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Evaluation of Fruit Anatomy, Accumulation and Detection of Polyphenols in Black Crowberry (*Empetrum nigrum*) from NW Slovakia

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The aim of our research was to connect the detailed study of fruit anatomy of black crowberry (*Empetrum nigrum*) with identification and detection of the main non-anthocyanin polyphenolic compounds. Our experimental results showed that the highest accumulation of anthocyanin bodies occurred in mature fruits in outer layers during fruit development. The shape of the anthocyanin bodies was most often globular, spherical, hemispherical and intermediate types were present only occasionally. Mature cells of the gynoecium and pericarp generally contain anthocyanin bodies incorporated inside vacuoles. The observed compounds accumulated in cells were rutin, quercetin and catechins, resveratrol, coumaric, p-coumaric, caffeic, ferulic acids, gallic, vanilic, syringic, cinnamic and caffeic acids. These compounds were selected because of their proposed positive effects on health. The analyses of the polyphenolic spectrum showed predominance of ferrulic acid together with gallic acid and catechins with quercetin.

Keywords: *Empetrum*, fruit development, polyphenols, Slovakia

INTRODUCTION

Nowadays, researches focus more on the study of bioactive compounds of lesser known fruit species (honeyberry, cornelian cherry, black crowberry, saskatoon berry) (Juríková et al., 2012, 2013; Skrovanková et al., 2015). Crowberry (*Empetrum nigrum* L.) is a wild berry commonly found in the northern hemisphere.

Black crowberry includes two subspecies – diploid *E. nigrum* subsp. *nigrum* (crowberry or black crowberry) and tetraploid *E. nigrum* subsp. *hermaphroditum* (Hagerup) Böcher (Fin, 2008; Juríková et al., 2016). It has been proved that the fruit of black crowberry controls inflammatory diseases, including cystitis, nephritis, and urethritis (Park et al., 2012; Hyun et al., 2016), exhibits significant inhibitory effect on angiogenesis (Bae et al., 2016) and is effective as a herbal antibiotic (Juríková et al., 2015). Health promoting property of the fruit is given by its high antioxidant activity. In both radical scavenging methods (DPPH, ABTS test) the crowberry showed the strongest antioxidant activity in comparison with other berry crops – bilberry, blackberry, blackcurrant, blueberry, cranberry, mulberry, raspberry and red currant (Halvorsen et al., 2002; Ogawa et al., 2008). Studies of black crowberry (*Empetrum nigrum*) are mainly focused on detection of prevailing bioactive compounds of the fruit, i.e., polyphenols (Juríková et al., 2016). The majority of phenolic compounds are represented by anthocyanins

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accumulated in the skin together with flavonol glycosides (galactosides, glucosides, arabinosides and xylosides) of myricetin, quercetin, laricitrin, isorhamnetin and syringetin (Laaksonen et al., 2011). Caffeic, gallic and protocatecheic acids predominate in the spectrum of phenolic acids (Ogawa et al., 2008). On the other hand, there is no information about accumulation of these compounds within the cells. The knowledge of the compartmentation is thus of importance in order to optimize the yield of phenolics in the processed products of berries, fruits and vegetables (Sapers et al., 1983; Hirota et al., 1998). Moreover, at different stages of fruit development, the microstructure of the fruit can provide important information about the changes that contribute to individual tissues forming the fruit and provide a better understanding of the physiological mechanisms and processes occurring in the developing fruit (Konarska, 2015a). Soluble phenolic compounds are mostly deposited in the cell walls and vacuoles. However, accumulation of soluble phenolic compounds is greater in the external tissues of a fleshy fruit (epidermal and subepidermal layers) than in the internal tissues (mesocarp and pulp) (Macheix et al., 1990; Wollenweber, 1994). Due to the fact that biosynthesis of phenolic compounds depends on the light, these compounds are mainly found in the skin of berries and fruits (Volf et al., 2014; Ali et al., 2018).

Although the chemical composition of black crowberry has been examined, there is still a lack of information about the accumulation of these substances. For this reason our research combined the study of predominant bioactive polyphenolic compounds with their accumulation within cells at 3 stages of maturation. Moreover, the analyses of phenolic compounds were compared in two closely related *Empetrum* taxa sampled in NW Slovakia – *E. nigrum* subsp. *nigrum* (Suchá Hora) and *E. nigrum* subsp. *hermaphroditum* (Veľký Kriváň hill in the Malá Fatra Mts).

MATERIALS AND METHODS

PLANT MATERIAL AND LOCALITIES

For detection of the polyphenolic profile of *Empetrum nigrum* L. as well as for anatomical studies of fruit development, the berries were collected from 2 different localities. The fruit samples of *E. nigrum* L. subsp. *nigrum* were collected in the Nature Reserve Rudné (NW Slovakia). The reserve is located in the administrative area of the village of Suchá Hora situated in the valley Oravská kotlina at an altitude of 750 m a.s.l. Fruit samples of *E. nigrum* subsp. *hermaphroditum* were picked on the western and south-western slopes of Veľký Kriváň hill at an altitude of 1650–1700 m (Polák, 1983). The site is located in the cadastral territory of three settlements: Terchová, Turany and Šútovo (Eliáš, 2004). According to phytogeographical classification, both localities are part of the flora of the West Carpathians (*Carpaticum occidentale*), the division of flora of the Central Carpathians (*Eucarpaticum*), and districts of the Západné Beskydy (Rudné) and the Fatra Mts (Veľký Kriváň hill), respectively (Futák, 1980).

