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Antioxidant capacity of small dark fruits: Influence of cultivars and harvest time

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Abstract.
BACKGROUND: Small dark fruits represent one of the most important sources of bioactive compounds with antioxidant capacity in the human diet. The content of health-promoting antioxidants in these fruits may be important information to take into account when a fruit producer has to choose which cultivar to grow.
OBJECTIVE: It is important to know how antioxidant capacity and antioxidant compounds as total phenolics and ascorbic acid vary between 9 small dark fruit species and for each species among cultivars (2 to 10 per species).
METHODS: The antioxidant capacity (ORAC assay), total phenolic (Folin-Ciocalteu) and ascorbic acid content were measured in 9 fruits (plums, blackcurrants, blackberries, blueberries, cherries, redcurrants, raspberries, white currants and gooseberries) / 42 cultivars harvested at maturity during their high production period.
RESULTS: The comparison of the average of the various cultivars of each small fruits showed that blackcurrants had the best antioxidant capacity (with plums), the highest ascorbic acid content and the highest total phenolic content (with blackberries). The present study shows that total phenolic compounds, ascorbic acid and antioxidant capacity strongly differed between genotypes of each small dark fruits. Other parameters as harvest time, culture conditions and maturity degree at the harvest may also influence the antioxidant capacity of small fruits.
CONCLUSIONS: Among small dark fruits, blackcurrants have high qualities. Choices of variety, harvest time and maturity degree are important for all fruits.

Keywords: ORAC, ascorbic acid, blackcurrant, phenolics, antioxidants

1. Introduction

Fruit and vegetables are important components of a healthy diet, and their sufficient daily consumption could help prevent major diseases, such as cardiovascular diseases, stroke, type 2 diabete, obesity and certain cancers [1, 2]. Encouraging the consumption of fruits and vegetables can help people increase their intake of micronutrients, dietary fibers and non-nutrient substances.

Berries are indeed good sources of bioactive compounds [3–5], including vitamin C and phenolic compounds [6, 7] from 5 major groups: phenolic acids, stilbenes, flavonoids (flavonols or catechins, flavonols, flavones, flavonones, isoflavonoids, anthocyanins), tannins and lignans [8, 9]. The health properties of some of them are recognized:

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- The biological properties of resveratrol and analogues include anti-inflammatory, antiallergenic, antiaging, algicidal, antimutagenic, anticancerigen, and other activities [10].
- Some studies indicate that biophenolic compounds (flavonoid and tannins) may act as a new type of antimicrobials which may control the pathogens and may help to overcome the problems with antibiotic resistance [7].
- Another potential use of bioactive phenolic compounds present in small fruits is the damage prevention by ROS and free radicals which are produced in an extensive range during human metabolic processes [11]. These phenolic compounds exhibit many biologically significant mechanisms of action, such as scavenging or detoxification of ROS, blocking ROS production, impacting cell cycle, suppression of tumors, modulation of signal transduction, apoptosis, detoxifying enzymes and metabolism [12].

Anthocyanins, a class of natural flavonoids are responsible for the colors of most small fruits. The abundance of anthocyanin pigments in berries makes these fruits good sources of antioxidants for a healthy diet or for the production of dietary supplements. The composition of anthocyanins in different berries varies according to plant species, cultivation conditions and production area. Moreover, the concentration of bioactive compounds in berries is influenced by the genotype [4, 11, 13]. So, some cultivars are preferably used as raw materials to prepare health-promoting food due to their favorable combination of bioactive compounds. Different phenolic compounds occur in different cultivars and their relative quantities and proportion may also vary [14]. These differences affect the total antioxidant capacity but also anticarcinogenic and antimutagenic activities [15].

The biosynthesis of a number of phytochemicals in fruits and vegetables is the result of genotypic and environmental interactions [16]; furthermore, a growing number of studies have shown that cultivation practices (conventional, organic, biodynamic) also influence the concentration of some bioactive molecules in fruits and vegetables [17]. There is no doubt that the application of good agricultural practices is one of the main determinants of food quality, and the food quality concept therefore currently needs an approach that starts from the management of soil and then takes into account the whole production chain.

