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Evaluation of Bioactive Phenols in Blueberries from Different Cultivars

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***Abstract*—**Blueberries are widely valued for their high content in phenolic compounds with antioxidant activity, and hence beneficial for the human health. In this way, a study was done to determine the phenolic composition (total phenols, anthocyanins and tannins) and antioxidant activity of blueberries from three cultivars (Duke, Bluecrop, and Ozarkblue) grown in two different Portuguese farms. Initially two successive extractions were done with methanol followed by two extractions with aqueous acetone solutions. These extracts obtained were then used to evaluate the amount of phenolic compounds and the antioxidant activity. The total phenols were observed to vary from 4.9 to 8.2 mg GAE/g fresh weight, with anthocyanin's contents in the range 1.5-2.8 mg EMv3G/g and tannins contents in the range 1.5- 3.8 mg/g. The results for antioxidant activity ranged from 9.3 to 23.2 μmolTE/g and from 24.7 to 53.4 μmolTE/g, when measured, respectively, by DPPH and ABTS methods. In conclusion it was observed that, in general, the cultivar had a visible effect on the phenols present, and furthermore, the geographical origin showed relevance either in the phenols contents or the antioxidant activity.

***Keywords*—**Anthocyanins, antioxidant activity, blueberry cultivar, geographical origin, phenolic compounds.

I. Introduction

BLUEBERRY is a tree species native from the northern hemisphere, belonging to the genus *Vaccinium* and family *Ericaceae*. The blueberry is known as the "longevity fruit" due to its health properties, mostly associated to the phenolic composition [1]. Hence, fruit quality in blueberries has become associated with its levels of phenolics and flavonoids as well as the overall antioxidant capacity. It has been shown that blueberries have among the highest antioxidant capacity of all the fruits and vegetables, although this varies greatly among cultivars [2]. In fact, published studies have compared the phytochemical content of blueberries and have found considerable levels of variability [3], [4]. Besides, blueberries are also particularly rich in vitamin C, which has long been associated with healthy properties of fruit.

Blueberry has recently been recognized as a functional food because of emerging evidence of its health-promoting properties, including nutrient richness and antioxidant potential [5]. Among berry fruits, blueberries are considered a good source of phenolic compounds, such as anthocyanins, which are related to their high antioxidant activity [6]. These phenols are antioxidant substances that help preventing degenerative diseases. It has been reported that blueberries have been widely used as sources of natural antioxidants in a diversity of applications for preventing diseases of the nervous system [7] and chronic disorders, such as coronary heart disease, stroke, and certain types of cancer [8].

The content and profile of phenolic compounds in blueberries has been studied by the scientific community[9]. The phenolic compounds have been reported for their biological activities, such as anti-oxidant [10]; anti-inflammatory [11]–[13]; anti-proliferative [14], [15]; anti-obesity properties [16], [17] and neuroprotective actions [18].

The phenolic compounds, such as flavonoids and anthocyanins, are the major pigments in blueberries and are partly responsible for their high antioxidant activity [19], [20]. However, other antioxidants have been reported in blueberries, like carotenoids and ascorbic acid in small amounts, along with elevated levels of tocopherols [20]–[22]. There is considerable variability in the antioxidant capacity of different *Vaccinium* species [23]–[25].

The content of phenolics in berries is affected by genetic differences, pre-harvest environmental conditions, by the degree of maturity at harvest [26] but also by differences in growing locations and storage conditions [27]. Blueberries are much appreciated but their quality may be deteriorated after harvest because the shelf life of this fruit is very short at ambient temperature due to susceptibility to microbial spoilage or mechanical damage, and also to moisture and nutritional loss [5].

The aim of this work was to characterize blueberries from three cultivars (Duke, Bluecrop, and Ozarkblue) harvested in two different farms, located in the North of Portugal, in terms of phenolic composition (total phenols, anthocyanins and tannins) as well as to evaluate their antioxidant activity by two different methods (ABTS and DPPH).

II. Materials and Methods

A. Samples

This work studied three blueberry cultivars, Duke (D), Bluecrop (B), and Ozarkblue (O), from two different Portuguese producers, named P1 and P2. The fruits were collected in the end of maturation.

B. Extraction

The phenolic compounds were extracted from fruits

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according adaptation of the method described [28]. Briefly, two successive extractions with methanol followed by two successive extractions with a acetone solution (60% v/v) were performed. For each of the 4 extractions performed, the sample was left for 1 hour in an ultrasonic bath at room temperature. This procedure resulted in two methanol extracts (Me-E) and two acetone extracts (Ac-E).

The extracts obtained were then used to quantify the phenolic composition and the antioxidant activity.

C. Quantification of Total Phenols

The total phenolic content in the fruit extracts were determined using gallic acid as a standard, by the Folin–Ciocalteu method according to [29]. Each sample (0.125 mL) was added to 0.5 mL of deionized water and 0.125 mL of Folin-Ciocalteu reagent (Sigma). After 6 min, 1.25 mL of 7.5% solution of sodium carbonate and 1.0 mL of deionized water were added. The mixture was left 90 min at room temperature in the dark and the absorbance at 760 nm was measured. A calibration curve was made with standard solutions of gallic acid. The results were expressed in equivalents of the standard used. All analyses were done in triplicate. The results were expressed as milligrams gallic acid equivalents (GAE) per gram of fresh matter.

