

Comparative Nutrient Element and Antioxidant Characterization of Berry Fruit Species and Cultivars Grown in Hungary

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Abstract. Fruits of four berry species (strawberry, raspberry, red and black currants) were compared in their elemental composition (Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn) and redox parameters involving total phenol content (TPC), ferric reducing ability (FRAP), 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, and total radical scavenger activity (TRSA). Berry cultivars contained significantly higher ($P \leq 0.05$) amounts of most detected elements as compared with apple with many maximum elemental values demonstrated by the black currant 'Otelo'. Black currant also had the greatest antioxidant capacity as demonstrated through all conducted assays. The results obtained through FRAP, TPC, and TRSA assays were closely correlated, whereas TRSA and DPPH varied independently. Our study provides valuable information on the antioxidant capacity of several berry species grown in Hungary and highlights the crucial influence of cultivar on elemental content and antioxidant power of berry fruits. This study demonstrated the ability to select berry cultivars for specific nutritional purposes or assign parental lines in functional breeding programs.

World berry fruit production totaled 6,750,749 Mt in 2006 (Faostat, 2006), from which strawberries (*Fragaria ×ananassa* Duch.), raspberries (*Rubus idaeus* L.), and currants (*Ribes rubrum* L. and *R. nigrum* L.) accounted for ≈80% of the total. In Hungary, these are also the main species, representing 96.1% of Hungarian berry production, and their importance is expected to increase fur-

ther as the concept of "functional food" becomes more popular. Enhanced functional properties of berries are the result of phytonutrients having high antioxidant capacity (Beekwilder et al., 2005; Häkkinen et al., 1999; Moyer et al., 2002; Tulio et al., 2008; Tulipani et al., 2008; Wu et al., 2006). Recently, several reviews were published on the bioactive compounds in berry fruits that affect human health (Beekwilder et al., 2005; Scalzo et al., 2005a, 2005b).

Antioxidant capacity (AOC) is the capability of a compound to inhibit oxidative degradation, e.g., lipid peroxidation (Roginsky and Lissi, 2005). In addition to tocopherols, carotenes, and ascorbic acid, phenolics are the main antioxidant components of fruits, vegetables, tea, wine, and so on. Although AOC of polyphenols is associated with various mechanisms, the elevated reactivity of phenolics toward free radicals is considered the primary mechanism (Robards et al., 1999).

Rates of cardiovascular diseases, stroke, and cancers are very high in Hungary (Hungarian Central Statistical Office, 2007). To combat this trend, increased fruit and vegetable consumption are recommended. Artificial antioxidant supplements cannot compensate for fresh fruit consumption (Halliwell, 2000), because potent antioxidant and anticancer activity of fruits can be attributed to additive and synergistic combinations in a complex mixture of several thousand phytochemicals (Liu, 2003).

Berries are known to possess outstanding antioxidative power (Cho et al., 2004; Hanum, 2004; Moyer et al., 2002). The antioxidant capacity of berries was shown to be influenced by several factors with cultivar variability being one of the most important (Scalzo et al., 2005c; Wang and Lin, 2000). The aim of this research was to assess the variability of elemental content and antioxidant properties in several cultivars of important berry species (strawberry, raspberry, red and black currant) as compared with apple, a more intensively consumed fruit. This study revealed correlations between several redox parameters and provides information relative to specialized dietary use of berry fruits as functional foods for the future.

Materials and Methods

Fruit and sample preparation for redox assays. Experiments were carried out using fully ripe fruits of strawberry ('Elsanta', 'Onebor', and 'Honeyoye'), raspberry ('Glen Ample', 'Malling Exploit', and 'Fertődi zamatos'), red currant ('Detvan', 'Jonkheer van Tets', and 'Rondom'), and black currant ('Fertődi 1', 'Otelo', and 'Titania') as well as apple cultivars (Jonathan, Golden Delicious, and Granny Smith). Each berry species was cultivated under identical growing conditions at Nagyréde, Hungary. Samples were prepared using 100 g of berry or apple fruits (peel and flesh together). Selected fruit were homogenized and centrifuged in a Hettich Zentrifugen (Mikro 22 R; Tuttlingen, Germany) device (4 °C, 35 min, 18,750 g_n) with supernatants being used for redox assays.