Greater knowledge on fruit cultivars may influence their market value and their processing opportunities to provide healthier food products [15]. This information could be important for the food industry to decide which cultivars to use up and for consumers to decide which products to choose.

For these reasons, we carried out a systematic study on antioxidant capacity of 9 different small dark fruits and among them of a lot of varieties or cultivars to explore the possible variations due to genotype. Comparisons with cultivars of strawberries [18], apples and pears [19] will be discussed. Culture conditions and harvest time will also be compared.

2. Materials and methods

2.1. Plant material

All fruits were obtained from two Belgium fruit auctions (Belgische Fruitveiling of St Truiden and Veiling Borgloon): 4 cultivars of plums, 4 of blackcurrants, 4 of blackberries, 4 of blueberries, 10 of cherries, 5 of redcurrants, 6 of raspberries, 2 of white currants and 3 of gooseberries. All cultivar names are extensively listed in Table 2. Auctions have taken a particular attention to choice material at the same maturity degree during their high production period. The materials were taken out the day of harvest at the date indicated on the Tables 2 and 3, stored at 4°C and used the day after for analyses. For analysis, the edible part was used according to usual Belgian consumer habits. Stalks and stone were discarded for all the fruits before analysis. The fruits were always harvested during the period where the production was higher except for the experiments on blackberry harvested at various time along the years. For this experiment, blackberry was chosen because, as raspberry, it was possible to obtain fruits during a large part of the year. For this reason, plants were cultivated in tunnel and greenhouse. Maturity degree was tested on 2 fruits: gooseberry (cultivar Winham industry) and cherry (cultivar Kordia). In this case, fruits of the same species were harvested on the same day by 2 different producers.

2.2. Sample preparation

For each fruit, 3 samples of 4 g of fresh material were used. To collect 4 g, a set or a part of fruits was needed. In all the cases, at least 5 different fruits were used. The samples were ground in a blender with 80 mL of extraction solvent: acetone (70%), water (28%), acetic acid (2%) [19]. The mixture was shaken for 1 hour at 4°C and centrifuged at 17000 g for 15 min. The supernatant was used for the assays. Each sample was independently extracted in triplicate or more and analyses were performed the same day except for ORAC assay.

2.3. Total phenolics

Total phenolic contents were determined by the Folin-Ciocalteu method [19]. Appropriately diluted extracts (3.6 mL) were mixed with 0.2 mL Folin-Ciocalteu reagent and 3 min later, 0.8 mL sodium carbonate (20% w/v) was added. The mixture was heated at 100°C for 1 min. After cooling, the absorbance at 750 nm was measured. Chlorogenic acid (Sigma) was used as standard, and results were expressed as mg of chlorogenic acid equivalents (CAE) per 100 g of fresh weight (FW). Analyses were performed in duplicate on each sample.

2.4. Hydrophilic antioxidant capacity

ORAC assays were carried out on a victor3 (PerkinElmer) plate reader. The temperature of the incubator was set to 37°C. Procedures were based on the method of Wu et al. [20]. Briefly, AAPH was used as peroxyl radical generator, trolox as standard and fluorescein as fluorescent probe. Fluorescence filters were used for an excitation wavelength of 485 nm and an emission wavelength of 520 nm. Twenty-five μL of diluted sample, blank, or trolox calibration solutions were mixed with 150 μL of 4 μM fluorescein and incubated for 15 min at 37° C before injection of 25 μL AAPH solution (173 mM). The fluorescence was measured every 2 min for 4 h. All samples were analyzed in duplicate at three different dilutions. The final ORAC values were calculated using the net area under the decay curves and were expressed as μM of trolox Equivalents (TE) per 100 g FW.