LIGHT MICROSCOPY

The collected flowers and fruits were fixed in FAA (formaldehyde-acetic acid-ethanol) or Navashin's fixative, according to the following formula: mixing of the part I (CrO_3-acetic acid-water) and part II (formalin-ethanol-water) in a ratio 1:1. The common methods of dehydration, infiltration and paraffin embedding were based on Erdelská (1986). Serial sections of the ovaries and fruits were cut at the thickness of 7–10 μm using a rotary microtone CUT 4055 MICROTEC. The slides were stained with Heidenheim´s haematoxylin or safranin and fast-green (Němec, 1962; Erdelská, 1986). The fresh fruits were hand-sectioned using a razor blade, placed on a slide in a drop of distilled water. The microscopic and macroscopic sections were carried out with a light microscope Olympus BX 41 and photographed using a camera Olympus E-520.

The results of anatomical observation were set up according to the stages of fruit maturation – described as stages I–III.

SAMPLE EXTRACTION

Immediately after culling, the fruits were frozen and stored at -40°C. The extraction was performed according to Hakimuddin et al. (2008) with modifications as provided below. The frozen fruits were homogenized in 90% methanol (2 ml/g) and subsequently extracted at 4°C for 30 minutes. After the extraction, centrifugation at 1990 rpm for 10 minutes was used to separate the supernatant and the sediment was subjected to a new extraction. This process was repeated three times. The supernatants containing phenolic compounds were dried using a Laborota 4011 digital Rotary Evaporator (Heidolph, Germany), and stored at -20°C.

DETERMINATION OF TOTAL POLYPHENOL CONTENT (TPC)

A standard solution of tannin (Sigma Aldrich) was prepared from tannin (50 mg) dissolved in water (100 ml). It was placed, using a pipette, in six flasks (50 ml) in volumes of 0.2, 0.3, 0.4 and 0.5 ml. The extract (1 ml) was added to seven flasks and dissolved as needed. Distilled water (20 ml) and the

Folin-Ciocalteu reagent (1 mL) were added to every flask. After three minutes 20% solution Na_2CO_3 (5 ml) was added. The solutions were mixed and distilled water was added to a volume of 50 ml. After 30 minutes the color intensity compared to the control (no tannin) was measured at 700 nm.

HPLC ANALYSIS OF INDIVIDUAL POLYPHENOLIC COMPOUNDS

The amount of polyphenolic compounds – phenolic acids and flavonoids – was detected by HPLC method. For analysis of individual polyphenols we used the method described by de Quiros et al. (2010) with some modifications: polyphenols were analyzed by HPLC Dionex 3000, USA with UV-VIS detection and an Chromeleon 7 (system software). Chromatographic separation was carried out on a Column: Phenomenex Kinetex C18 150 × 4.6 mm. The flow rate was 1 $ml.min^{-1}$. The injection volume was 10 µl. The separation was performed at room temperature (30°C). The detector was set at 275 nm.

ANTIOXIDANT ACTIVITY OF FRUITS

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was done according to the previously described methods (Brand-Wiliams et al., 1995) with some modifications. Stock solutions were prepared by dissolving DPPH (24 mg) in methanol (100 ml), and then stored at -20°C until needed. Working solutions were prepared by mixing the stock solution (10 ml) with methanol (45 ml) to obtain an absorbance of 1.1 ± 0.02 units at 515 nm using a LIBRA S6 Spectrophotometer. Fruit extracts (150 µl) were allowed to react with the DPPH solution (2,850 µl) for 1 hour in the dark. Following this, absorbance was measured again at 515 nm. Antioxidant activity was calculated as a decrease of the absorbance value using the formula: (%) = (A0 – A1/A0) × 100%, where A0 is the absorbance of the blank (without the sample), and A1 is the absorbance of the mixture containing the sample. The absorbance results were converted using a standard calibration curve and expressed as ascorbic acid equivalents (AAE) (Rupasinghe et al., 2006). This protocol was repeated three times for each fruit extract.

RESULTS

FRUIT ANATOMY

The results of anatomical observation were evaluated at three stages.

Stage I: Anthesis and fertilization

Generally, the wall of the gynoecium at the stage of anthesis consists of ten layers: an epidermal layer, three hypodermal layers, three-layer middle part and three layers in the inner part (Fig. 1a). The epidermal cells of the gynoecium of this species have typical regular shapes, their anticline walls are twice as long. The distribution of colored vacuoles in these cells has a regular character; the vacuoles are always located in the apical part of cells, pressed to the outer wall (Fig. 1b). These vacuoles occupied approximately one-third of the epidermal cells. Small anthocyanin globules were observed sporadically on the basal part of the cells. Inside some hypodermal layer cells, we recorded fully developed colored vacuoles, however, most of the cells at this stage contained only small vacuoles or clustered irregular type of anthocyanin bodies. Colored vacuoles of 2nd, 3rd and 4th cell layers were usually fully developed at the period of maturity of the female gametophytes, including the process of fertilization. The 5th layer of hypodermal cells did not develop color vacuoles. The 6th layer corresponded with the carpel's suture; the protoplast of its cells contained color vacuoles or anthocyanin globules, as in the case of the hypodermal cells. Toward the inside of the gynoecium, the layer (7th) without color vacuoles followed. Three inner layers (8th, 9th, 10th) of the gynoecium were developed by most striking vacuoles in almost all cells (Fig. 1c). Moreover, we found colored vacuoles inside the epidermal cells of the integument during maturation of the female gametophyte (Fig. 1d). We observed numerous colored vacuoles and anthocyanin bodies in the tissues of the style and in the cells enclosing the vascular bundles. Anthocyanin bodies of various sizes were accumulated inside the vacuoles while they usually have a globular shape. However, in non-fertilized flowers with signs of senescence, the colored vacuoles were absent inside all gynoecium cells. The cells contained only numerous anthocyanin globules of various sizes (Fig. 2a).