Biochemical assays. Dried fruit samples (0.2 g) were digested in a mixture of 2 mL HNO₃ and 2 mL H₂O₂ in a Teflon bomb (PTFE) for inductively coupled plasma analysis (Stefanovits-Bányai et al., 2006). The digested samples were diluted with deionized water to a total volume of 10 mL. The following elements were determined by ICP-OES (ICAP 61; Thermo Jarrell Ash Co., Franklin, MA): Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, P, Pb, and Zn.

Total phenolic content (TPC) was measured using Folin-Ciocalteu's reagent according to the method of Singleton and Rossi (1965). The content of soluble phenols was calculated from a standard curve based on gallic acid concentration.

Antioxidant capacity was determined by the ferric-reducing ability of plasma (FRAP) method (Benzie and Strain, 1996).

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Total radical scavenger capacity was measured using a recently developed chemiluminescence assay (Blázovics et al., 1999). A Lumat 9501 luminometer (Berthold, Bad Wildbad, Germany) was used for total scavenger capacity determination of the different berry extracts. The emitted light was measured in the 390 to 620 nm range. Reaction mixture contained H₂O₂ (0.30 mL, 10⁻⁴ dilution of 33% H₂O₂), micropoxidase (0.30 mL of 1 mM) as a catalyst, and alkaline luminol solution (pH 9.8) (in 0.050 mL of 0.07 mM). The emitted photons were accumulated during a 30-s exposure and expressed in relative light unit (RLU %). Fruit juice samples were diluted 100-fold; and 0.050 mL was added to the luminol solution and vortexed (10 s) before analysis.

The measurement of DPPH radical-scavenging activity was carried out according to the method of Blois (1958) and modifications by Hatano et al. (1988). Absorbance of the methanolic DPPH dye was assessed spectrophotometrically at 517 nm. For characterization of the activity, DPPH degradation was measured in undiluted (cc) as well as 10-fold and 100-fold fruit juice dilutions.

Statistical analyses. Data presented for each cultivar represent the mean values determined from three independent homogenates. Correlation analyses of redox parameters and one-way analysis of variance were carried out in Microsoft Excel 2003 (Microsoft, Redmond, WA). Significant differences were calculated according to a Duncan's multiple range test with $P \leq 0.05$ being considered significant in all analyses.

Results and Discussion

Heavy metals were not detected in the tested berries. In general, elemental analysis demonstrated that nine of the 12 detected elements exhibited lower levels in one or more apple cultivars as compared with berries (Table 1). It was established that considerable variation occurred among different cultivars of the same species, and fruits of black currant cultivar Otelo contained the highest quantities of most detected elements. The fruits of black currant cultivars contained the highest levels of macrominerals such as Ca, K, Mg, and P. These minerals are dietary requirements in human nutrition and have various physiological effects. When comparing nutrient contents with the standard values reported for these fruits [U.S. Department of Agriculture (USDA), Agricultural Research Service, 2007], all apples contained more Ca. Similarly, strawberry cv. Onebor and the red currant 'Rondom' also showed outstanding Ca levels. The Na content of all Hungarian-grown apple and berry cultivars exceeded the standard USDA levels. The K/Na ratio was the lowest in apple and highest in strawberry, which might be useful to compensate for high Na levels in a typical human diet (John et al., 2002). The Mg content of berries was similar those set in the USDA database.

Table 1. Elemental content of apple and four berry species recorded on a fresh weight basis (mg/100 g⁻¹).