2.5. Ascorbic acid

The 2,6-dichloroindophenol (DCIP) method was used to measure only reduced ascorbic acid [19]. Briefly, each molecule of vitamin C converts a molecule of DCIP into a molecule of $DCIPH_2$, and that conversion can be monitored as a decrease in the absorbance at 520 nm. A standard curve was prepared using a series of known ascorbic acid concentrations. Diluted samples in 5 % metaphosphoric acid or ascorbic acid calibration solutions (600 μL) were mixed with 500 μL 10% metaphosphoric acid, 300 μL citrate buffer (pH 4.15) and 300 μL DCIP (0.1 mg. Ml^{-1}). The optical density blanching was used; for each sample, the blank value was determined after addition of 60 μL ascorbic acid (1 mg. ml^{-1}) in the aim to measure the interference due to the sample color. The results were expressed as mg of AA per 100 g FW.

All results presented are the means (± SE) of three independent extractions (=3 biological replicates) and two analyses per extraction (=2 technical replicates). Differences among data for each technique or condition were compared by ANOVA, using Tukey HSD's post test $P < 0.05$.

3. Results and discussion

3.1. Antioxidant capacity of various cultivars of small dark fruits

The comparison of the average data obtained for the various cultivars of each species studied in the present work is presented in Table 1. Results obtained during the same study on strawberry, apples and pears [18, 19] are added to improve the comparison between the most currents Belgian fruits. The antioxidant capacity of white currants and gooseberries were the lowest among the small fruits, close to the level of apples and pears. Namiesnik et al. [21] have already reported that the antioxidant capacity and bioactivity of gooseberry was lower than blueberries

Table 1
Average of the antioxidant capacity (μmol TE/100g FW), ascorbic acid (mg AA/100g FW) and phenolic (mg CAE/100g FW) contents of various cultivars of different fruits compared to values of strawberries [18] and apples and pears [19]

Fruits	Phenolics mg CAE/100g FW	Ascorbic acid mg AA/100g FW	ORAC μmol TE/100g FW
Plums	$348 \pm 81^{c,d,e}$	3.0 ± 0.4^{g}	5911 ± 342^{a}
Blackcurrants	705 ± 99^{a}	293.8 ± 29.8^{a}	$5824 \pm 77^{a,b}$
Blackberries	803 ± 183^{a}	72.5 ± 11.9^{c}	$4371 \pm 797^{b,c}$
Blueberries	491 ± 38^{b}	13.3 ± 1.7^{f}	$3951 \pm 257^{c,d}$
Cherries	324 ± 35^{d}	$21.3 \pm 9.1^{e,d}$	3625 ± 333^{d}
Strawberries	335 ± 44^{d}	106.0 ± 14.1^{b}	3516 ± 300^{d}
Pears	191 ± 31^{f}	18.8 ± 3.8^{e}	$2516 \pm 391^{e,f}$
Redcurrants	387 ± 22^{c}	110.2 ± 15.0^{b}	2431 ± 149^{e}
Apples	256 ± 22^{e}	23.8 ± 2.2^{e}	$2309 \pm 270^{e,f}$
Raspberries	333 ± 24^{d}	$64.3 \pm 12.9^{c,d}$	$2255 \pm 238^{e,f}$
Gooseberries	$375 \pm 58^{c,d}$	$57.6 \pm 10.6^{c,d}$	$2028 \pm 139^{f,g}$
White currants	$323 \pm 194^{d,e,f}$	43.1 ± 17.4^{d}	1537 ± 674^{g}

and cranberries. The antioxidant capacity of redcurrants and raspberries was similar to that previously obtained for apples and pears [19]. However phenolic and ascorbic acid contents of apples and pears were lower than those of the small fruits (except plums). The antioxidant capacity of blueberries, blackberries and cherries was close to that of strawberries known as good source of micronutrients, especially antioxidant phenolics [22]. The phenolic content of these four fruits was also similar except for blueberries and mainly blackberries where it was twice of the values observed in the other three fruits. The content in ascorbic acid was much higher in strawberry.

Blackcurrants had the best antioxidant capacity (with plums) and blackberries, the highest ascorbic acid content (while plums contained very little ascorbic acid) and the highest total phenolic content (with blackberries).

Overall results showed that red fruits can serve as good sources of bioactive compounds for the human diet, but due to its high concentrations in phenolic compounds and ascorbic acid and its strong antioxidant capacity, blackcurrant can specially be regarded as good candidate for the preparation of nutritional supplements.

