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EVALUATION OF ANTIOXIDANT POTENTIAL OF ALBANIAN FIG VARIETIES "KRAPS ZI" AND "KRAPS BARDHE" CULTIVATED IN THE REGION OF TIRANA

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Abstract

Phenolic compounds in recent years are well known for their beneficial effect in human health, as they are featured by antioxidant properties. Figs are sources of phenolic compounds more than red wine and tea. The phenolic content is influenced by the cultivar and is different in the skin and the pulp of the fruit also. The aim of this study was the evaluation of the antioxidant potential of two autochthonous Albanian fig varieties cultivated in the region of Tirana, as they are widely consumed in the local market for fresh consuming.

Selected varieties "Kraps Zi" dark type and "Kraps Bardhe" light type, which produce twice a year, respectively first (breba) and second (main) crop, were collected and compared. Phenols extracted separately from peel and pulp of fresh fruits, were analyzed for total phenolic content, flavonoid and anthocyanins content. Antioxidant activity was estimated with 1,1-diphenyl-2-picrylhydrazyl - DPPH, and 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid - ABTS radical scavenging assays.

Analysis revealed that the main crop of both varieties had higher phenolic content compared to breba crop. Among all fruit fractions the highest phenolic content was observed in peel of dark variety (up to 226.4 mg gallic acid 100 g^{-1} fresh weigh - FW). An appreciable content of anthocyanins (up to 135.1 mg cyanidin-3-rutinoside 100 g^{-1} FW) showed the peel of dark variety too. Antioxidant activity evaluated with ABTS assay resulted up to 2.89 mol ascorbic acid 100 g^{-1} FW, and with DPPH method antioxidants activity, expressed as equivalents of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), resulted up to 3.9 mol TE100 g^{-1} FW. The dark variety "Kraps Zi" resulted with higher antioxidant potential compared to light variety.

The results were in accordance to other similar studies.

***Key words**: Antioxidant potential, Anthocyanins, Fig fruit, Phenols.*

1. Introduction

Fig (*Ficus carica L.*) is among the oldest fruit trees and is known from ancient times. Today, it is an important fruit crop cultivated around the world in subtropical and tropical regions and to some extent in moderate climatic regions of the temperate zones (Aksoy [1]).

In Albania fig fruit trees are widespread and may be found in the north-west, mainly in the centre and in the south of the country, occupying about 15 % of total fruit trees.

As a seasonal food, figs represent an important constituent of the Mediterranean diet (Solomon *et al.*, [2)]. There is plenty of literature on the antioxidant activity of polyphenols, including references to many fruits and vegetables (Del Caro and Piga [3]). Among all common fruits and vegetables in the diet, berries, and figs, especially those with dark blue or red colours, have the highest antioxidant capacity (Liu *et al.*, [4], Solomon *et al.*, [2], Celik *et al.*, [5]). Figs are an excellent source of phenolic compounds, such as proanthocyanidins, whereas red wine and tea, which are two good sources of phenolic compounds, contain phenols lower than those in fig (Vinson *et al.*, [6]).

Fig fruits are often consumed peeled by removing skin; however, fig fruit skins contain healthful nutrients that should not be discarded (Caliskan and Polat [7]). Solomon *et al.*, [2] reported that fig fruit skin is a major source of anthocyanins and polyphenols.

Phenolic compounds in addition to antioxidative roles, possess a wide spectrum of biochemical properties and can also have a beneficial effect in preventing the development of diseases like cancer and cardiovascular diseases, also have antimutagenic, anticarcinogenic, antiinflammatory, or antimicrobial activities (Eberhardt *et al.*, [8], Kim *et al.*, [9]).

Phenolics, flavonoids, anthocyanins, and related total antioxidant activities based on chemical extraction

have typically been measured using methanol or methanol/water mixtures (Solomon *et al.,* [2]; Veberic *et al.,* [10], Caliskan and Polat [7], Del Caro and Piga [3], Duenas *et al.*, [11]).