Stage II: Immature fruit without endocarp

Distribution of colored vacuoles in different parts of the gynoecium after successful fertilization remained similar to the previous stage. The protoplasts of the epidermal cells retained colored vacuoles in the apical part, but some vacuoles were present in the basal part of these cells, too. The inside the cells of the three hypodermal layers, three middle layers and three inner layers remained well developed with colored vacuoles during the first stages of the post-fertilization development, mainly at the early stages of the division of endospermal cells (Fig. 2b). The situation in distribution of the colored vacuoles was different at the later stages. The beginning of the embryogenesis was the reason for obvious changes in the structure of the gynoecium converting to the pericarp in the process of fruit and seed ripening. We found out

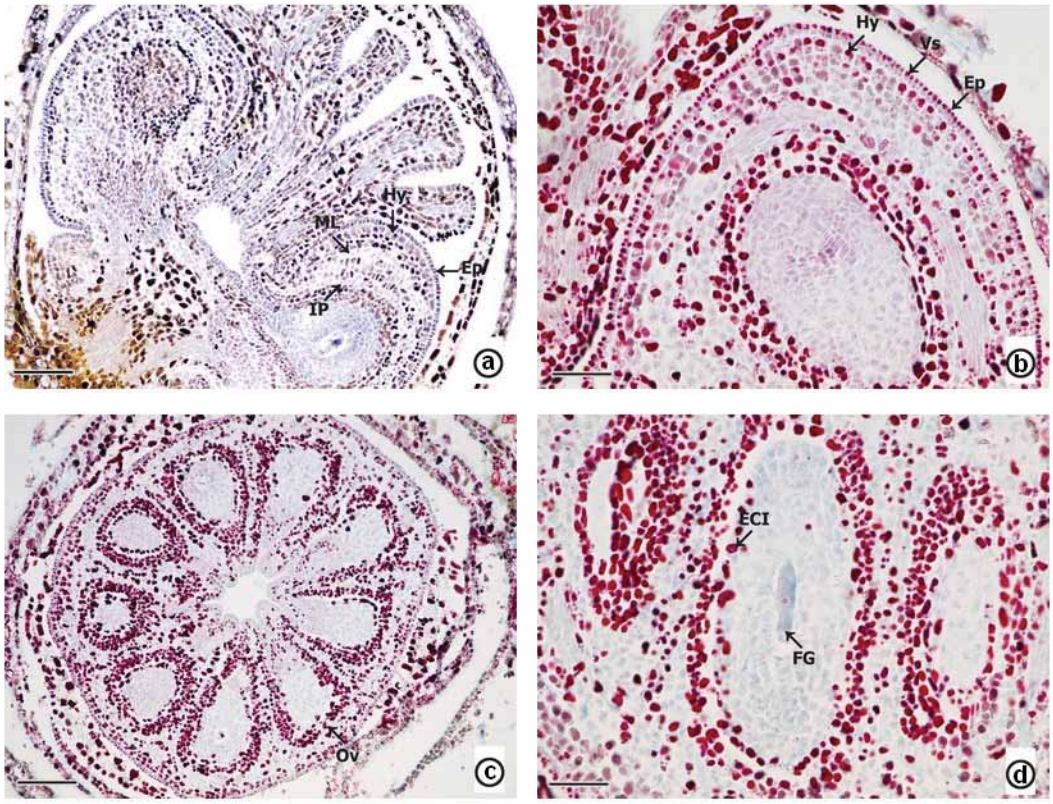

Fig. 1. Fruit anatomy of *Empetrum nigrum* (**a**) Longitudinal section of the buds just before opening (bar = 100 μm), (**b**) Detail of the ovary wall (bar = 50 μm), (**c**) Cross section of the gynoecium with ovules (bar = 100 μm), (**d**) Ovule detail with a mature female gametophyte (bar = 50 μm). IP – inner part, Ep – epidermis, Hy – hypodermis, Vs – vacuoles, Ov – ovule, ECI – epidermal cells of the integument, FG – female gametophyte.

disintegration of vacuoles in hypodermal layers; protoplasts of these cells contained only anthocyanin globules of various sizes. The degradation of vacuoles occurred in the inner layers, as well. The complete degradation of the protoplasts and sclerification of the cell walls followed this process, leading to the formation of the sclerenchymatous endocarp of the mature fruits (Fig. 2c).

