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# SEEDS OF SELECTED FRUITS AS A GOOD SOURCE OF FLAVAN-3-OLS

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Key words: proanthocyanidins, fruit seeds, sea buckthorn, flowering quince, quince, rowanberry, hawthorn, rosehip, elderberry

The aim of the study was to determine the content of flavan-3-ols of selected fruit seeds. The investigated seeds have a very different level of flavan-3-ols and polymeric procyanidins. Seeds rowanberry and hawthorn contained the highest quantity flavan-3-ols (517.48 and 221.49 mg/100 g). However elderberry (24.15 mg/100 g) and quince (16.12 mg/100g) contained the lowest amount of flavan-3-ols. In other seed, buckthorn was measured 95.27 mg/100 g of monomeric catechin, but in flowering quince and rosehip have 165.25 and 168.37 mg/100 g. (-)-Epicatechin was dominated in seeds from flowering quince, quince, rowanberry and elderberry. The content of (-)-epicatechin in rowanberry was 460.10 mg/100 g, which was about 89% for total content of flavan-3-ols. However (+)-catechin predominated in seeds of roseship (96%). Buckthorn seeds are claimed to be rich in a complex mixture of catechins, ranging from (-)-epicatechin (54%) and (+)-catechin (46%). The contents sum of proanthocyanidins in investigated seeds ranged from 219.16 to 4263.67 mg/100 g. The lowest content of proanthocyanidins was obtained for elderberry, roseship C and B. The investigated seeds, especially rowanberry and hawthorn have excellent sources of flavan-3-ols. This result suggests that the berry seeds should be further utilized rather than just discarded as waste.

## INTRODUCTION

Phenolic compounds are commonly found in both edible and non-edible parts of plants, and they have been reported to have multiple biological effects, including antioxidant activity. Potential sources of antioxidant compounds have been searched for in several types of plant material such as vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots, spices and herbs, and crude plant drugs [Kähkönen *et al.,* 1999].

Seeds, apart from their physiological function connected with the reproduction of plants, together with pomace are the waste products of fruit-processing. Difficulties connected with implementing the farm wastes can prompt researchers to experiment with utilization of biologically active components present in wastes, and their possible use as antioxidants in cosmetic and pharmaceutical industry. It would be beneficial for the improvement of seeds' complete utilization, if they could be used as a source of natural food additives and ingredients. Seeds showed a much higher antioxidant activity and phenolic content than the edible parts of tested fruits.

Berry seeds have not generally received much attention as the sources of antioxidants and this could be due to their lack of popularity and lack of commercial applications. They are commonly being used for the production of oil. Raspberry and blackberry seeds, which are byproducts of juice processing, because of their unique chemical composition and mostly due to fatty acids, tocopherols, and proanthocyanidins contained in them, are used in the production of oil [Xu *et al.,* 2006]. Grape seeds are used as a dietary supplement and natural food additive in the USA, Australia, Japan, Korea, and many European countries. The results reported by Chell *et al.* [2007] show that strawberry seeds removal during juice processing and home cooking results in a significant loss of important phenolics, especially catechins and flavonols. The highest level of total ellagic acid was found in strawberry leaves followed by achenes, and finally, flesh [Maas *et al.,* 1991].

In strawberry pulp with achenes the total ellagic acid content for red fruit (cv. Camarosa) was more than 6 times higher as opposed to pulp without achenes [Williner *et al.,* 2003]. The study on antifungal activity of strawberries found that antifungal compounds included phenolics and the highest activity was found in achenes [Terry *et al.,* 2004]. When strawberry fruits are processed to obtain juice and puree, substantial waste material containing high levels of achenes is generated. Instead of being fed to livestock or sent to sanitary landfill, this processing waste could be a potential source of nutraceuticals. It is important to know phenolic composition and antioxidant properties of strawberry achenes in order to evaluate them as a source of natural antioxidants.

There has been an increasing consumer demand for this product due to its powerful antioxidant properties and other beneficial biological activities [Bakkalbasa *et al.,* 2005]. A phytochemical investigation of coca has led to the isolation of (+)-catechin and (-)-epicatechin [Azizah *et al.,* 2002]. Antioxidative activity of tamarind seed coat is due to the presence of epicatechin [Tsuda *et al.,* 1994]. Avocado seeds are claimed

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to be rich in a complex mixture of polyphenolic compounds, ranging from (+)-catechin and (-)-epicatechin to highly polymeric substances; a proanthocyanidins has also been identified [Geissman & Dittmar, 1965].