Solomon *et al.*, [2] showed that the higher the polyphenol content, particularly anthocyanins, in fig fruit, the higher their antioxidant activity. The functionality of these compounds is mainly expressed in their scavenging free oxygen radicals, which are involved in many pathological conditions (Briviba and Sies [12], Tadić *et al.*, [13], Hasan *et al.*, [14]).

There are few researches describing the distribution of the phenols content between fig pulp and peel (Solomon *et al.*, [2], Del Caro and Piga [3]). However reports for Tirana region on bioactive content of fig fruit are not available. Therefore the aim of this work is to evaluate and compare antioxidant potential of different fraction of two fresh autochthonous Albanian fig varieties, which are widespread and locally known for fresh consuming.

2. Materials and Methods

2.1 Plant material

This study was conducted in Tirana region, in Albania. Two fresh fig varieties belonging to the variety "Kraps" and named after their skin color "Kraps Zi" (purple color) and "Kraps Bardhe" (green color), were used as plant material. These varieties produce twice a year first (breba) crop and second (main) crop, and were collected in the period June - August 2015. Ten kg of mature figs were randomly chosen and manually picked. After collection samples were transported immediately to Laboratory of Agrifood Technologies, Faculty of Biotechnology and Food.

For each analysis replicate were randomly chosen 10 fresh figs and manually separated the peel from the pulp.

2.2 Preparation of extracts

For extraction, the sample homogenate obtained for whole fruit and pulp with a Waring blender (Commercial, USA, and for peel with Ultra Turrax (IKA T 25, GR) were extracted by adoption of the method of Kim *et al.,* [15]. The extraction was carried out by using 5 ± 0.001 g (fresh weight) of sample, with 22.5 mL extraction buffer (HCl:methanol:water, 2:80:18, v/v/v) at room temperature. After 15 min. vortexing (VV3, VWR International**)** were centrifuged (Eba 21, Hettich) for 30 min. at 3000 rpm. After centrifugation clear supernatants were used for next analyses. All analyses were done in triplicate.

2.3 Reagents

All reagents used were analytical grade, and purchased from different sources (Fisher, Sigma-Aldrich, Fluka, Merck, and VVR).

2.4 Total phenolics

The total phenolic contents were measured using Folin-Ciocalteu assay according to Singleton and Rossi's [16]. Folin-Ciocalteu (Fluka) reagent diluted five times was mixed with 0.2 mL of sample and 0.5 mL 7.5% Na_2CO_3. The reaction took 30 min. at room temperature in darkness. After reaction time, the absorption was measured at 760 nm using UV-Vis spectrophotometer (Libra S22, Bichrom UK). The results were expressed in mg equivalent of gallic acid (GAE) per 100 g fresh weight (FW), according to calibration curve, build in range of 0.02 - 0.10 mg gallic acid (Fluka) used as standard.

2.5 Total flavonoids

Total flavonoids content was determined colorimetrically by the method described previously by Zubair *et al.,* [17]. Each obtained extracts (1 mL) was placed to 10 mL volumetric flasks then added distilled water 5 mL and 0.3 mL of 5% $NaNO_2$ (Merck), after 5 min was added 0.3 mL of 10% $AlCl_3$ (Merck). After another 6 min., 2 mL of 1 M NaOH (Merck) was added and made up to 10 mL the volume with distilled water. The reaction mixture absorbance was measured at 510 nm using a UV-Vis spectrophotometer (Bichrom, UK,). The results were expressed in mg equivalent of (+) catechin per 100 g FW, according to the calibration curve, linear in range of 10 - 100 µg/mL (+) catechin (Sigma) as standard.

2.6 Total anthocyanins

Total anthocyanin contents were determined according to the pH differential method (Cheng and Bren, [18]). Absorbance was measured at 520 and 700 nm, where absorbance of sample was calculated: $A = (A_{520} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH\ 4.5.,}$ and expressed as mg equivalent of cyanidin-3-rutinoside (molar extinction coefficient of 28800 and molecular weight of 595.2) per 100 g FW.