Species/cultivars	Al	B	Ba	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
Apple												
Golden Delicious	0.64 a ^y	0.38 d	0.03 a	9.95 a	0.05 a	0.17 a	95.70 a	5.88 a	0.12 a	3.35 b	9.79 a	0.08 a
Granny Smith	0.84 b	0.38 d	0.03 a	11.58 a	0.07 a	0.13 a	89.70 a	6.43 a	0.09 a	4.42 c	11.88 a	0.05 a
Jonathan	0.70 a	0.61 e	0.03 a	10.22 a	0.21 d	0.10 a	100.06 a	6.50 a	0.07 a	3.23 b	12.93 a	0.06 a
<i>Means across cvs.</i>	0.73	0.46	0.03	10.58	0.11	0.13	95.15	6.27	0.09	3.67	11.53	0.06
Strawberry												
Elsanta	0.62 a	0.09 a	0.05 b	18.54 b	0.05 a	0.38 b	140.57 b	12.26 b	0.33 d	2.61 a	21.05 b	0.11 b
Honeoye	0.56 a	0.11 a	0.04 a,b	16.79 b	0.05 a	0.28 b	141.76 b	12.16 b	0.28 c	2.47 a	21.71 b	0.08 a
Onebor	1.01 c	0.14 a	0.06 b	31.23 d	0.06 a	0.95 e	147.25 b,c	15.41 b	0.34 d	2.59 a	24.15 b	0.10 b
<i>Means across cvs.</i>	0.73	0.12	0.05	22.19	0.05	0.54	143.19	13.28	0.32	2.56	22.31	0.10
Raspberry												
Glen Ample	1.02 c	0.18 b	0.03 a	21.99 c	0.11 b	0.62 d	153.43 c	17.56 b,c	0.16 a,b	3.86 b	29.18 c	0.29 d
Fertódi zamatos	1.17 c	0.16 b	0.06 b	39.90 e	0.11 b	0.52 c	153.52 c	22.17 c	0.24 c	5.06 d	33.00 c	0.32 d
Malling Exploit	1.11 c	0.21 c	0.04 a,b	29.88 d	0.09 b	0.60 c,d	171.84 d	20.14 c	0.28 c	4.66 c	35.06 c	0.27 d
<i>Means across cvs.</i>	1.10	0.19	0.05	30.59	0.10	0.58	159.60	19.96	0.23	4.53	32.42	0.29
Red currant												
Detvan	1.12 c	0.23 c	0.06 b	42.19 e	0.09 b	0.55 c	176.64 d	14.67 b	0.19 b	4.67 c	35.33 c	0.17 c
Jonkheer van Tets	2.25 e	0.20 b,c	0.07 b	49.59 e,f	0.10 b	1.73 f	232.54 e	16.54 b	0.28 c	5.70 d	45.15 d	0.16 b,c
Rondom	1.00 c	0.17 b	0.11 c	61.50 f	0.13 c	0.64 d	162.73 d	16.12 b	0.26 c	3.93 b	59.10 e	0.23 c
<i>Means across cvs.</i>	1.46	0.20	0.08	51.10	0.10	0.97	190.64	15.78	0.24	4.77	46.52	0.19
Black currant												
Fertódi 1	1.46 d	0.28 c	0.09 c	70.01 g	0.13 c	0.55 c	309.62 g	21.20 c	0.18 b	6.54 e	62.74 f	0.21 c
Otelo	2.21 e	0.20 b,c	0.13 d	79.87 h	0.32 e	1.20 e	297.55 f	26.08 d	0.21 b	7.46 e	53.70 e	0.23 c
Titania	1.02 c	0.16 b	0.10 c	40.63 e	0.12 b,c	0.47 c	225.19 e	16.54 b	0.22 b	4.86 c	36.51 c	0.20 c
<i>Means across cvs.</i>	1.56	0.21	0.11	63.51	0.19	0.74	277.45	21.27	0.21	6.29	50.99	0.22

^aValues represent the mean of three replicates.

^yMean values followed by the same letter within a column do not differ significantly ($P \leq 0.05$) according to a Duncan's multiple range test.