3.2. Antioxidant capacity of various cultivars of small dark fruits

For the 10 cultivars of cherries (Table 2), the antioxidant capacity was comprised between 2283 (Sweetheart) and 5548 μmol TE/100g FW (Br Biggareau), close to the values obtained for strawberries [18]. The content of ascorbic acid was close (between 8.2 and 22.6 mg/100g FW) for all the cultivars except Samba (showing a higher content, 111.4 mg/100 FW). The content of phenolic compounds was well correlated with the antioxidant capacity ($R^2 = 0.720$), as previously observed by Chaovanalikit and Wrolstad [23] and Damar and Eksi [24]. Khoo et al. [25] also indicated that selection of cultivars is important to obtain cherries with better potential health promoting effects because in addition, they have shown differences in bioactivity experiments (Caco-2 cancer cell proliferation inhibitory activity and effect on prostaglandin E2 production).

The antioxidant capacity of the various plum cultivars was often higher than that of cherries and the differences between cultivars (Table 1) were lower. It varied from 6639 ± 571 (Altesse Double) to 4990 ± 276 μmol TE/100g FW (Stanley). Unlike observations of Mubarak et al. [26], there was a correlation between the phenolic content and the antioxidant capacity in plums ($R^2 = 0.717$). The ascorbic acid content of all the plum cultivars was very low compared to other fruits.

Even better correlation between antioxidant capacity and phenolic compounds was observed for the cultivars of gooseberries ($R^2 = 0.969$) and blueberries ($R^2 = 0.935$) (Table 2).

The antioxidant capacity of raspberries and blackberries varied of the simple to the double according to cultivar with generally no correlation with total phenol content ($R^2 = 0.236$ and 0.476 for raspberries and blackberries respectively). The differences were even more important for ascorbic acid, some cultivars having a high content of this vitamin.

Table 2
Antioxidant capacity (μmol TE/100g FW), ascorbic acid (mg AA/100g FW) and phenolic (mg CAE/100g FW) contents of cherries (C), plums (P), gooseberries (GB), blueberries (BL), raspberries (RB), blackberries (BB), blackcurrants (BC), redcurrants (RC), and white currants (WC) from various cultivars. Significant differences between cultivars of the same species as determined by ANOVA ($p < 0.05$) are indicated by different letters

