

# Peer seeds - rich source of antioxidants

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During fruit processing, a large amount of solid residues such as seeds, remains underutilized. There is a need to find method for use of these by-products and to convert them into products with significant health, environmental and/or economic benefits. This study provides data about content of lipophilic antioxidants (carotenoids and tocopherols) and hydrophilic antioxidant potential of seeds of four autochthonous Balkan pear cultivars (Kajzerica, Kaluđerka, Turšijara and Karamanka). The content of vitamin E ranged from 20.8 to 41.2 mg/100 g of seeds. In all of the investigated cultivars  $\gamma$ -tocopherol was dominant tocopherol, followed by  $\alpha$ -tocopherol. Concerning carotenoids, their concentration ranged between 0.24 and 0.30 mg/100 g of seeds, with a predominance of lutein. The highest content of vitamin E and carotenoids was recorded in Kajzerica seeds. Antioxidant capacity of investigated samples was evaluated by hydrophilic Trolox Equivalent Antioxidant Capacity (TEAC) assay and it ranged from 2.3 mmol TE/100 g in Turšijara seeds to 14.6 mmol TE/100 g in Kaluđerka seeds. Our results provide useful information for potential use of pear seeds.

**Key words:** pear, seed, tocopherols, carotenoids

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## 1. INTRODUCTION

Pear (*Pyrus communis* L.) fruit is one of the most important stone fruits (Kolniak-Ostek, 2016). It is consumed worldwide as fully mature fresh fruit and also, as component of processed products such as drinks, preserved fruits, candies and jams (Reiland and Slavin, 2015). There are various reports on health-promoting properties of polyphenolic compounds present in pears (Khurana et al., 2013). Still there is a limited number of studies dealing with other groups of bioactive compounds in pears such as tocopherols and carotenoids. During fruit processing, a large amount of waste such as pear seeds, core and peel, remains underutilized. Current waste management practices have two main limitations: they are costly and with unfavorable impact on the environment. There is a constant need to develop a method for the conversion of by-products into useful products of higher value in an environmentally friendly way (Kowalska et al., 2017). Finding new sources of phytochemicals among byproducts of fruit industry is of great economic importance. Ingredients naturally present in daily life products have greater acceptability for most people over their synthetic analogs. Quantities of seeds generated during pear processing are subsequently significant. The present study was intended to analyze carotenoid and vitamin E contents in pear seeds of Balkan autochthonous varieties. Their antioxidant potential was also evaluated. The obtained results provide useful information for the utilization of seeds of selected pear cultivars.

## 2. MATERIALS AND METHODS

### 1. Sample

Fruits of four cultivars (Kajzerica, Kaluđerka, Turšijara and Karamanka) were collected at optimum ripening stage recommended for consumption. Seeds were separated and dried at the room temperature. Dried seeds were pulverized using laboratory mill and powder was kept in a refrigerator until further analysis.

### 2. Extraction of carotenoids and tocopherols

Extraction of carotenoids and tocopherols from the seed samples of four pear cultivars was conducted under subdued light conditions according to previously published methodology (Živković et al., 2017). After addition of anhydrous  $\text{Na}_2\text{SO}_4$ ,  $\text{Mg}_2\text{CO}_3$  and 100  $\mu\text{L}$  of  $\alpha$ -tocopherol acetate and  $\beta$ -apo-8'-carotinal (as internal standards), one gram of pulverized sample was homogenized with a mixture of methanol and tetrahydrofuran (1/1, v/v, containing 0.1% BHT) using an ultra turrax, followed by filtration on a Büchner funnel. The procedure was repeated two more times until the sample was colorless. The filtrated solutions were combined and the solvent was evaporated using a rotary evaporator and a water bath at 35°C. The dry residue was redissolved in 5 mL of methanol/tetrahydrofuran (1/1, v/v, + 0.1% BHT). The solution was centrifuged for 5 min at 14000 rpm. Finally, 50  $\mu\text{L}$  of the solution were injected into a reversed-phase HPLC system to analyze carotenoids concentrations. For analysis of vitamin

E, an aliquot of this solution was evaporated under a nitrogen stream and redissolved in n-hexane/methyl tert-butyl ether (98/2, v/m). After centrifugation (5 min, 14000 rpm), the samples were used for vitamin E analysis. Twenty microliters were injected into a normal-phase HPLC system.