Flavan-3-ol monomers and polymers (proanthocyanidins – known better as condensed tannins) are ubiquitous in the plant kingdom and are present in many foods, especially in fruits and berries. Flavan-3-ols are also present as esters of gallic acid and have some glycosidated structures. Proanthocyanidins are oligomers consisting of flavan-3-ol subunits linked through C4→C8 or C4→C6 bonds (B-type). An additional ether bond can also be formed between C2→C7 (A-type). Proanthocyanidins consisting only of (epi)catechin subunits are called procyanidins, whereas prodelphinidins are oligomers and polymers of (epi)gallocatechin [Manach *et al.,* 2004]. Proanthocyanidins have attracted increasing attention in the field of nutrition and medicine due to their potential health benefits observed *in vitro* and *in vivo*. It has been reported that proanthocyanidins possess various biological activities, such as anti-inflamation, anti-carcinogenic, anti-mutagenic, anti-ulcer, anti-atherogenic, anti-microbial effect and serve as the inhibitors of human low density lipoprotein oxidation [Santos-Buelga & Scalbert, 2000].

Proanthocyanidins are of great interest to nutrition and medicine because of their potent antioxidant capacity [Santos-Buelga & Scalbert, 2000]. It has been recently hypothesized that the free radical scavenging properties of PAs may reduce the risk of cardiovascular diseases, cancer [Bagchi *et al.,* 2000], and blood clotting and that certain types of trimeric PAs may protect against urinary tract infections [Santos-Buelga & Scalbert, 2000].

Proanthocyanidins in flowering quince, quince, rowanberry, hawthorn, rosehip and elderberry have not yet been reported. The objective of the present study was to determine the content of flavan-3-ols and proanthocyanidins in seeds of selected fruit sea buckthorn, flowering quince, quince, rowanberry, hawthorn, rosehip, and elderberry.

## MATERIALS AND METHODS

The seeds of following berry and fruits: two variety of sea buckthorn (A- *Hippophäe rhamnosides* L. and B- *Hippophäe salicifolia*), flowering quince (*Chaenomeles japonica*), quince (*Cydonia oblonga*), rowanberry (*Sorbus aucuparia* L.), hawthorn (*Crataegus oxyacanta*), three variety of rosehip (A- *Rosa rugosa,* B- *Rosa canina,* C- *Rosa hybrida*) and elderberry (*Sambucus nigra* L.) were used in the experience. Berry and fruits were collected in the middle of October at a plantation near Wrocław, Poland, and immediately stored at -20°C. The ten selected berries and fruits with seeds were freeze-dried (-50°C, 24 h) (ALPHA 1-4 LSC, Germany), and after lyophilizing process, separately seeds was grounded in laboratory mill.

Direct thiolysis of freeze-dried seeds was performed as described by Guyot *et al*. [2001]. Portions (0.5 g) of milled seeds were precisely measured in 1.5 mL Eppendorf vials, then acidic methanol (3.3% (v/v), 400 $\mu$L) and toluene α-thiol (5% in methanol, 800 $\mu$L) were added. Vials were closed and incubated at 40°C for 30 min with agitation on a vortex every 10 min. Next, the vials were cooled in ice water and centrifuged immediately at 4°C at 14,000 rpm (20.000g) for 10 min. Samples were stored at 4°C until RP-HPLC analysis. All incubations were done in triplicate. Thiolysis products were separated on a Merck Purospher RP 18 end-capped column 250x4 mm, 5$\mu$m (Merck, Darmstadt, Germany). The liquid chromatograph was a Waters (Milford, MA) system equipped with DAD and Scanning Fluorescence detectors. The solvent A (aqueous acetic acid, 2.5% (v/v)) and solvent B (acetonitrile) were used as the following gradient: initial 3% B, 0-5 min, 9% B linear; 5-15 min, 16% B linear; and 15-45 min, 50% B linear, followed by washing and reconditioning of the column. Flow rate 1 mL/min, and oven temperature 30°C were used. The compounds for which reference standards were available (synthesized or isolated previously), were identified on chromatograms according to their retention times and UV-vis spectra. Fluorescence was recorded at excitation wavelength of 278 nm and emission wavelength of 360 nm. Calibration curves were established using flavan-3-ol and benzylthioether standards prepared in our laboratory. The average degree of polymerization (DP) was measured by calculating the molar ratio of all the flavan-3-ol units (thioether adducts + terminal units) to (-)-epicatechin and (+)-catechin.

## RESULT AND DISCUSSION

The result for (+)-catechin, (-)-epicatechin, total of proanthocyanins and data obtained by thiolysis degradation of proanthocyanidin content of each of the selected berries and fruits seeds are presented in Table 1.