2.7 ABTS radical scavenging assay

The antioxidant activity of extracts was determined with ABTS radical scavenging assay (Re R. *et al.,* [19]). The ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical cation was generated by mixing 7.0 mM ABTS (Sigma) in dd H_2O and 2.45 mM potassium persulfate (Merck) in dd H_2O, and the reaction was performed for 16 h at room temperature in the dark before use. The stock solution was diluted in methanol (VVR) until the absorption at 734 nm was 0.7 ± 0.02. For the assay 990 µL of $ABTS^+$ solution was mixed with 10 µL of extract. The absorption of sample was measured after 6 min. of reagent addition using spectrophotom-

eter (Bichrom, UK). Antioxidant activity of extract was expressed as mol equivalent ascorbic acid (AAE) per 100 g FW.

2.8 DPPH radical scavenging assay

DPPH radical scavenging activity of fig extracts was performed according to the methods of Sun *et al.,* [20] with some modifications. 15, 30, 45 and 60 µL of sample extracts were completed to 2 mL with 0.1 mM DPPH (Sigma). The mixture was vortexed for 20 sec. The absorbance was measured at 515 nm using spectrophotometer (Libra S22, Bichrom UK), after 20 min. incubation at room temperature and in darkness. 2 mL of 80% methanol was used as a blank solution. The absorbance of DPPH (2 mL) was $A_{control}$. The inhibition percentage of the absorbance was calculated as follows: Inhibition % = $(A_{control} - A_{sample})/A_{control}$. The amount of sample necessary to decrease the absorbance of DPPH by 50% (IC_{50}) was calculated graphically. The antioxidant activity was expressed as mol equivalent of Trolox (TE) (Sigma) per 100 g FW.

3. Results and Discussions

In the Figure 1 are shown selected autochthonous fig varieties.

"Kraps Zi" a dark type, middle-sized, purple coloured, with deep rose pulp, and with very sweet tasting; and "Kraps Bardhe" a light type, with green skin colour, slightly rose pulp, middle sized, sweet-tasting and juicy.

The moisture content (MC) of samples is reported in the Table 1.
Pulp of dark type fig variety showed the highest MC (82.54%) compared to light variety (81.12%). MC was decreased in second crop for both varieties about 14%, this may related to climatic condition.

Total phenolic (TP) content of all fractions is expressed in fresh weight (FW) basis (Figure 2). Among the fresh figs, main crop of "Kraps Zi" variety fig had the highest amount of total phenolic content, while breba crop of "Kraps Bardhe" fig variety had the lowest phenolic content.

Caliskan and Polat [7], and Bucic-Kojic *et al.,* [24] measured similar TP values (28.6 - 211.9 mg GAE/100 g FW)) in figs compared to our fig results. Del Caro & Piga [3] found similar amounts of TP compared with those found in this study. Higher values have been reported by Djuric *et al.,* [25] (till 536.4 mg GAE/100 g FW). Lower results of TP (56.0 to 74.9 mg GAE/100 g FW) have been reported in fresh fig fruits by Solomon *et al.,* [2], Pande and Akoh [21], and Slatnar *et al.,* [22].

Results showed that the highest TP content among all fractions was observed in skin of dark figs (83.28 to 285.28 mg GAE/100g FW), and the pulp showed the lower phenolic content (56.34 to 92.97 mg GAE/100g FW).