Black and gray background colors show the highest and lowest values respectively for each element among all tested species and cultivars.

Trace elements are essential regulators of cell redox homeostasis because they are cofactors for several antioxidant enzymes as well as contributors to signal transduction pathways and regulators of NF- κ B activity (Blázovics, 2007). The quantity of trace elements was also higher in berries than in apples. However, as compared with standard values (USDA, 2007), Hungarian-grown apples contained more Cu, Mn, and Zn. 'Onebor' strawberry and the red currant 'Jonkheer van Tets' showed outstanding Fe content, whereas all raspberries and black currants contained much lower levels of Fe, Zn, and especially Mn.

Redox active elements (e.g., Fe and Cu) may induce oxidative hazard and thereby contribute to the pathogenesis of serious diseases (Bush, 2002; Dreher and Junod, 1996; McMurray et al., 1990). Iron content was significantly higher in all berries as compared with apples. A serving of 100 g of 'Otelo' black currant covers 36% of the daily recommended Cu intake for middle-aged men, whereas a single serving of 'Jonkheer van Tets' covers 22% of the recommended Fe intake (DRI, 2001). Considering that dietary intake of minerals is influenced by several

factors, including bioavailability and regulation of blood levels by the kidneys and other organs (McDowel, 2003), the elemental content of berries is not expected to have unfavorable health effects.

However, in some neurodegenerative diseases, increased metal levels in the brain contribute to free radical reactions-induced damage (Halliwell and Gutteridge, 1990). Recently, berries have been suggested to have protective effects against certain brain disorders (Shukitt-Hale et al., 2008). Therefore, strawberry cultivars Elsanta and Honeoye or the black currant 'Titania' that accumulate relatively lower concentrations of Al, Cu, Fe, Mn, and Zn in fruits might be more beneficial as dietary supplements for patients with such diseases. Cultivars such as Jonkheer van Tets or Otelo would be less useful for this purpose as a result of their higher levels of specific metallic ions. Furthermore, the raspberry and currant cultivars possessed significantly higher ($P \leq 0.05$) amounts of Al than apples, making them somewhat less desirable in neurodegeneratively affected patients. To further demonstrate the importance of metallic ion concentration in foodstuffs or dietary supple-

ments, Stefanovits-Bányai et al. (2006) proposed the use of aqueous extracts from female *Ginkgo* leaves (characterized by lower antioxidant properties but more favorable metal ion constitution) as opposed to male leaves for curative purposes in neurodegenerative disorders.

Polyphenols are present only in plant food sources of the human diet and epidemiological studies support the fact that a higher intake of flavonoids is associated with lowered risk for cancer, heart disease, and stroke (Block et al., 1992; Hannum, 2004). Compared with apple, total phenolic content was slightly but significantly higher ($P \leq 0.05$) in strawberry cultivars Elsanta and Honeoye, whereas the TPC in all three black currants was considerably higher (Fig. 1A). The great difference in the TPC of red and black currant cultivars agrees with the fact that black currant contains roughly 37-fold higher concentrations of total anthocyanins than red currants (Wu et al., 2006). In our study, total phenolic content varied in the relative order of apple < raspberry = red currant < strawberry < black currant. Balasundram et al. (2006) compiled TPC of several fruit species in which berries and apples could be ranked according to the