Harvest date	Cultivars	Phenolics mg CAE/100g FW	Ascorbic acid mg AA/100g FW	ORAC μmol TE/100g FW
06-29	Br Biggareau (C)	523 ± 27^{a}	22.6 ± 1.4^{b}	5548 ± 175^{a}
07-06	Kordia (C)	406 ± 16^{b}	15.3 ± 0.8^{c}	5472 ± 345^{a}
07-06	Kelleriis (C)	517 ± 21^{a}	11.3 ± 0.8^{d}	4149 ± 328^{b}
07-13	Regina (C)	376 ± 9^{b}	$9.3 \pm 0.7^{e,d}$	3868 ± 301^{b}
07-06	Lapins (C)	$285 \pm 15^{c,d}$	11.2 ± 1.0^{d}	3848 ± 242^{b}
06-29	Hedelfinger (C)	313 ± 11^{c}	$13.2 \pm 1.7^{c,d}$	3025 ± 158^{c}
06-29	Summit (C)	262 ± 21^{d}	$13.2 \pm 3.4^{c,d}$	$2819 \pm 177^{c,d}$
06-29	Samba (C)	252 ± 18^{d}	111.4 ± 6.1^{a}	2655 ± 86^{d}
07-06	Schneider (C)	196 ± 13^{e}	8.2 ± 0.5^{e}	$2644 \pm 132^{d,e}$
07-20	Sweetheart (C)	181 ± 7^{e}	10.5 ± 1.0^{d}	2283 ± 208^{e}
08-31	Altesse Double (P)	458 ± 18^{a}	3.0 ± 0.4^{b}	6639 ± 571^{a}
08-31	Anna Spath (P)	424 ± 18^{a}	4.2 ± 0.5^{a}	6071 ± 245^{a}
08-31	President (P)	407 ± 19^{a}	2.6 ± 0.5^{b}	$5943 \pm 550^{a,b}$
08-31	Stanley (P)	403 ± 15^{a}	2.2 ± 0.3^{b}	4990 ± 276^{b}
06-29	Winham Industry (GB)	534 ± 15^{a}	57.1 ± 3.1^{b}	2307 ± 119^{a}
06-29	Achiller (GB)	371 ± 27^{b}	43.1 ± 4.6^{c}	1870 ± 145^{b}
06-29	Madlett (GB)	256 ± 17^{c}	87.7 ± 4.3^{a}	1720 ± 131^{b}
08-17	Pemperton (BL)	579 ± 14^{a}	13.8 ± 1.0^{b}	4467 ± 288^{a}
08-17	Brigitta (BL)	529 ± 20^{a}	9.9 ± 1.7^{c}	4274 ± 292^{a}
07-20	Bleu Crop (BL)	435 ± 19^{a}	17.9 ± 1.3^{a}	$3719 \pm 241^{a,b}$
07-20	Bleu Gay (BL)	422 ± 15^{a}	$11.5 \pm 0.8^{b,c}$	3344 ± 109^{b}
06-08	Sugana (RB)	313 ± 31^{b}	74.1 ± 5.0^{b}	2783 ± 273^{a}
06-22	Lagorai (RB)	$283 \pm 16^{b,c}$	87.4 ± 1.9^{a}	2684 ± 145^{a}
06-08	Tulameen (RB)	386 ± 14^{a}	93.6 ± 2.4^{a}	2677 ± 197^{a}
06-22	HF22 (RB)	269 ± 22^{c}	$82.0 \pm 7.0^{a,b}$	2300 ± 140^{a}
07-13	Polka (RB)	328 ± 8^{b}	20.2 ± 1.3^{c}	1695 ± 156^{b}
08-17	Himbotop (RB)	417 ± 18^{a}	28.5 ± 3.7^{c}	1391 ± 148^{b}
06-08	Obsidian (BB)	1352 ± 61^{a}	$86.5 \pm 9.0^{a,b}$	6037 ± 645^{a}
06-22	Karacha Black (BB)	615 ± 43^{b}	90.6 ± 5.5^{a}	5432 ± 206^{a}
06-08	Loch Ness (BB)	615 ± 33^{b}	38.4 ± 4.1^{c}	3027 ± 266^{b}
06-08	Lochtay (BB)	629 ± 56^{b}	74.6 ± 3.8^{b}	2989 ± 184^{b}
06-22	Tsema (BC)	660 ± 32^{b}	292.4 ± 14.3^{b}	7974 ± 263^{a}
06-29	Bon Gairn (BC)	993 ± 56^{a}	212.6 ± 6.7^{c}	5884 ± 356^{b}
06-22	Cocea Black (BC)	541 ± 15^{c}	352.9 ± 2.4^{a}	$4913 \pm 368^{b,c}$
06-22	Titania (BC)	627 ± 34^{b}	317.1 ± 4.6^{b}	4524 ± 346^{c}
06-15	Jonkheer (RC)	$409 \pm 23^{a,b}$	75.2 ± 8.5^{d}	2875 ± 552^{a}
06-15	Rotet (RC)	393 ± 13^{b}	133.1 ± 4.3^{b}	2687 ± 263^{a}
06-15	Rolan (RC)	341 ± 11^{c}	88.0 ± 2.2^{d}	$2293 \pm 222^{a,b}$
06-29	Junifer (RC)	456 ± 34^{a}	156.2 ± 5.8^{a}	2167 ± 197^{b}
06-29	Rovada (RC)	337 ± 15^{c}	98.4 ± 4.6^{c}	2131 ± 209^{b}
08-03	Victoria (WC)	515 ± 20^{a}	60.3 ± 0.8^{a}	2204 ± 137^{a}
06-29	Blanca (WC)	130 ± 11^{b}	25.9 ± 2.9^{b}	870 ± 72^{b}