Figure 1. "Kraps Zi" and "Kraps Bardhe" fig varieties

Table 1. Moisture content (expressed in %) of breba and main crop for fresh fig varieties [mean values and standard deviations (SD)]

Variety	Breba crop		Main Crop	
	Mean	SD	Mean	SD
Kraps Zi				
Whole fruit	82.54	0.04	70.64	0.13
Pulp	82.84	0.5	70.94	0.4
Peel	80.34	0.9	68.44	0.6
Kraps Bardhe				
Whole fruit	81.12	0.01	76.16	0.01
Pulp	81.52	0.3	76.56	0.5
Peel	79.98	0.4	75.02	0.3

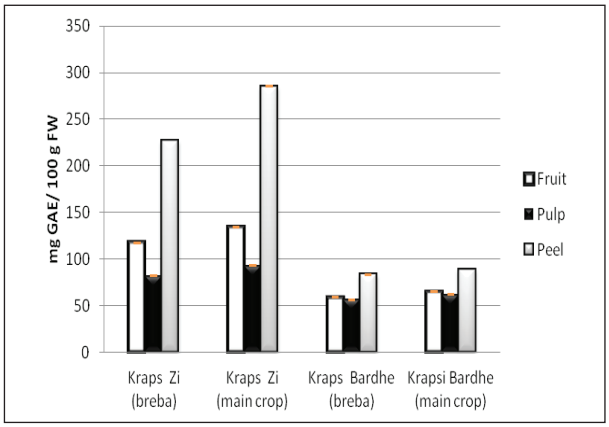

Figure 2. Total phenolic content in fig varieties

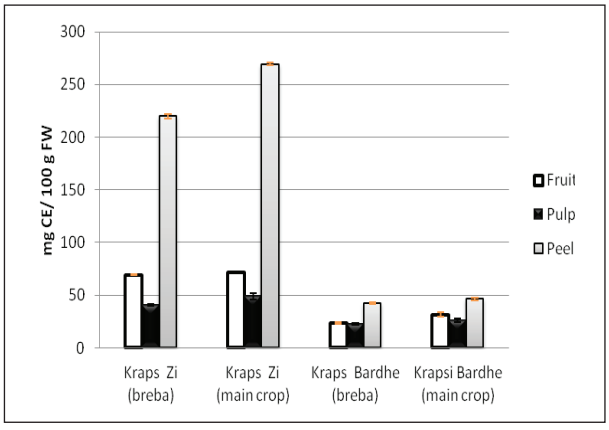

Figure 3. Total flavonoid content

Total flavonoid content of the two fig varieties is shown in Figure 3.

Total flavonoid content of the two fig varieties found to be higher in dark variety and located in the fruit skin from 42.47 - 269.54 mg CE/100 g FW, respectively the highest values resulted in skin of main crop of "Kraps Zi", and the lowest in breba crop of "Kraps Bardhe". In pulp of dark type were approximately two folds higher compared to light variety.

In Figure 4 is shown total anthocianin content in two fig varieties.