Stage III: Mature fruits

During the maturation of fruits we observed that the anthocyanins were accumulated particularly in the outer layers of the pericarp. The epidermal cells contained colored vacuoles, many of them had well-visible anthocyanin globules. Numerous anthocyanin bodies occurred inside the first hypodermal layer cells. These structures were present occasionally in the second hypodermal layer, too (Fig. 2d). Anthocyanin bodies showed various sizes and shapes. Most often we observed clusters of a spherical type, further hemispherical and indeterminate types. Except for the surface layers, the presence of vacuoles with tannins was observed in the apical part of the mature fruits representing remains of the style tissue. The same vacuoles were also localized in the epidermal cells of the outer seed coat (*testa*). The occurrence of intensively colored vacuoles was not found in deeper lying parts of the mature fruits. These cells of the medium part of the pericarp contained significantly smaller amounts of anthocyanins. Vacuoles with the highest concentration of anthocyanins in the mature fruits were present, especially within the protoplast of epidermal cells.

BIOACTIVE COMPOUNDS IN *EMPETRUM NIGRUM*

In the second part of the experiment, the attention was focused on quantification of the main bioactive compounds of *E. nigrum* – polyphenols. The samples originated from two localities – Suchá Hora (locality I) and Malá Fatra Mts (locality II).

The experimental results showed that the antioxidant activity reached relatively similar values

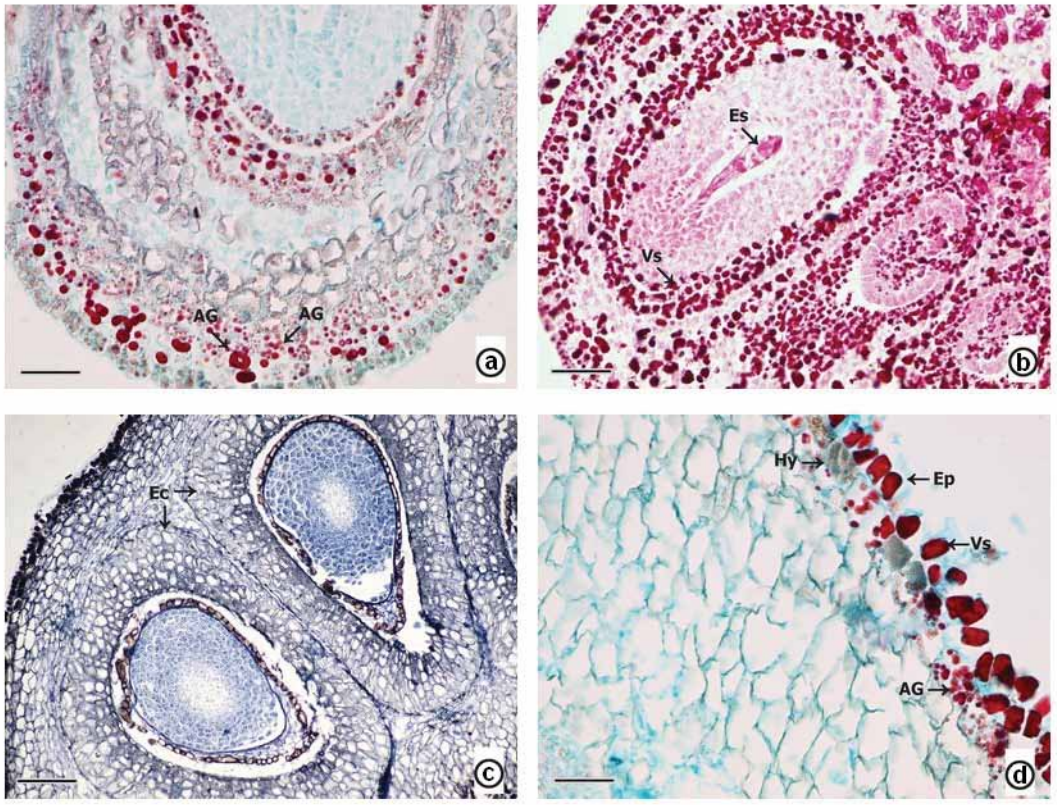

Fig. 2. Fruit anatomy of *Empetrum nigrum* (**a**) Gynoecium wall detail from unfertilized flowers (bar = 50 μm), (**b**) Cross section of the ovary with young developing seeds (bar = 50 μm), (**c**) Two seeds enclosed in the sclerenchymatous endocarp (bar = 100 μm), (**d**) Segment of the fruit coat (bar = 50 μm). AG – anthocyanins globules, Es – endosperm, Vs – vacuoles, Ec – endocarp, Ep – epidermis, Hy – hypodermis, Ov – ovule.

for both sampled taxa (7.87 ± 0.34 mmol TROLOX equivalent/g in *E. nigrum* subsp. *nigrum* fruits from locality I; 6.99 ± 0.33 mmol TROLOX equivalent/g in fruits of *E. nigrum* subsp. *hermaphroditum* from locality II) (Table 1).

