative properties of buckwheat grain, hull and flours. *Planta Medica*, vol. 75, no. 9, p. 86. http://dx.doi.org/10.1055/s-0029-1234411

Oomah, B. D., Mazza, G. 1996. Flavonoids and antioxidative activities in buckwheat. *Journal of Agricultural and Food Chemistry*, vol. 44, no. 7, p. 1746-1750. http://dx.doi.org/10.1021/jf9508357

Oparin, P. B., Mineev, K. S., Dunaevsky, Y. E., Arseniev, A. S., Belozersky, M. A., Grishin, E. V., Egorov, T. A., Vassilevski, A. A. 2012. Buckwheat trypsin inhibitor with helical hairpin structure belongs to a new family of plant defence peprides. *The Biochemical Journal*, vol. 446, no. 1, p. 69-77. http://dx.doi.org/10.1042/BJ20120548, PMid:22612157

Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., Cazin, M., Cazin, J.-C., Bailleul, F., Trotin, F. 2000. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *Journal of Ethnopharmacology*, vol. 72, no. 1-2, p. 35-42. http://dx.doi.org/10.1016/S0378-8741(00)00196-3

Re, R., Pellegrini, N., Proteggente, A., Pannala, A:, Yang, M., Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, vol. 26, no. 9-10, p. 1231-1237. http://dx.doi.org/10.1016/S0891-5849(98)00315-3

Sedej, I., Mišan, A., Sakač, M., Mandić, A., Pestorić, M. 2008. Antioxidative properties of buckwheat grain, hull and flour. *Planta Medica*, 74, no. 9, p. 52. http://dx.doi.org/10.1055/s-0028-1084897

Sedej, I., Sakač, M., Mandić, A., Mišan, A., Tumbas, V., Čanadanović-Brunet, J. 2012. Buckwheat (*Fagopyrum esculentum* Moench) grain and fractions: antioxidant compounds and activities. *Journal of Food Science*, vol. 77, no. 9, p. C954-C959. http://dx.doi.org/10.1111/j.1750-3841.2012.02867.x, PMid:22888949

Sun, T., Chi-Tang, H. 2005. Antioxidant activities of buckwheat extracts. *Food Chemistry*, vol. 90, no. 4, p. 743-749. http://dx.doi.org/10.1016/j.foodchem.2004.04.035

Tsai, H., Deng, H., Tsai, S., Hsu, Y. 2012. Bioactivity comparison of extracts from various parts of common and tartary buckwheats: evaluation of the antioxidant- and angiotensin-converting enzyme inhibitory activities. *Chemistry Central Journal*, vol. 6, no. 1, p. 78. http://dx.doi.org/10.1186/1752-153X-6-78, PMid:22853321

Tsybina, T. A., Dunaevsky, Y. E., Musolyamov, A. K., Egorov, T. A., Belozersky, M. A. 2001. Cationic inhibitors of serine proteinases from buckwheat seeds. *Biokhimiia*, vol. 66, no. 9, p. 941-947. http://dx.doi.org/10.1023/A:1012388805336

Tsybina, T., Dunaevsky, Y., Musolyamov, A., Egorov, T., Larionova, N., Popykina, N., Belozersky, M. 2004. New protease inhibitors from buckwheat seeds: properties, partial amino acid sequences and possible biological role. *Biological Chemistry*, vol. 385, no. 5, p. 429-434. http://dx.doi.org/10.1515/BC.2004.049, PMid:15196004

Verardo, V., Arráez-Román, D., Segura-Carretero, A., Marconi, E., Fernández-Gutiérrez, A., Caboni, M. F. 2010. Identification of buckwheat phenolic compounds by reverse phase high performance liquid chromatography-electrospray ionization-time of flight-mass spectrometry(RP-HPLC-ESI-TOF-MS). *Journal of Cereal Science*, vol. 52, no. 2, p. 170-176. http://dx.doi.org/10.1016/j.jcs.2010.04.009

Wang, Z.-H., Zhao, Z.-H., Zhang, Z., Yuan, J.-M., Noback, D., Wieslander, G. 2006. Purification and characterization of a protease inhibitor from *Fagopyrum tartaricum* Gaertn seeds and its effectiveness against insects. *Chinese Journal of Biochemistry and Molecular Biology*, vol. 22, no. 12, p. 960-965.

Watanabe, M., Ohshita, Y., Tsushida, T. 1997. Antioxidant compounds from buckwheat (*Fagopyrum esculentum* Moench) hulls. *Journal of Agricultural and Food Chemistry*, vol. 45, no. 4, p. 1039-1044. http://dx.doi.org/10.1021/jf9605557

Wieslander, G., Fabjan, N., Vogrinčič, M., Kreft, I., Janson, C., Spetz-Nnyström, U., Vombergar, B., Tagesson, C., Leanderson, P., Norbäck, D. 2011. Eating buckwheat cookies is associated with the reduction in serum levels of myeloperoxidase and cholesterol: A double blind crossover study in day-care centre staffs. *The Tohoku Journal of Experimental Medicine*, vol. 225, no. 2, p. 123-130. http://dx.doi.org/10.1620/tjem.225.123, PMid:21931228

Xiong, S.-L., Li, A.-L., Ren, F., Jin, H. 2009. Study on extraction of total flavonoids from powder or husks of different cultivars of buckwheat and analysis on their free radical scavenging activities. *Food Science*, vol. 30, no. 3, p. 118-122.

Yildiz, G., Bilgiçli, N. 2012. Effects of whole buckwheat flour on physical, chemical, and sensory properties of flat bread, Lavaş. *Czech Journal of Food Sciences*, vol. 30, no. 6, p. 534-540.

Yongyan, H., Baili, F., Tao, D., Pengtao, L., Jinfeng, G., Yan, C. 2007. Antioxidant activity of ethanol extracts of different buckwheat. *Proceedings of the 10th International Symposium on Buckwheat*, China, p. 465-468. ISBN 9787810923583.

Zhao, G., Peng, L.-X., Wang, S., Hu, Y.-B., Zou, L. 2012. HPLC fingerprint – antioxidant properties study of buckwheat. *Journal of Integrative Agriculture*, vol. 11, no. 7, p. 1111-1118. http://dx.doi.org/10.1016/S2095-3119(12)60104-X

Zielińska, D., Syawara-Nowak, D., Zieliński, H., 2010. Determination of the antioxidant activity of rutin and its contribution to the antioxidant capacity of diversifed buckwheat origin material by updated analytical strategies. *Polish Journal of Food and Nutrition Sciences*, vol. 60, no. 4, p. 315-321.

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