The investigated seeds had different levels of flavan-3-ols. Seeds of rowanberry and hawthorn contained the highest quantity from the group of flavan-3-ols (517.48 and 221.49 mg/100 g). However, elderberry (24.15 mg/100 g) and quince (16.12 mg/100 g) contained the lowest amount of flavan-3-ols. In the buckthorn A and B seeds, sum of (+)-catechin and (-)-epicatechin were measured to be 46.38 and 95.27 mg/100 g, respectively. Seeds of rosehip have very similar content of catechins, ranging from 104.91 to 168.37 mg/100 g. The investigated seeds had more catechins than other seeds studied by Xu *et al.* [2006]. The seed of raspberry and blackberry contained only from 6.81 to 17.6 mg/g of total catechins [Xu *et al.,* 2006].

(+)-Catechin is present in almost all analyzed berries and fruit, with one significant exception. In hawthorn, we could not find (+)-catechin. Our results for catechins in hawthorn are similar to those reported by Cui *et al.* [2006]. The content of these compounds ranged from 160.99 mg/100g for rosehip B to 0.86 mg/100g for quince. Rosehip B has more (+)-catechins than roseship A and C (160.99 > 111.35 > 99.59, respectively). Ercisli [2007] found that *Rosa canina* (rosehip B) has more phenolic compounds than other investigated species of roses. Sea buckthorn B has two times higher levels of (+)-catechins than sea buckthorn A (Table 1). In general, the results obtained for sea buckthorn correspond well with the data obtained by Fan *et al.* [2007]. This difference could be explained by cultivar variation of samples. However, various environmental factors determine the extent to which genetic potentialities are achieved. Ecology, drought, soil type/structure, disease, herbivore damage, and farming practices (*i.e.*

TABLE 1. The content of flavan-3-ols and proanthocyanidins in selected fruits seeds.

| Fruit seeds | (+)-Catechin (mg/100 g) | (-)-Epicatechin (mg/100 g) | (+)-Catechin-4−benzylthioether (mg/100 g) | (-)-Epicatechin-4−benzylthioether (mg/100 g) | Total proanthocyanidins (mg/100 g) | Degree of polymerization (DP) |
|---|---|---|---|---|---|---|
| Sea buckthorn A | 29.20±0.18 | 17.18±1.06 | 239.94±3.65 | 222.51±2.67 | 508.83 | 2.1 |
| Sea buckthorn B | 44.05±0.23 | 51.21±2.05 | 321.87±1.03 | 300.28±2.85 | 717.42 | 2.0 |
| Flowering quince | 6.21±0.45 | 159.04±1.67 | 12.27±0.56 | 1383.34±1.84 | 1560.86 | 1.3 |
| Quince | 0.86±0.06 | 15.26±0.56 | 2.50±0.21 | 425.11±3.01 | 443.73 | 2.0 |
| Rowanberry | 57.38±1.01 | 460.10±2.76 | 9.65±0.65 | 3736.54±6.05 | 4263.67 | 1.8 |
| Hawthorn | 0.00±0.00 | 221.49±1.34 | 0.00±0.00 | 376.06±7.93 | 597.55 | 5.6 |
| Roseship A | 111.35±1.34 | 4.65±0.21 | 100.83±1.54 | 196.25±1.22 | 413.08 | 2.0 |
| Roseship B | 160.99±2.04 | 7.38±0.34 | 81.51±2.94 | 632.77±2.81 | 882.65 | 12.9 |
| Roseship C | 99.59±1.45 | 5.32±1.11 | 170.55±2.65 | 108.21±0.54 | 383.67 | 3.0 |
| Elderberry | 4.56±0.21 | 19.60±0.87 | 9.09±1.13 | 185.92±2.99 | 219.16 | 1.8 |

pruning, application of pesticides, *etc.*) do have an influence on secondary plant metabolism.

(+)-Catechin level in flowering quince, elderberry, and quince is low (6.21 mg/100 g). The result obtained for quince is similar to the result described by Silvana *et al.* [2004].

Rowanberries are the richest sources of (-)-epicatechins among investigated berries and fruit. (-)-Epicatechins constitute about 89% of the total content of flavan-3-ols. Haukkanen *et al.* [2006] reported that rowanberries, apart from flavan-3--ols, have also other significant contents of hydroxycinnamic acids and flavonols. Hawthorn and flowering quince have about 221.49 and 159 mg of (-)-epicatechin per 100 g. Hawthorn is a traditional European medical plant. Dried flowers, leaves, and fruits of hawthorn are used as crude drugs. Several studies have shown that aqueous and alcoholic extracts have beneficial effects on the heart and blood circulation including cardiovascular protective and hypotensive effects; and oligomeric proantocyanidins are considered to be the main active constituents in hawthorn [Rohr *et al.,* 1999]. Despite their high (+)-catechin content, rosehips are very poor in (-)-epicatechin, ranging from 4.65 to 7.38 mg/100 g.