The results of the experiment showed that the content of total polyphenols was 5.573 ± 0.01 mg/g gallic acid (locality I – Suchá Hora) and 5.741 ± 0.01 mg/g gallic acid (locality II – Malá Fatra Mts), respectively. In the spectrum of the observed phenolic acids, ferrulic acid predominated together with gallic acid (75.74 ± 3.988; 79.72 ± 4.12 and 20.58 ± 1.53; 23.08 ± 1.67 μg/g). Other phenolic acids were present only in trace amounts – vanilic acid (6.24 ± 0.24; 6.57 ± 0.34 μg/g), syringic acid (3.89 ± 0.459; 3.77 ± 0.39 μg/g), trans *p*-coumaric acid (0.57 ± 0.13; 0.32 ± 0.10 μg/g), cinnamic acid (0.65 ± 0.11; 0.54 ± 0.10 μg/g) and protocatechic acid (0.11 ± 0.03; 0.14 ± 0.034 μg/g). With respect to detected flavonoids, catechin predominated with quercetin (8.08 ± 1.35; 5.99 ± 1.09 and 2.10 ± 0.27; 2.56 ± 0.31 μg/g). Rutin together with resveratrol were detected in lower amounts

TABLE 1. Content of total phenolics (mg gallic acid/g) and antioxidant activity by DPPH method (mmol TROLOX/g) in *Empetrum nigrum* fruit originated from two localities (locality I – Suchá Hora and locality II – Malá Fatra).

	Locality I	**Locality II**
Total polyphenols (mg gallic acid/g)	5.57 ± 0.01	5.74 ± 0.01
Antioxidant activity (mmol TROLOX equivalent/g)	7.87 ± 0.34	6.99 ± 0.33

TABLE 2. Polyphenolic profile of *Empetrum nigrum* samples originated from locality I (Suchá Hora) and locality II (Malá Fatra).

Polyphenolic compounds	Locality I content of detected polyphenolic compounds (μg/g FW)	Locality II content of detected polyphenolic compounds (μg/g FW)
gallic acid	20.59 ± 1.53	23.08 ± 1.68
catechins	8.08 ± 1.35	5.99 ± 1.10
vannilic acid	6.25 ± 0.24	6.57 ± 0,35
caffeic acid	0.65 ± 0.09	0.67 ± 0.10
syringic acid	3.90 ± 0.46	3.77 ± 0.40
trans- *p*- coumaric acid	0.57 ± 0.13	0.32 ± 0.11
coumaric acid	1.52 ± 0.24	1.82 ± 0.35
ferrulic acid	75.74 ± 3.99	79.72 ± 4.12
rutin	1.78 ± 0.93	2.64 ± 1.24
protocatecheic acid	0.11 ± 0.03	0.14 ± 0.03
resveratrol	0.26 ± 0.16	0.41 ± 0.30
cinnamic acid	0.65 ± 0.11	0.54 ± 0.10
quercetin	2.10 ± 0.27	2.56 ± 0.31

(1.78 ± 0.93; 2.63 ± 1.23 and 0.26 ± 0.16; 0.41 ± 0.30 μg/g) (Table 2).

The statistical evaluation of total phenolics, antioxidant activity and phenolic spectrum of *Empetrum nigrum* proved significant differences between locality I (Suchá Hora) and locality II (Malá Fatra) with respect to total polyphenols and antioxidant activity of *Empetrum* fruits ($P = 0.0001$; $P = 0.0328$). No differences were determined among the assayed polyphenolic compounds.

DISCUSSION

We found that intensively colored vacuoles in the walls of the gynoecium during fertilization and first stages of post-fertilization were located in the surface layers of *Empetrum* fruits. Anthocyanins in epidermal cells were present (persisted) all the time, in a typical position in the apical part of the cells. The epidermis cells of mature fruits of *Empetrum* are characterized by a polygonal shape, while the mesocarp consists of cells radially elongated (Nikitin and Pankova, 1982). The presence of anthocyanin-containing vacuoles in epidermal cells was also observed in other species. In the epidermal cell vacuoles of *Vaccinium corymbosum*, anthocyanins were visible in the form of large, spherical globules in the living protoplast or granularities in the fixed cells. Simultaneously, all layers of cells forming the hypanthium wall exhibited tannin deposits located in the vacuoles that had oval shapes and varied sizes (Konarska, 2015b). Wiltshire and Collings (2009) found that the central vacuole in epidermal cells of red onion is connected with vacuolar tubules that greatly increase the surface area of the tonoplast and might increase transport rates between the cytoplasm and vacuole.

On the contrary, in mature fruits of *Vitis* cultivar the anthocyanins were represented as uniformly dark red colored vacuoles localized in the first shallow sub-epidermal layer, while epidermis was devoid of them. Vacuoles were observed to occur sporadically in the 4th, 5th and 6th cell layers, and were not observed in deeper lying cells. Anthocyanins were in a non-complexed form (Moskowitz and Hrazdina, 1981). Konarska (2015a) observed that the vacuoles of the epidermal and hypodermal cells in the fruits of all analyzed *Prunus domestica* cultivars contained anthocyanins, frequently in the form of large, spherical globules. She supposed that accumulation of the granular deposits in vacuoles indicates fruit ripening and senescence. Anatomical observations of anthocyanin rich cells in the apple skin revealed that the density of red pigment was high in cells of

the outer layer of the fruit, and gradually decreased toward the inside of the flesh. Anthocyanins were frequently found in clusters or in agglomerations. They accumulated in the inner side of developed vacuoles. The shape of anthocyanins was of three morphological types: an indeterminate type, a hemispherical type, and a spherical type (Bae et al., 2006). Anthocyanins seemed to be synthesized around the tonoplast and condensed on the inward side of the vacuole. Chanoca et al. (2015) found that cytoplasmic anthocyanin aggregates in close contact with the vacuolar surface are directly engulfed by the vacuolar membrane in a process reminiscent of microautophagy. The engulfed anthocyanin aggregates are surrounded by a single membrane derived from the tonoplast and eventually become free in the vacuolar lumen like an autophagic body.