Table 3
Antioxidant capacity (μmol TE/100g FW), ascorbic acid (mg AA/100g FW) and phenolic (mg CAE/100g FW) contents of blackberries, cultivar Loch Ness harvested at various time during the year (DP = delayed planting). Significant differences as determined by ANOVA ($p < 0.05$) are indicated by different letters

Harvest date	Culture	Phenolics mg CAE/100g FW	Ascorbic acid mg AA/100g FW	ORAC μmol TE/100g FW
05-11	Greenhouse	454 ± 30^{c}	15.9 ± 1.0^{d}	$3692 \pm 564^{a,b}$
06-08	Greenhouse	615 ± 33^{b}	38.4 ± 4.1^{b}	$3027 \pm 266^{c,b}$
08-03	Tunnel - substrate	$407 \pm 28^{c,d}$	154.9 ± 3.2^{a}	3333 ± 245^{b}
08-17	Tunnel - soil	680 ± 15^{a}	22.7 ± 1.2^{c}	4516 ± 319^{a}
11-22	DP - greenhouse	381 ± 16^{d}	5.9 ± 1.1^{e}	3294 ± 214^{b}

Among the currants, the blackcurrants had the highest antioxidant capacity, the highest content in phenolic compounds and ascorbic acid. The antioxidant capacity of blackcurrant varied from 4524 (Titania) to 7974 (Tsema) μmol TE/100g FW. The percentage of variation was similar for ascorbic acid and phenolic compounds but there were no correlation between them ($R^2 = 0.035$ and 0.114 between antioxidant capacity and phenolic compounds or ascorbic acid respectively). Significant differences in ascorbic acid content and antioxidant capacity (ORAC) between blackcurrant cultivars were already reported by Khoo et al. [25]. The antioxidant capacity of redcurrants was lower and close of that of gooseberries. Their ascorbic acid content was higher than the previous fruits except blackcurrant.

It seems clear from the previous results that these parameters (antioxidant capacity, ascorbic acid and total phenolic contents) could be used to characterize and classify the different cultivars of each fruits as already proposed by Bordonaba and Terry [27] for blackcurrant or Zhang et al. [28] for Citrus.

3.3. Antioxidant capacity varied with harvest time, maturity and culture conditions

In the case of blackberry (Loch Ness), the normal harvest period is August. To obtain blackberry earlier, the plants are grown in greenhouses. Late harvest is also possible by using cold-stored plants in greenhouses. The fruits were harvested by the same producer at the same ripening stage. The highest antioxidant capacity, phenolic and ascorbic acid contents were observed in August but the differences were not very important, lower than 33% (Table 3), except for ascorbic acid in November. The observed variations of antioxidant capacity were not so high than these observed in strawberries harvested at different dates [18].

Moreover, to test the influence of maturity degree, 2 producers have harvested their fruits on the same day but with a different maturity degrees (Fig. 1), as illustrated by a clearer or darker color for gooseberries (Winham industry) and cherries (Kordia). In gooseberries, the difference in maturity didn't correlate with variations in antioxidant capacity while it was the case in cherries. The dark cherries had a higher content in phenolics and ascorbic acid and a higher antioxidant capacity. Total phenolic content and antioxidant capacity of cherries increased as maturity progressed from un-ripened to fully ripened stage [29]. The influence of ripening on antioxidant capacity was different from fruit to fruit. Ripe red raspberries had also stronger antioxidant activities and higher total anthocyanin content when compared with the pink stage (50% maturity) [30].

Environmental factors have also a great influence on the antioxidant capacity. Numerous works have shown that environmental parameters (light conditions, temperature, irrigation, fertilization or cultivation systems) can affect the antioxidant capacity in strawberries, blackcurrant and other fruits [31–34]. Blackberries harvested in Augustus and grown in tunnel on soil have higher antioxidant capacity and phenolic content than those grown on substrate (Table 3). On the contrary, the content in ascorbic acid was lower. Differences were also observed in strawberry cultivated on sand or loam [18] in tunnel or greenhouse. Moreover, Zheng et al. [35] have showed that genotype can strongly influence the composition of fruit (blackcurrant) as a response to weather conditions [28].