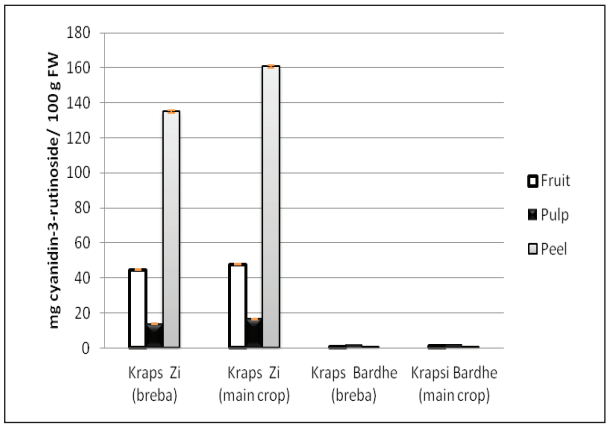

Figure 4: Total anthocianin content in two fig varieties

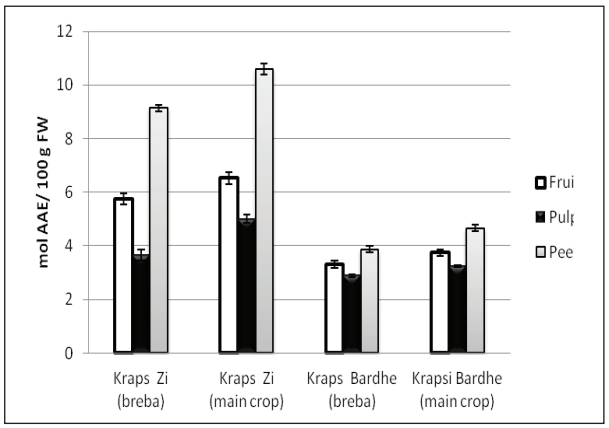

Figure 5. ABTS scavenging activity, in pulp, whole fruit and skin

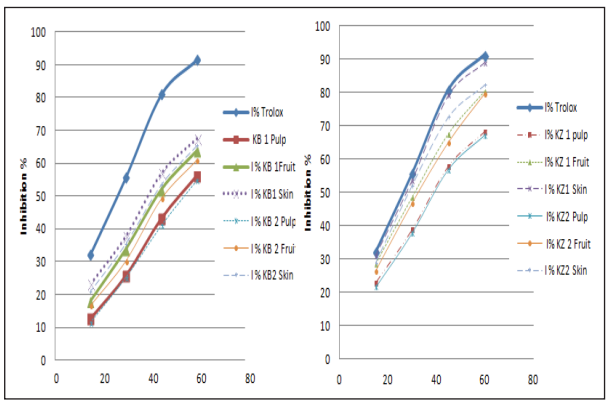

Figure 6. DPPH scavenging activity expressed as percent of inhibition, for pulp, whole fruit and skin

Dark fig variety showed the highest anthocyanin content, almost accumulated in the fruit skin (from 135.09 -160.76 mg cyanidin-3-rutinoside/ 100 g FW). Results for purple figs were found to be ten-fold higher than those reported by Solomon *et al.*, [2], Kamiloglu and Capanoglu [23]. Green variety had anthocianins content only in slight amounts in pulp (0.06 - 1.9 mg cyanidin-3-rutinoside/100g FW), while pulp of dark figs showed small amounts of anthocianins (13.65 - 16.57 mg cyanidin-3-rutinoside /100g FW).

Total antioxidant activity were measured with two different methods (ABTS and DPPH) and resulted higher in extracts of dark fig varieties (Figures 5, and 6). ABTS results ranged from 2.88 to 10.60 mol acid ascorbic equivalent (AAE)/100 g of FW, also from DPPH results ranged from 4.51 - 15.38 mol Trolox equivalents (TE)/100 g of FW. "Kraps Zi" variety had significantly higher antioxidant activity with about 2.5 fold higher compared to light variety tested.

The skin was the major contributing tissue to the total antioxidant activity compared to the pulp, having 2.5 fold higher activity. Antioxidant activity of deep rose pulps were higher compared with those of the lighter rose pulps.

Main crop of "Kraps Zi" variety, which is characterized by dark purple fruits, containing the highest levels of polyphenols and flavonoids among the tested varieties. The green-fruited variety, "Kraps Bardhe", has been found to contain low levels of polyphenols and flavonoids.

It was noted that the second crop had somewhat higher the content of total analysed phenolics. This could be explained by the fact that the fruit develop in warmer, drier and sunnier environmental conditions than the first crop. These weather conditions could be the trigger for higher phenolic synthesis.

4. Conclusions

-The results of this paper represent the first published data for selected "Karps Zi" and "Kraps Bardhe" autochthonous Albanian fig varieties evaluating antioxidant potential.

-Breba and main crop of dark type fig had more TP, TA, and TF compared to light type fig variety and varies from one fruit part to the other. According to our results, the second crop had somewhat higher concentrations than the first one, perhaps due to more stressful conditions during the ripening period.

-The differences in phenolic content in figs may be due to differences in type of variety used, weather condition and growing technology in the orchard.

-This study is in agreement with other studies suggesting that figs, especially those with dark colors, have the highest antioxidant potential, and can contribute to the local diet as a typical seasonal fruit.

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