D. Quantification of Anthocyanins

Total anthocyanins were determined using the SO_2 bleaching method [30]. Each sample (1 mL) was added to 1 mL of ethanol acidified by 0.1% HCl and 20 mL of 2% HCl solution. In one tube, 2 mL of previous solution was added to 0.8 mL of water (t1). In another tube (t2) were mixed 2 mL of previous solution and 0.4 mL of HNaSO3 solution (15% w/v). After 20 min at dark room temperature, the absorbance at 520 nm was measured. The results were expressed as milligrams of malvidin 3 glucoside equivalents by gram of fresh fruit.

E. Quantification of Tannins

Total tannins were estimated by modification of method described by [31]. Briefly, the sample was diluted to 1/50 in water. 2.0 mL of the previous solution was added to 1.0 mL of water and 3.0 mL of 12 M HCl. The content was divided into two tubes. One of them was heated for 30 min in boiled water and cooled (tube A), while the last one stayed at room temperature (tube B). A 0.5 mL amount of 95 % ethyl alcohol was added. The absorbance was read at 550 nm for each tube. The results were expressed as milligrams by gram of fresh fruit.

F. Determination of Antioxidant Activity

The antioxidant capacity was determined by the methods using the free radicals 2,2-Diphenyl-1-picrylhydrazyl (DPPH*) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) ABTS, respectively described by [32] and [33]. The results were based on the percentage of inhibition of each fruit, compared with a standard antioxidant (Trolox) in a dose–response curve being expressed in Trolox equivalents.

In the first method, to a tube were added 100 µL of sample and 2 mL of DPPH previously prepared and then was placed placed in a dark place at room temperature for 30 minutes. Finally absorbance was measured in a spectrophotometer at a wavelength of 515 nm. The analysis was performed in triplicate.

In the second method, in the tube 100 µL of sample were combined with 2 ml of ABTS, previously prepared, and again the samples were placed in the dark at room temperature, this time for 15 minutes. Finally the absorbance of the samples was read at a wavelength of 734 nm. Three repetitions were also carried out in each sample.

The results were expressed as µmol of trolox equivalents (TE) by gram of fresh fruit.

III. Results and Discussion

A. Phenolic Composition

Fig. 1 shows the amount of phenolic compounds present in the methanol (Me-E) and acetone (Ac-E) extracts, as a whole, expressed as gallic acid equivalents (GAE) per gram of fresh fruit.

The methanolic extracts contained between 3.5 and 6.5 mg GAE/ g of phenolic compounds, while the acetone extracts contained between 1.1 and 1.7 5 mg GAE/ g. These results showed that the phenolic compounds were preferentially recovered in methanol extracts (75-83%) when compared to acetone extracts. The same tendency was observed by [34] for the cultivar Rabbiteye.

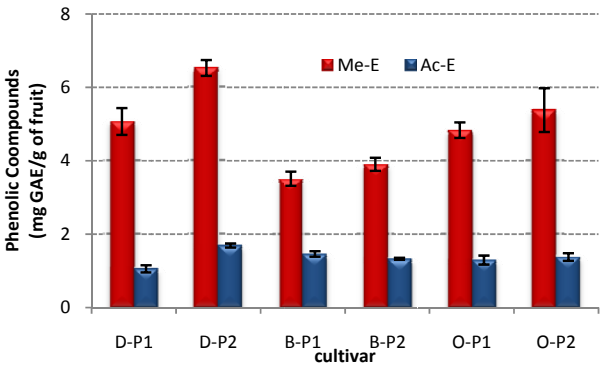

Fig. 1 Amount of total phenols present in the methanol (Me-E) and acetone (Ac-E) extracts for the different cultivars

The total phenols quantified ranged from 6.1-8.2 mg GAE/g for Duke (D), 4.9-5.2 mg GAE/g for Bluecrop (B), and 6.1-6.8 mg GAE/g for Ozarkblue (O). For each producer, the Duke cultivar contained the highest values, followed by Ozarkblue and Bluecrop. Dragovic-Uzelac et al. [35] described values of total phenols slightly lower for Duke (2.8-3.6 mg GAE/g) and Bluecrop (2.9-3.7 mg GAE/g) from Croatia, having used an ethanolic solution as extractor solvent. For the three cultivars, the samples harvested in the farm P2 presented higher content of phenolic compounds. In case of Duke cultivar, this difference was more evident. However the same tendency was observed for both producers. Many factors such as pre-harvest environmental conditions, the degree of maturity or growing

location are described in [26], [27] to affect the content of phenolic compounds.

The amount of total anthocyanins (expressed as milligrams of malvidin 3 glucoside equivalents by gram of fresh fruit) and tannins (expressed as milligrams by gram of fresh fruit) present in the three cultivars are shown in Table I.