### 2.1. HPLC analysis of carotenoids

Chemical analysis of carotenoid rich extracts of seeds and oils of two *Salvia* species controls were carried using reversed-phase HPLC with diode array detector at 450 nm according to methods previously described (Bauerfeind et al., 2014). The chromatographic separation was performed on a Develosil® RPAQUEOUS C30 column (250 x 4.6 mm i.d., 5 µm particle size). During analysis, the column temperature was maintained at 13±1°C. The mobile phase consisted of solvent A (methanol) and solvent B (methyl tert-butyl ether). The fractions were separated using gradient elution according to the following scheme: 10-50% B 0-40 min; 50-60% B 40-42 min; 60% B 42-65 min; 60-10% B 65-70 min; 10% B 70-75 min. Flow was adjusted to 1 mL/min, and the detection wavelength was set at 450 nm. Quantification of carotenoids was conducted based on comparison of peak areas and their specific absorption maxima with those obtained for external standards with defined concentrations considering the recovery of the internal standard ( $\beta$ -apo-8'-carotinal). All experiments with oils were repeated three times, while for seed extracts analysis was conducted in duplicate.

### 2.2. HPLC analysis of tocopherols

Tocopherols and tocotrienols were analysed using a normal-phase HPLC equipped with a fluorescence detector (excitation: 292 nm, emission: 330 nm) (Merk-Hitachi, Darmstadt, Germany) according to the previously published method (Kschonsek et al., 2016). An Eurospher 100 Diol analytical column (250 x 4 mm i.d., 7 µm particle size) was used as stationary phase and n-hexane/methyl tert-butyl ether (98+2, v/m) was used as mobile phase with a flow rate of 1.5 mL/min. Column temperature was maintained at 30±1°C during isocratic analysis. Quantification of tocopherols was conducted based on comparison of peak areas with those obtained for external standards with defined concentrations considering the recovery of the internal standard ( $\alpha$ -tocopherol acetate). Stock solutions of each tocopherol and tocotrienol standard were prepared in ethanol and stored at -30°C.

### 2.3. Determination of hydrophilic antioxidant capacity using TEAC assay

Hydrophilic antioxidant capacity was determined according to a previously published procedure (Müller et al., 2010). For the seeds, a previous hydrolysis was necessary to release bound antioxidants. Therefore 0.5 g of ground pear seed samples were mixed with hydrochloric acid (1 M) and incubated at 37°C for 30 min. After that, the samples were mixed with sodium hydroxide solution (2 M in 75% methanol) and incubated again for 30 min. The samples were mixed with metaphosphoric acid (0.75 M) and centrifuged (5 min, 5000 rpm). The supernatant was transferred into a 25 mL volumetric flask. The residue was mixed with 5 mL methanol/water (1/1, v/v), shaken for 1 min and centrifuged at 5000 rpm for 5 min. This extraction procedure was repeated twice. For the final step, the volumetric flask was filled up with ethanol/water (1/1, v/v). Subsequently, hydrophilic antioxidant capacity was measured. The ABTS•+ solution was prepared by mixing 7 mmol/L of ABTS salt solution with 2.45 mmol/L of potassium persulfate. This mixture was held at room temperature in the dark for 16 h. Thereafter, the ABTS•+ solution was diluted with phosphate buffered saline (75 mM, pH 7.4) to an absorbance of 0.70 ± 0.05 at 730 nm (Zhou et al., 2011). For the assay, 20 µL of extract was mixed with 200 µL of diluted ABTS•+ solution and

the decrease in absorbance at 730 nm was measured using a 96-well-microplate reader (FluoStar Optima, BMG Labtech, Germany). Calibration curve was constructed using freshly prepared trolox solutions. Values obtained from three replicates are expressed as mmol Trolox equivalents (TE)/100 g of seed sample.