Only the seeds of sea buckthorn B are claimed to be rich in a complex mixture of flavan-3-ols; they contain 54% of (-)-epicatechin and 46% (+)-catechin. Sea buckthorn is a fascinating plant growing widely in various regions of the world. It has many diverse uses, from controlling soil erosion to being a source of horse fodder, nutritional foods, drugs, and skin-care products. Although high contents of natural antioxidants including ascorbic acid, tocopherols, carotenoids, and polyphenols have been detected in sea buckthorn fruits [Heinonen *et al.,* 1998] and leaves [Chumbalov *et al.,* 1976], only sea buckthorn had epigallocatechin. The content of epigallocatechin in sea buckthorn A and B was 652.79 and 747.67 mg/100 g, respectively (data not presented). This result corresponds well with the data obtained by Fan *et al.* [2007]. Fan *et al.* [2007] in their study on sea buckthorn seeds found, apart from (+)-catechin and (-)-epicatechin, also gallocatechin, epigallocatechin, and two dimeric procyanidins, catechin(4α-8)catechin, and catechin(4α-8)epicatechin.

The contents sum of proanthocyanidins in investigated seeds ranged from 219.16 to 4263.67 mg/100 g. The lowest content of proanthocyanidins was obtained for elderberry, roseship C and B. Generally, for all tested seeds, rowanberry seeds had 19.5 times more oligomers and polymers of the flavan-3-ol monomer unit than elderberry and 2.8 times more than seeds of flowering quince (Table 1). Xu *et al.* [2006] demonstrated that the total proanthocyanidin contents in the typically bramble seed *i.e.* blackberry and raspberry vary from 681 to 1760 mg/100 g, respectively.

Among the methods available to investigate the structures of proanthocyanidin polymers, acid-catalysed degradation in the presence of toluene-α-thiol is of particular interest, as it distinguishes between extension units and terminal units. The average composition of proanthocyanidin polymers in seeds was determined with the use of acid-catalysed degradation in the presence of toluene-α-thiol, followed by reverse-phase HPLC analysis with UV-visible detection. A typical HPLC chromatogram of the thiolysis products is shown in Figure 1. In thiolysis reactions, all the extension subunits of proanthocyanidins are attacked by benzyl mercaptan to form the corresponding benzylthioether. Only the terminal unit is released as the free flavan-3-ol. Variability with respect to tannins composition exists among analyzed seeds. (-)-Epicatechin was the main constitutive unit of the proanthocyanidins of flowering

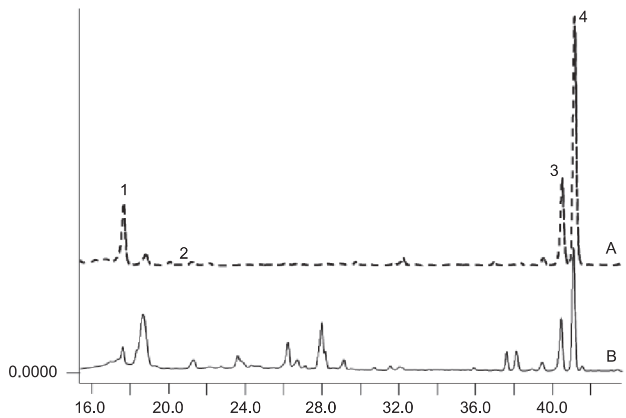


FIGURE 1. HPLC-FD (A) and HPLC-DAD (B) chromatograms of thiolysis methods of rosehip C catechins detected at 280 nm: 1- (+)-catechin; 2- (-)-epicatechin; 3- (+) catechin-4-benzyl thioether; 4- (-)-epicatechin--4-benzyl thioether.

quince, quince, rowanberry, hawthorn, and elderberry. The main constitutive units of the proanthocyanidins of sea buckthorn and rosehip, present in equal amounts, were (+)-catechin and (-)-epicatechin.