Except the walls of the gynoecium and pericarp, colored vacuoles are also present in cells surrounding vascular bundles. Jiang et al. (2007) notes that the idioblast in the specialized connectives of *Lonicera* contains phenolic compounds protecting the developing of vascular bundles from damage in anthers, thus the normal development of pollen grains and pollination can be ensured. However, following this stage, phenolic compounds began degrading. We suppose that colored vacuoles present in the epidermal cells of the ovule integument play a similar protective role during development of the female gametophyte. Besides *Empetrum* species, maximum accumulation of anthocyanins in epidermal cells of the mature ovules was observed in other plants, e.g., *Lonicera* (Ďurišová et al., in press), and *Cerasus* (Chudíková et al., 2012). We also assume that anthocyanins play the same protective role within colored vacuoles in the inner part of the gynoecium during the first stages of fruit development in *Empetrum*. Later, these layers are transformed into the sclerenchymatous endocarp that immediately protects seeds. The vacuoles containing tannin material remain in the epidermal cells of the testa. Other tissues of seeds contain storage materials, mainly starch and fat (Altan and Özdemir, 2004). Distribution of anthocyanins in the pericarp of mature fruits of *Empetrum* is characterized by accumulation of epidermal cells in the surface layers, especially in the central vacuole. The contents of these vacuoles have a potential commercial value as densely packed bodies of stabilized anthocyanins can be used as food additives (Chanoca et al., 2015).

The total polyphenol content was higher than in the research of Lacramioara and Ciprian (2016) who detected 4.3 ± 0,09 mg GAE/g DW polyphenols and Park et al. (2012) found 3.9 ± 2 mg GAE/g FW in samples of *Empetrum nigrum* from Korea. According to Ogawa et al. (2008), phenolic acids – caffeic, gallic and protocatechuic predominated in *E. nigrum* but in our experiments we also determined the high level of ferrulic acid, which reached up to 75.74 ± 3.988; 79.72 ± 4.12 μg/g.

Quercetin levels in the edible parts of most vegetables and fruits were generally below 10 mg/kg (Hertog et al., 1992; Manach et al., 2004) which corresponded with our results. Park et al. (2012) determined the average content of quercetin in *E. nigrum* berries – 2.3 ± 0.15 μg/g which is in accordance with the results of our experiments. Dudonne et al. (2015) determined higher concentration of quercetin – 3.72 ± 0.29 μg/g. On the contrary, our results were in contrast with Justesen et al. (1998), who reported quercetin levels higher than 20 mg/kg in several berries and fruits (cowberry, lingonberry, cranberry, blueberry, black currant, blue grapes, rosebud, apple and apricot). According to the results of Juríková et al. (2015), quercetin was the main phenolic compound in sea buckthorn berry and crowberry.

The total antioxidant activity of the crowberry (*E. nigrum* subsp. *hermaphroditum*) fruit reported by Halvorsen et al. (2006) represented 9.63 mmol/l, which was a higher value in comparison with the results of our research.

CONCLUSION

Occurrences of colored vacuoles with anthocyanin bodies indicate its protective role during earlier stages of the female gametophyte development as well as seed development in fruit maturation. In addition, colored vacuoles enhance the protective effect against the negative influence of the environmental conditions and play a significant role as an attractant for frugivorous animals. The presented results of our research show that black crowberry is a valuable source of ferrulic acid together with gallic and catechins with quercetin.

AUTHORS' CONTRIBUTIONS

TJ designed the article's goals, drafted the paper. LD collected plant material, made anatomical procedures and wrote some parts of the manuscript (Materials and Methods, Results, Discussion), PE collected plant material, wrote some parts of the manuscript (Materials and Methods) and edited the manuscript into its final form. JM critically revised the manuscript and conducted the chemical analyses of phenolic compounds in fruits. JS wrote the manuscript (Introduction and Discussion). MO conducted the chemical analyses of phenolic compounds in fruits. The authors declare no conflict of interest.

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REFERENCES

Ali A, Chong CH, Mah SH, Abdullah LC, Choong TSY, and Chua BL. 2018. Impact of storage conditions on the stability of predominant phenolic constituents and antioxidant activity of dried *Piper betle* extracts. *Molecules* 23(2): 484. DOI: 10.3390/molecules23020484

Altan Y, and Özdemir C. 2004. Morphological and anatomical studies on economically important *Empetrum nigrum* L. subsp. *hermaphroditum* (Hagerup) Böcher (*Empetraceae*). *Economic Botany* 58 (4): 679–683.

Bae RN, Kim KW, Kim T Ch, and Lee SK. 2006. Anatomical observation of anthocyanin rich cells in apple skins. *Horticultural Science* 41(3): 733–736. DOI: 10.21273/HORTSCI.41.3.733

Bae H-S, Kim HJ, Jeong da H, Hosoya T, Kumazawa S, Jun M, Kim O-Y, Kim SW, and Ahn M-R. 2016. In vitro and in vivo antiangiogenic activity of crowberry (*Empetrum nigrum* var. *japonicum*). *Natural Product Communications* 11(4): 503–506.