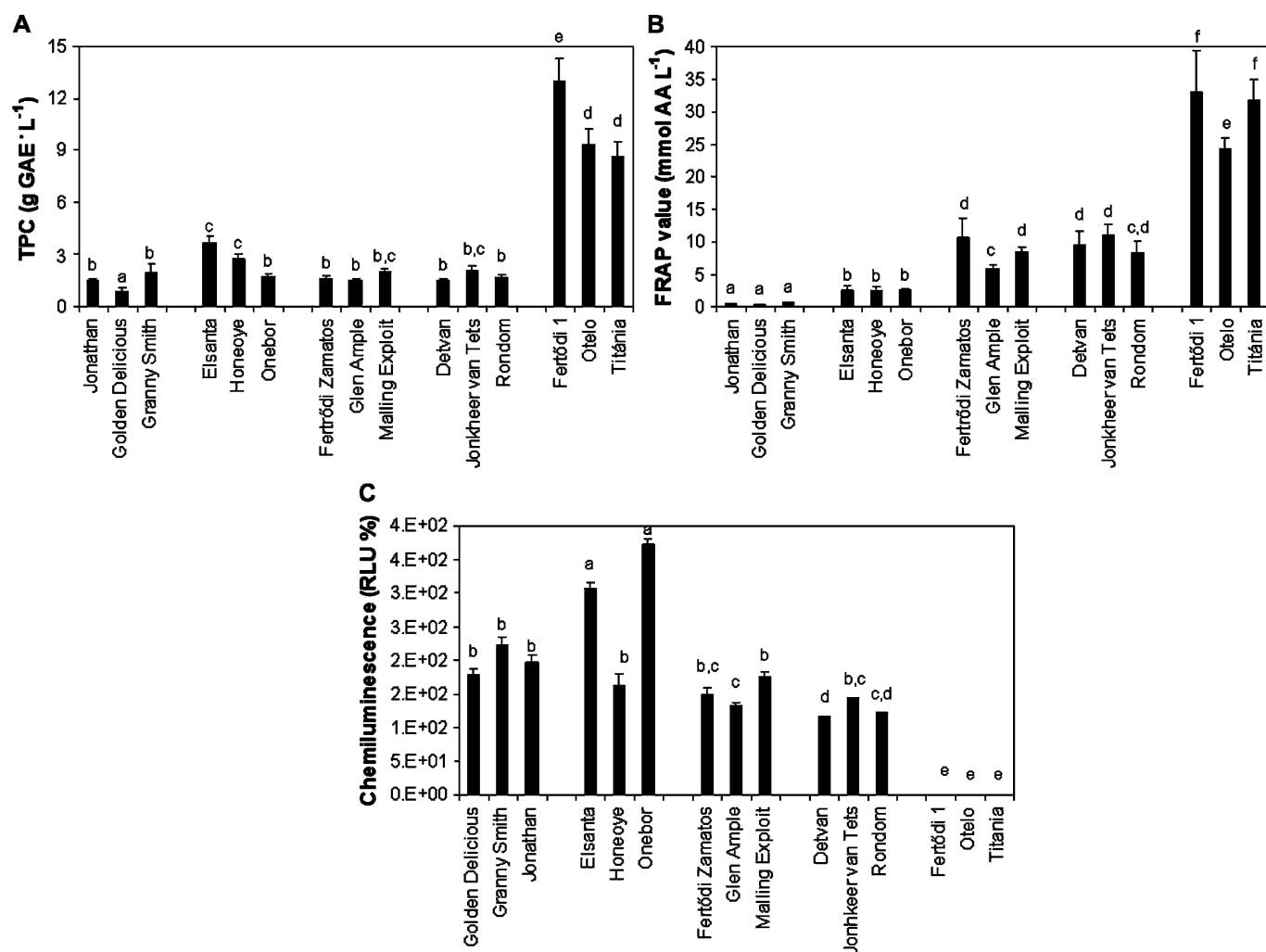


Fig. 1. Total phenolic content (A), ferric-reducing ability (B), and total radical scavenger capacity in squeezed fruit juices of apple, strawberry, raspberry, red and black currant cultivars. Mean values followed by the same letter do not differ significantly ($P \leq 0.05$) according to a Duncan's multiple range test.

following order: raspberry < strawberry < apple < blackberry < cranberry. Inconsistencies between these data can be attributed to the analysis of different cultivars as well as diverse agronomic and/or environmental factors (Balasundram et al., 2006). Phenolic antioxidants ameliorate degenerative diseases through a variety of mechanisms, but their benefits are likely to be partially as a result of their antioxidant capacity (Liu, 2003).