TABLE I
TOTAL ANTHOCYANINSAND TANNINS IN THE DIFFERENT CULTIVARS

Sample	Anthocyanins (mg EMv3G/g)	Tannins (mg/g)
D-P1	1.9±0.1	1.5±0.4
D-P2	2.8±0.1	3.8±0.8
B-P1	1.9±0.0	2.3±0.4
B-P2	1.5±0.0	2.9±0.3
O-P1	1.6±0.1	2.1±0.2
O-P2	1.8±0.1	3.5±0.7

The results show the mean value and the standard deviation

The anthocyanins were obtained in the methanolic extract due to their high solubility in that solvent. The amount ranged from 1.5 to 2.8 mg EMv3G/g of fresh fruit, whereas the higher value was obtained for Duke Cultivar from farm P2. In contrast, for Bluecrop a lower mean value was obtained for P2. These values were higher than the 0.56 mg/g obtained by [36].

Concerning the tannins, their content varied according to the producer, so that the amounts obtained for the fruits from P2 were higher when compared with P1. The values of total tannins obtained for Duke (D) were 1.5 and 3.8 mg/g, for Bluecrop (B) were 2.3 and 2.9 mg/g, and for Ozarkblue (O) were 2.1 and 3.5 mg/g. It was further observed that the taninns were recovered in a similar way in the methanolic and acetone extracts.

B. Antioxidant Activity

The antioxidant activity, determined by DPPH and ABTS assays, expressed as micromole of trolox equivalents (TE) per gram of fresh fruit, is shown in Fig. 2.

The results measured by DPPH method ranged from 9.3 to 23.2 µmolTE/g, whereas measured by ABTS method ranged from 24.7 to 53.4 µmolTE/g. For DPPH, the highest value was obtained for D-P2, while for ABTS was obtained for O-P2. For all samples, the absolute values quantified by ABTS method were higher than those by DPPH which is in accordance with [35]. However, the values quantified by these authors were higher than those obtained in this work.

As observed for phenolic composition, the fruits harvested from producer 2 exhibited higher values of antioxidant activity. The correlation between antioxidant activity determined by DPPH method and phenolic compounds was 0.5052 and 0.7442, for Me-E and Ac-E, respectively. For ABTS method, the R^2 value was 0.8449 for Me-E, and 0.7225 for Ac-E, showing a high correlation between these two parameters.

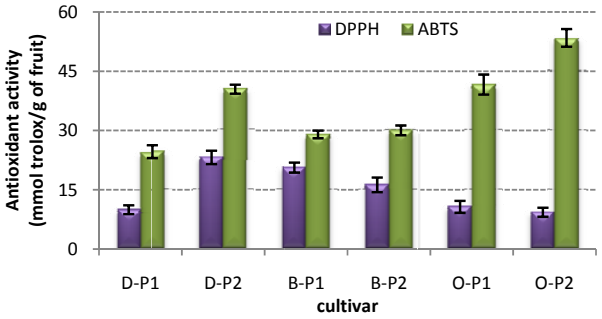

Fig. 2 Antioxidant activity of the different cultivars assayed by DPPH and ABTS methods

C. Correlation between Phenolic Compounds and Antioxidant Activity

Fig 3 shows the correlations obtained between the total phenolic compounds and antioxidant activity as determined by DPPH and ABTS assays. The results show that in both cases the correlations are fairly strong, with regression coefficients of 0.76 and 0.85 for DPPH and ABTS data, respectively. These results indicate that around 80% of the antioxidant activity quantified in blueberries is owing to the presence of phenolic compounds.

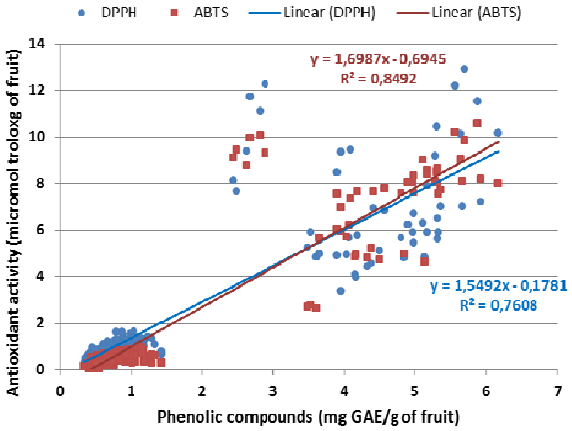

Fig. 3 Correlations between phenolic compounds and antioxidant activity determined by both methods (DPPH and ABTS)

IV. CONCLUSION

The values quantified of total phenols ranged from 4.9-8.2 mg GAE/g of fresh fruit, whereas the higher values were obtained for cultivar Duke harvested at producer 2.

Total anthocyanins and tannins they ranged from 1.5 to 2.8 mg EMv3G/g and from 1.5 to 3.8 mg/g, respectively.

The results showed that the amount of phenolic compounds present in blueberries depends not only on the cultivar, but also on the conditions of production.

Furthermore, the antioxidant activity varied according to the method applied. The absolute values measured by DPPH (9.3 to 23.2 mmolTE/g) were, in all samples, lower than those quantified by ABTS method (24.7 to 53.4 mmolTE/g).

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