Brand-Williams W, Cuvelier ME, and Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology* 28: 25–30. DOI: 10.1016/S0023-6438(95)80008-5

Dudonne S, Dube P, Anhe FF, Pilon G, Marette A, Lemire M, Harris C, Dewailly E, and Desjardins Y. 2015. Comprehensive analysis of phenolic compounds and abscisic acid profiles of twelve native Canadian berries. *Journal of Food Composition and Analysis* 44: 214–224. DOI: 10.1016/j.jfca.2015.09.003

Chanoca A, Kovinich N, Burkel B, Stecha S, Bohorquez-Restrepo A, Ueda T, Eliceiri KW, Grotewold E, and Otegui MS. 2015. Anthocyanin vacuolar inclusions form by a microautophagy mechanism. *Plant Cell* 27(9): 2545–59. DOI: 10.1105/tpc.15.00589

Chudíková R, Ďurišová Ľ, Baranec T, and Eliáš P jun. 2012. The reproductive biology of selected taxa of the genus *Cerasus* Duham. *Acta Biologica Cracoviensia Series Botanica*, 54(2): 11–20. DOI: 10.2478/v10182-012-0023-x

Eliáš P. 2004. *Populačná a reprodukčná biológia vybraných ohrozených druhov flóry Slovenska*. PhD. Thesis, Slovak University of Agriculture, Nitra.

Erdelská O. 1986. Embryo development in the dogwood *Cornus mas*. *Phytomorphology* 36(1): 23–28.

Fin C. 2008. *Empetrum nigrum* – crowberry. In: Janick J and Paull RE [eds.], *The encyclopedia of fruits and nuts*, 348. CAB International, Wallingford, Oxfordshire, UK.

Futák J. 1980. Fytogeografické členenie. In: Mazúr E [ed.], *Atlas Slovenskej Socialistickej Republiky*, 88. Veda, Bratislava, SK.

Hakimuddin F, Tiwari K, Paliyath G, and Meckling K. 2008. Grape and wine phenolic compounds downregulate the expression of signal transduction genes and inhibit the growth of estrogen receptor-negative MDA-MB231 tumors in nu/nu mouse xenografts. *Nutrition Research* 28(10): 702–713. DOI: 10.1016/j.nutres.2008.06.009

Halvorsen B, Holte K, Myhrstad MC, Barikno I, Hvattum E, Remberg SF, Wold AB, Haffner K, Baugerod H, Andersen LF, Moskaug Ø, Jacobs DR Jr, and Blomhoff R. 2002. A systematic screening of total antioxidants in dietary plants. *The Journal of Nutition* 132(3): 461–471. DOI: 10.1093/jn/132.3.461

Halvorsen BL, Carlsen MH, Phillips KM, Bøhn S, Holte K, Jacobs DR Jr, and Blomhoff R. 2006. Content of redox-active compounds in foods consumed in US. The American Journal of Clinical Nutrition 84(1): 95–135. DOI: 10.1093/ajcn/84.1.95

Hirota S, Shimoda T, and Takahama U. 1998. Tissue and spatial distribution of flavonol and peroxidase in onion bulbs and stability of flavonol glucosides during boiling of the scales. *Journal of Agriculture and Food Chemistry* 46(9): 3497–3502. DOI: 10.1021/jf980294w

Hertog MGL, Hollman PCH, and Katan MB. 1992. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *Journal of Agriculture and Food Chemistry* 41(12): 2379–2383. DOI: 10.1021/jf00024a011

Hyun TK, Kim H-C, Ko Y-J, and Kim J-S. 2016. Antioxidant, α-glucosidase inhibitory and anti-inflammatory effects of aerial parts extract from Korean crowberry (*Empetrum nigrum* var. *japonicum*). *Saudi Journal of Biological Sciences* 23(2): 181–188. DOI: 10.1016/j.sjbs.2015.02.008

Jiang XS, Wu H, Lun X, and Lu DW. 2007. Morphological characteristics and biological significance of specialized connectives in *Lonicera* (Caprifoliaceae). *Journal of Systematics and Evolution* 45(1): 39–51. DOI: 10.1360/aps06053

Jurikova T, Rop O, Mlcek J, Sochor J, Balla S, Szekeres L, Hegedusova A, Hubalek J, Adam V, and Kizek R. 2012. Phenolic profile of edible honeysuckle berries (genus *Lonicera*) and their biological effects. *Molecules* 17(1): 61–79. DOI: 10.3390/molecules17010061

Jurikova T, Sochor J, Rop O, Mlček J, Balla S, Szekes L, Zitný R, Zitka O, and Kizek R. 2012. Evaluation of polyphenolic profile and nutritional value of non-traditional fruit species in the Czech Republic – a comparative study. *Molecules* 17(8): 8968–8981. DOI: 10.3390/molecules17088968

Jurikova T, Balla S, Sochor J, Pohanka M, Mlcek J, and Baron M. 2013. Flavonoid profile of saskatoon berries (*Amelanchier alnifolia* Nutt.) and their health promoting effects. *Molecules* 18(10): 12571–12586. DOI: 10.3390/molecules181012571

Jurikova T, Mlcek J, Skrovankova S, Balla S, Sochor J, Baron M, and Sumczynski D. 2016. Black crowberry (*Empetrum nigrum* L.) flavonoids and their health promoting activity. *Molecules* 21(12): 1685. DOI: 10.3390/molecules21121685

Justesen U, Knuthsen P, and Leth T. 1998. Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photo-diode array and mass spectrometric detection. *Journal of Chromatography A* 799(1–2): 101–110.