Anthocyanins are a class of natural polyphenolic compounds that are responsible for the colors of most fruits. They also exhibit antioxidant activities. Small dark fruits represent one of the most important sources of bioactive compounds with antioxidant capacity, especially blackcurrants. Several genetic and environmental factors affect the production and accumulation of bioactive compounds in fruits, and the effect of cultivar in affecting the nutritional

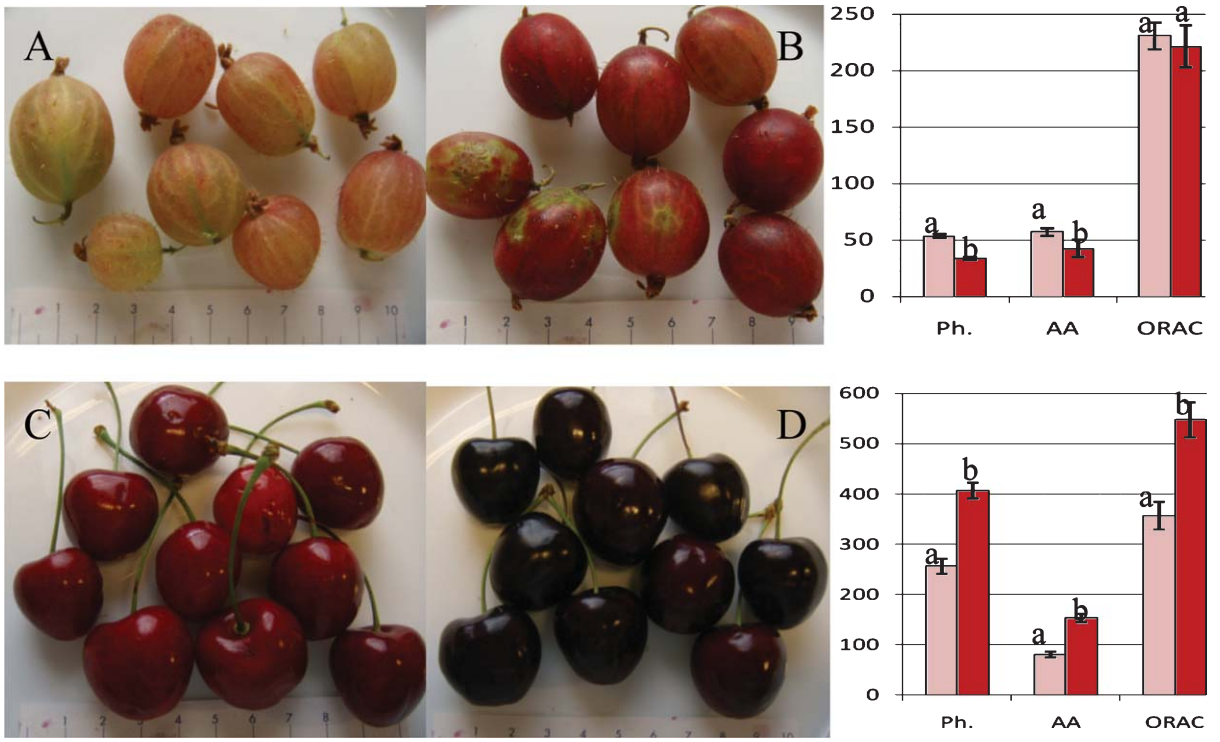

Fig. 1. Antioxidant capacity (10^{-5} mol TE/100g FW), ascorbic acid (mg AA/100g FW, except for cherries 0.1 mg AA/100g FW) and phenolic (mg CAE/100g FW) contents of gooseberries, variety Winham industry harvested on June 29 (A and B) and cherries, variety Kordia, harvested on July 6 (C and D) by various producers. □: Half ripened fruits; ■: fully ripened fruits. Different letters indicated significant differences ($p < 0.05$).

quality of fruits is well known [36], even if still few genotypes are well characterized for these important features [37]. Regardless of its environmental or physiological drivers, point-source variation in fruit phytonutrient contents may be a relevant interest in health-related studies. Moreover, it may impact the nutritional benefits to consumer and affect the quality advantages associated with direct-marketed fruits [38].

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