The squeezed juice samples exhibited antioxidant capacity, which was measured by the FRAP method on the basis of Fe^{3+} to Fe^{2+} redox reaction. The AOC expressed in ascorbic acid equivalents was higher in all berries than in apples and changed between 0.3 and 33.1 $\text{mmol}\cdot\text{L}^{-1}$ measured in 'Golden Delicious' apple and 'Fertődi 1' black currant, respectively (Fig. 1B). This represents a 110-fold variation among all tested berry cultivars that can primarily be attributed to the considerable differences among species, whereas variations within species were restricted. The FRAP values ranked on the order of apple < strawberry < raspberry = red currant < black currant, which is very similar to that obtained by Halvorsen et al. (2002) using the same FRAP assay.

The radical scavenging activities of samples were determined in a H_2O_2 /OH-microperoxidase-luminol system with luminometry. The chemiluminescence light intensity given in RLU % is reduced in the presence of free radical scavenger compounds (Blázovics et al., 1999). The system's emitted light diminished as a function of juice concentration. The most notable difference occurred in the 100-fold diluted samples; black currant cultivars had the highest and the strawberries 'Onebor' and 'Elsanta' had the lowest scavenger capacities (Fig. 1C).

The apple cultivars exerted the poorest DPPH radical scavenging capacity as a function of concentration, which was exceeded by all berries (Fig. 2). The greatest differences occurred in comparison between the 100-fold diluted samples. The raspberry (Fig. 2A) and red currant cultivars (Fig. 2B) showed quite similar DPPH scavenging capacities, which

were slightly exceeded by the best strawberry cultivars, Elsanta and Honeoye. The black currant cultivars, especially Fertődi 1, possessed outstanding DPPH scavenging ability.

The greatest differences occurred between the black currant and apple cultivars showing the highest and lowest antioxidant capacity values, respectively. The differences were reliably expressed with all redox assays; however, variations could be detected in the ranking order of several other cultivars obtained with different methods. The closest correlation, $r = 0.918$, was found between the FRAP and TPC. It indicates that a considerable fraction of AOC is the result of phenolics in the berry fruits as was previously shown for apple and all tested berry species (Beekwilder et al., 2005; Connor et al., 2005; Häkkinen et al., 1999; Hannum, 2004; Kondo et al., 2002). However, other explanations attribute the close correlation between these assays to the similarity of the chemistry behind them (Huang et al., 2005).

It is interesting that strawberry cultivars possessed low FRAP values and relatively high polyphenol contents as compared with raspberry or red currant. Variable correlations between antioxidant assays and TPC can be explained by a range of influencing factors, including the diversity among samples tested (Wu et al., 2004), the different phenolic compounds exhibiting variable reaction kinetics (Ozgen et al., 2006), and the hydroxylation, methoxylation, acylation, or glycosylation associated with the phenolic structures (Cho et al., 2004, 2005; Ecklund et al., 2005). Furthermore, the presence and activity level of enzymes responsible for mediating oxidative stress (Jaio and Wang, 2000) as well as the different chemistry behind the antioxidant procedures used (Ecklund et al., 2005; Ozgen et al., 2006) can similarly account for variable correlation coefficients.

The total radical scavenger activity (TRSA) values were also well correlated with the FRAP and TPC data having correlation coefficients of 0.821 and 0.681, respectively. This confirms that phenolics have a crucial

contribution to the radical scavenging capacity of berries. Even if flavonoids are devoted to metal chelation, they retain their radical scavenging capacity (Lugasi et al., 2003). Considerable, although weaker, correlations were also evident between DPPH and FRAP ($r = 0.544$) or TPC ($r = 0.540$). In addition, TRSA and DPPH showed a much lower correlation ($r = 0.297$) indicating the different nature of chemical reactions involved in the two radical scavenger assays (Prior et al., 2005).