## 3. Statistical analysis

Results are presented as the mean value ± standard deviation of three independent replicate experiments (n = 3). Statistical analysis was based on a one-way ANOVA test. Statistically significant effects were further analyzed and means were compared using Boniferroni test. A level of P<0.05 was taken as statistically significant.

## 3. RESULTS AND DISCUSSION

### 1. Vitamin E

Vitamin E, as the most significant chain-breaking antioxidant in lipophilic environment (Werner and Böhm, 2011), includes two groups of compounds, tocopherols and tocotrienols, together known as tocopherols. Tocopherols are important in regulation of metabolic processes such as inflammation and cancer in humans. In addition, they have numerous essential physiological functions such as anticoagulant (Cook-Mills and McCary, 2010). In investigated seeds of autochthonous pear cultivars four tocopherols and two tocotrienols were detected (Table 1). Independent of cultivar,  $\gamma$ -tocopherol was the dominant one and its content ranged from 11.45 in Kaluderka seed to 34.30 in Kajzerica seed. This finding is consistent with previous findings for pear seeds (Górnaś et al., 2015a). The significant predominance of  $\gamma$ -tocopherol was also reported in previous studies for oils extracted from pear seeds (Górnaś et al., 2016), as well as for other fruit seeds from Rosaceae family such as apricots (Górnaś et al., 2015b) and plums (Górnaś et al., 2015c). Content of  $\gamma$ -tocopherol obtained in our study was slightly lower compared with some commercial cultivars: Beurre d' Amanlis, Conference, Latgale, Mramornaja, Suvenirs and Williams Bon Chretien (Górnaś et al., 2015a). Epidemiological studies show that higher intake of  $\gamma$ -tocopherol from food is associated with significant reduction of possible risk of prostate cancer occurrence (Wright et al., 2007). Also, previous investigations show that an increased dietary consumption of vitamin E, mainly in the form of  $\gamma$ -tocopherol, could be protective against cervical, breast and colon cancer (Kosowski and Clouatre, 2008). In investigated cultivars significant level of  $\alpha$ -tocopherols was also noticed ranging from 2.88 (Karamanka) to 7.19 mg/100 g of seed (Kaluderka). While  $\alpha$ -tocopherol is well known as strongest antioxidant,  $\gamma$ -tocopherol is considered as a stronger anti-inflammatory agent (Tang et al., 2015). Other tocopherols were quantified in amounts lower than 0.5 mg/100 g DW. The concentration of  $\sigma$ -tocopherol in seeds of different pear cultivars ranged between 0.31 and 1.10 mg/100 g dw (Kaludjerka and Kajzerica, respectively), while the level of  $\beta$ -tocopherol was between 0.06 mg/100 g dw (Karamanka) and 1.02 mg/100 g dw (Kaludjerka).

In the last decade, the interest in tocotrienols significantly increased. They possess numerous beneficial effects such as hypocholesterolemic, anti-cancer and neuroprotective properties (Tang et al., 2015). Today, it is well known that they are pharmacologically superior compared to tocopherols. Due to their unsaturated chain their cell penetration is significantly improved which make them highly antioxidative (Kua et al., 2016). The predominant tocotrienol in the investigated seeds was  $\gamma$ -tocotrienol. Besides,  $\beta$ -tocotrienol was determined, while  $\alpha$ -tocotrienol was present only in Kajzerica seeds.