Konarska A. 2015a. Characteristics of fruit (*Prunus domestica* L.) skin: structure and antioxidant content. *International Journal of Food Properties* 18(11): 2487–2499. DOI: 10.1080/10942912.2014.984041

Konarska A. 2015b. Morphological, anatomical and ultrastructutal changes in *Vaccinium corymbosum* fruits during ontogeny. *Botany* 93(9): 589–602. DOI: 10.1139/cjb-2015-0050

Laaksonen O, Sandell, M, Järvinen R, and Kallio H. 2011. Orosensory contributing compounds in crowberry (*Empetrum nigrum*) press-by products. *Food Chemistry* 124: 1514–1524.

Lacramioara O, and Ciprian M. 2016. Antioxidants content in *Empetrum nigrum* fresh and dried fruits. *Iranian Journal of Public Health* 45(2): 263–265.

Lee YT, Don MJ, Hung PS, Shen YC, Lo YS, Chang KW, Chen CF, and Ho LK. 2005. Cytotoxicity of phenolic acid phenethyl esters on oral cancer cells. *Cancer Letters* 223(1): 19–25. DOI: 10.1016/j.canlet.2004.09.048

Macheix J-J, Fleuriet A, and Billot J. 1990. *Fruit phenolics*. CRC Press, Boca Raton, USA.

Manach C, Scalbert A, Morand C, Remesy C, and Jimenez L. 2004. Polyphenols: food sources and bioavailability. *The American Journal of Clinical Nutrition* 79(5): 727–747. DOI: 10.1093/ajcn/79.5.727

Němec B [ed.]. 1962. *Botanická mikrotechnika*. ČSAV, Praha.

Nikitin AA, and Pankova IA. 1982. *Anatomičeskij atlas poleznych i nekotorych jagotovitych rastenij*. Nauka, Leningrad.

Moskowitz AH, and Hrazdina G. 1981. Vacuolar contents of fruit subepidermal cells from *Vitis* species. *Plant Physiology* 68: 686–692.

Ogawa K, Sakakibara H, Iwata R, Ishii T, Sato T, Goda T, Shimoi K, and Kumazawa S. 2008. Anthocyanin composition and antioxidant activity of the crowberry (*Empetrum nigrum*) and other berries. *Journal of Agriultural and Food Chemistry* 56(12): 4457–4462. DOI: 10.1021/jf800406v

Park SY, Lee ES, Han SH, Lee HY, and Lee S. 2012. Antioxidative effects of two native berry species, *Empetrum nigrum* var. *japonicum* K. Koch and *Rubus buergeri* Miq., from the Jeju island of Korea. *Journal of Food Biochemistry* 36(6): 675–682. DOI: 10.1111/j.1745-4514.2011.00582.x

Polák M. 1983. 2. Geologická stavba. In: Pagáč J and Vološčuk I [eds.], *Malá Fatra. Chránená krajinná oblasť*, 14–23. Príroda, Bratislava, SK.

de Quirós AR-B, Lage-Yusty M, and Lopez-Hernandez J. 2010. Determination of phenolic compounds in macroalgae for human consumption. *Food Chemistry* 121(2): 634–638. DOI: 10.1016/j.foodchem.2009.12.078

Rupasinghe HPV, Jayasankar S, and Lay W. 2006. Variation in total phenolic and antioxidant capacity among European plum genotypes. *Scientia Horticulturae* 108(3): 243–246. DOI: 10.1016/j.scienta.2006.01.020

Sapers GM, Jones SB, and Maher GT. 1983. Factors affecting the recovery of juice and anthocyanin from cranberries. *Journal of the American Society for Horticultural Science* 108: 246–249.

Skrovankova S, Sumczynski D, Mlcek J, Jurikova T, and Sochor J. 2015. Bioactive compounds and antioxidant activity in different types of berries. *International Journal of Molecular Sciences* 16(10): 24673–24706. DOI: 10.3390/ijms161024673

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, and Byrne DH. 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis* 19(6–7): 669-675. DOI: 10.1016/j.jfca.2006.01.003

Volf I, Ignat I., Neamtu N, and Popa VI. 2014. Thermal stability, antioxidant activity, and photo-oxidation of natural polyphenols. *Chemical Papers* 68(1): 121–129. DOI: 10.2478/s11696-013-0417-6

Wiltshire EJ, and Collings DA. 2009. New dynamics in an old friend: Dynamic tubular vacuoles radiate through the cortical cytoplasm of red onion epidermal cells. *Plant and Cell Physiology* 50(10): 1826–1839. DOI: 10.1093/pcp/pcp124

Wollenweber E. 1994. Flavones and flavonols. In: Harborne JB [ed.], *The flavonoids: Advances in research since 1986*, 259–335. Chapman & Hall, New York, USA.