All investigated seeds, apart from sea buckthorn, had more (-)-epicatechin-4-benzylthioether than (+)-catechin-4--benzylthioether (Table 1). The evaluation of the antioxidant activity of the standard solution of (+)-catechin and (-)-epicatechin, showed behaviour corresponding to the results of Lagune *et al.* [1996], the antioxidant activity of (-)-epicatechin being stronger than of (+)-catechin. The same authors explain that the stereochemical position of the C-3 in the (+)-catechin (2R: 3S) and (-)-epicataechin (2R: 3R) affects the antioxidant activity. They conclude that the hydroxyl group in C-3 is involved in the scavenging of free radical. Therefore, seeds which have more (-)-epicatechin than (+)-catechin probably possess stronger antioxidant activity.

Moreover, thiolysis allows by distinction between extension and terminal units of proanthocyanidins to assess the average degree of polymerization (DP). Rosehip B showed a DP of 12.9 (Table 1) which resulted from its low content of (-)-epicatechin and high content of (-)-epicatechin-4–benzylthioether. Flowering quince showed a DP of 1.3 due to its high content of (-)-epicatechin and (-)-epicatechin-4–benzylthioether.

The DP of 1.3 – 5.6 which corresponds to oligomeric procyanidins is present in sea buckthorn A and B, quince, rowanberry, rosehip A and C, elderberry, and hawthorn seeds. The degree of polymerization for buckhorn is the same as obtained by Rösch *et al.* [2004] in their study. The DP and the nature of the constitutive units are important structural features related to the bitterness and astringency of taste and biological activity of proanthocyanidins [Noble, 1998].

## CONLCUSIONS

The presented study demonstrates that investigated seeds, especially rowanberry and hawthorn contained high amounts of proanthocyanidins. Due to their high content of proanthocyandins the seeds have promising potential as natural antioxidants in the food industries or in the pharmaceutical and cosmetic industries. Future studies are required to determine the types of other bioactive compounds in seeds, as well as the efficiencies of individual phenolic compound, antioxidant and antimicrobial activity.

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## NASIONA WYBRANYCH OWOCÓW ŹRÓDŁEM ZWIĄZKÓW Z GRUPY FLAWAN-3-OLI

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Celem niniejszej pracy było określenie zawartości flawan-3-oli oraz procyjanidyn w nasionach wybranych owoców. W badaniach wykorzystano nasiona następujących owoców: rokitnika pospolitego (*Hippophae rhamnosides* L.), pigwowca japońskiego (*Chaenomeles japonica*), pigwy (*Cydonia oblonga*), jarzębiny pospolitej (*Sorbus aucuparia* L.), głogu jednoszyjkowego (*Crataegus oxyacanta*), dzikiej róży (*Rosa canina*) i czarnego bzu (*Sambucus nigra* L.). Zebrane owoce w stadium pełnej dojrzałości, poddano procesowi liofilizacji, i rozdrobniono w młynku laboratoryjnym. Zawartość procyjanidyn oznaczono za pomocą chromatografii cieczowej (HPLC) metodą tiolizy.

Stwierdzono, że badane nasiona owoców charakteryzowały się dużą zmiennością pod względem zawartości badanych związków. Nasiona jarzębiny pospolitej oraz głogu jednoszyjnkowego zawierały największe ilości oznaczonych związków z grupy flawan-3-oli (517,48 i 221,49 mg/100 g). Natomiast najuboższe w te związki były nasiona bzu czarnego (24,15 mg/100 g) oraz pigwy (16,12 mg/100 g). W pozostałych nasionach tj. rokitnika pospolitego oznaczono 95,27 mg/100g flawon-3-oli natomiast u pigwowca japońskiego i dzikiej róży 165,25 i 168,37 mg/100 g. W badanych nasionach pigwowca japońskiego, pigwy pospolitej, jarzębiny pospolitej oraz bzu czarnego z grupy flawan-3-oli dominowała (-)-epikatechina oraz jej pochodne tiolowe. Zawartość (-)-epikatechiny w nasionach jarzębiny wynosiła 460,10 mg/100g, co stanowiło 88,9% z ogólnej zawartości katechin. Natomiast (+)-katechina dominowała w nasionach dzikiej róży (95,6%). Nasiona rokitnika zwyczajnego są bardzo dobrym źródłem, zarówno (-)-epikatechiny (53,8%) jak i (+)-katechiny (46,2%). Całkowita zawartość oznaczonych proantocyjanidyn w badanych nasionach wahała się od 219,16 do 4263,67 mg/100 g. Najniższą zawartość proantocyjanidyn oznaczono w nasionach bzu czarnego oraz róży C i B.

Badane nasiona, w szczególności z jarzębiny pospolitej i głogu dwuszyjkowego są dobrym surowcem związków z grupy flavon-3-oli.