Our study showed that very similar ranking orders of fruits could be established with the use of redox assays. Black currants had the best antioxidant characters in all measured systems. Several epidemiological studies suggest that a higher flavonoid intake may assist in the prevention of some chronic diseases (Block et al., 1992; Knekt et al., 2002; Middleton et al., 2000). However, breeding for improved phytonutrient quality involves more than just increasing antioxidant capacity. Other attributes like elemental content may also have important health benefits. In addition, antioxidant activity does not always correlate with bioactivity against specific disease components, e.g., tumor proliferation, angiogenesis, and so on (Meyers et al., 2003).

Similar to other analyses (Scalzo et al., 2005c; Tulipani et al., 2008), our results highlighted the importance of genotype as a critical influence on the elemental content and antioxidant power of berry fruits. This enables the possibility to select cultivars for special nutritional purposes or assign parental lines in functional breeding programs. The quantification of several antioxidant phytonutrients may provide an opportunity to specialize a fruit diet in response to the varied requirements arising from differences in countries, ages, health conditions, risks for disorders, eating habits, and other demographic aspects. Developing such specialized fruit diets for specific health conditions or nutritional requirements would require that breeders have information from studies outlining the complex nature of how the complement

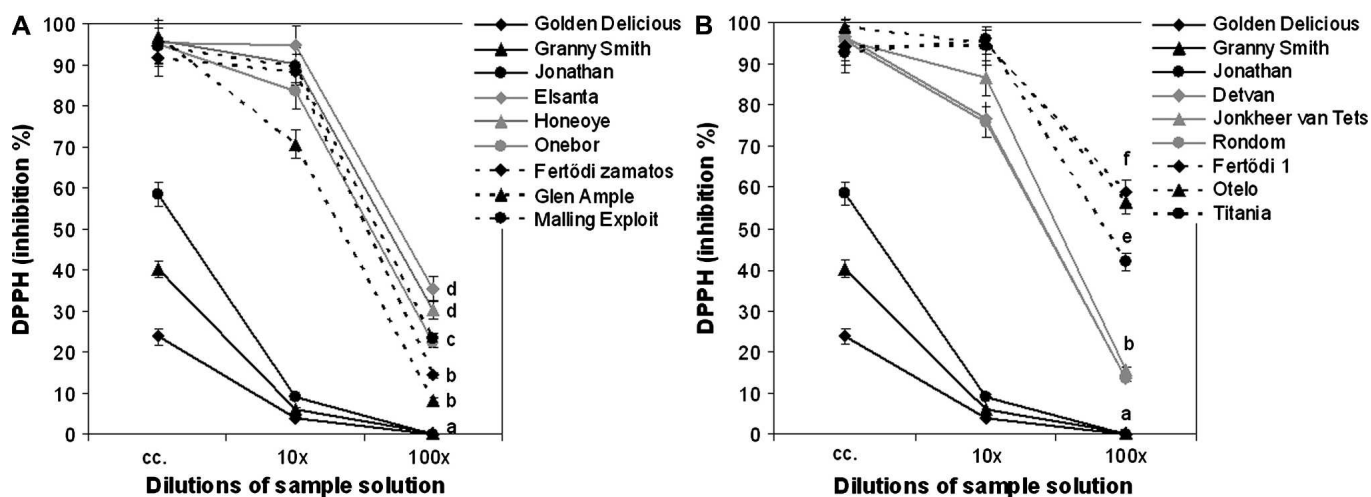


Fig. 2. DPPH radical scavenging activity in squeezed fruit juices of strawberry, raspberry (A), and red and black currant cultivars (B) in comparison with apple. Mean values followed by the same letter do not differ significantly ($P \leq 0.05$) according to a Duncan's multiple range test.

of antioxidants act synergistically or antagonistically to affect the various aspects of disease. Unfortunately, our knowledge is severely restricted at this point and hence, curative use of fruits with boosted antioxidant power should be initially subjected to medical control.

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