### 1.1. Carotenoids

Major source of carotenoids in human diet are fruits and vegetables. As lipid-soluble antioxidants, they have numerous benefits for human health. According to epidemiological studies there is the link between consumption of fruits and vegetables rich in carotenoids and reduced risk of various forms of cancer, cardiovascular diseases and age-related macular degeneration (Werner and Böhm, 2011). While  $\beta$ -carotene is precursor of vitamin A, lutein and zeaxanthine have been implicated in preventing macular degeneration Howitt and Pogson (2006). Having in mind health benefits of carotenoids, their production and consumption from natural sources, especially from seeds, constitutes an important step in building up the diet of malnourished people in developing countries Federico and Schmidt (2016).

There are no previous studies about carotenoid composition of selected pear cultivars. Six different carotenoid compounds were detected and lutein was dominant one with content ranging from 0.17 to 0.24 mg/100 g of seeds (\*descriptive\*). (All-E)- $\beta$ -carotene was on the second place in relation to its content, and it was detected in all of the investigated cultivars with the exception of Kajzerica cultivar.

**Table 2.** Antioxidant capacity of pear seeds

Pear cultivar	H-TEAC (mmol TE/100 g) <sup>a</sup>
Kajzerica	8.64 ± 0.43a
Karamanaka	5.75 ± 0.45b
Kaludjerka	14.56 ± 0.83c
Tursijara	2.28 ± 0.47d

<sup>a</sup>Values are means ± standard deviation. n = 3. Mean values with different letters are significantly different at P<0.05.

### 1.2. Hydrophilic antioxidant properties

Pear seeds are good sources of phenolic compounds which are well known for their antioxidant properties. In this study, H-TEAC assay was used for the determination of hydrophilic antioxidant capacity of seeds after hydrolysis which led to release of bound substances. This assay measures the ability of antioxidant compounds to reduce the ABTS•+ radical cation to its non-radical form Rahman et al. (2018).

After comparing the results, we found that Kaludjerka seeds exhibited the highest antioxidant capacity (14.6 mmol TE/100 g), while the lowest one was determined for Tursijara seeds (2.3 mmol TE/100 g) (Table 2). According to previous results (Kolniak-Ostek, 2016), one of the dominant polyphenols in pear seeds is arbutin. In previous study (Tai et al., 2016), arbutin showed a strong ABTS radical cation-scavenging activity. Also, pear seeds are rich source of polymeric procyanidins (Kolniak-Ostek, 2016), which are well known antioxidant compounds.

## CONCLUSION

In summary, this study offers results which support potential use of pear seeds as new and cheap source of valuable phytochemicals. Rich in antioxidants, they can be used in different sectors of food and pharmacy industry providing thus significant health, environmental and economic benefits.

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**Table 1.** Content of tococls in seeds of autochthonous pear cultivars (in mg/100 g of seeds)

Sample	$\alpha$ -T <sup>a</sup>	$\beta$ -T	$\gamma$ -T	$\sigma$ -T	$\alpha$ -T3	$\beta$ -T3	$\gamma$ -T3	$\sigma$ -T3	Total (Vitamin E)
Kajzerica	4.47±0.19	0.14±0.01ab	34.30±3.25a	1.10±0.10a	0.31±0.02a	0.35±0.01a	0.57±0.01a	nd	41.24
Karamanka	2.88±0.12b	0.06±0.00b	23.42±0.91b	0.72±0.03b	nd	0.11±0.00b	3.67±0.14b	nd	30.86
Kaludjerka	7.19±0.45c	1.02±0.09c	11.45±0.05c	0.31±0.01c	nd	0.15±0.02c	0.66±0.06a	nd	20.78
Tursijara	3.16±0.01b	0.23±0.01a	23.25±0.10b	0.50±0.01d	nd	0.67±0.01d	0.53±0.00a	nd	28.34

<sup>a</sup>Values are means ± standard deviation. n = 3; Mean values within a column with different letters are significantly different at P<0.05; nd – not detected.

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