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Antioxidant Properties of Berry Fruit Juices as Dependent on Raw Material Quality and Technological Processing: A Review

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ABSTRACT

Berry fruit juices are characterized by a broad spectrum of bioactive properties, among which particular attention should be paid to antioxidant capacity. The highest antioxidant activity is exhibited by secondary metabolites in plants, primarily by polyphenols (phenolic acids, tannins, a large group of flavonoids and anthocyanins), carotenoids, vitamin C, organic acids, calcium, selenium and other. The antioxidant capacity of fruit juices is influenced by a variety of factors, including raw material quality and the conditions of the technological process. This review focuses on the antioxidant properties of berry fruit juices and on the compounds responsible for those properties, contained in various berry fruit species. The significance of environmental factors, such as climate and agricultural conditions, as well as storage conditions prior to processing, are also discussed. The effect of the technological process of juice production on antioxidant properties was determined, with special emphasis on the conditions of fruit crushing, mash maceration, heat treatment and storage. The advantages and disadvantages of the applied procedures and unit operations are presented. Particular attention was paid to fruit mash maceration with the use of enzymatic preparations obtained from genetically modified microorganisms. It was demonstrated that the antioxidant properties of berry fruit juices may be improved by proper selection of raw materials and by control over the parameters of the technological process.

Keywords: antioxidant properties, bioactive substances, berries, storage, technological process

CONTENTS

INTRODUCTION

Recent years have witnessed growing consumer interest in products which, in addition to supplying nutritional building blocks and energy, deliver additional health benefits. Berries and berry juice play a very important role in that group of products. They are a rich source of bioactive components which are known for their antioxidant capacity (Deighton *et al*. 2000; Borowska and Szajdek 2003; Dietrich *et al*. 2004). Bioactive components neutralize free radicals and prevent many lifestyle diseases. Their antioxidant properties have been validated by *in vivo* research on volunteers (Netzel *et al*. 2002; Duthie *et al*. 2006; García-Alonso *et*. *al*. 2006). Bioactive components comprise mostly polyphenols, vitamins A and C, carotenoids, organic acids, calcium, selenium and others (Hannum 2004; Borowska *et al.* 2005; Amarowicz *et al*. 2009). A study of commercially available juices has shown that those products are abundant in bioactive compounds and show strong antioxidant properties; nonetheless, differences were observed between various brands (Zając and Podsędek 2002; Kalisz and Mitek 2003). Factors that affect the health benefits of fruit juice include fruit species, variety, growing conditions, types of treatment and unit operations during fruit processing. The most important processes are crushing, mash maceration with the involvement of enzymes, clarification, concentration, heat treatment and pulsed electric field treatment (Brownmiller *et al*. 2008; Oszmiański 2008; Amarowicz *et al*. 2009; Kozák *et al*. 2009; Odriozola-Serrano *et al*. 2009). Those processes support production, but they also lead to changes in antioxidant activity, which is not always beneficial (Czapski 1999; Grajek 2003; Dietrich *et al*. 2004; Landbo and Meyer 2004). A reduction in antioxidant capacity results from the loss of bioactive compounds, mainly phenolic compounds. According to Dietrich *et al*. (2004), commercially produced black currant juice shows 59% of the antioxidant activity of its raw material. Many authors also emphasize the importance of juice storage conditions before the product reaches the market (Garzón and Wrolstad 2002; Rein and Heinonen 2004; Walkowiak-Tomczak

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Invited Mini-Review

Table 1 Total phenols content, anthocyanin content and antioxidant activity (ORAC method) of berry fruits

Species	Total phenols[a]		Anthocyanins[a]		ORAC[b]	
Bilberry (*Vaccinium myrtillus*)	577-614	Giovanelli and Buratti 2009	330-344	Giovanelli and Buratti 2009	44.6	Prior *et al.* 1998
Blackberry	361	Heinonen *et al.* 1998	80-230	Moyer *et al.* 2002	14.8-22.6	Jiao and Wang 2000
(*Rubus fruticosus*)	275-650	Moyer *et al.* 2002	134.6-152.2	Pantelidis *et al.* 2007	26.7-78.8	Moyer *et al.* 2002
Blackcurrant (*Ribes nigrum*)	128-411	Moyer *et al.* 2002	128-411	Moyer *et al.* 2002	36.9-93.1	Moyer *et al.* 2002
Blueberry	181.1-473.0	Prior *et al.* 1998	62.6-235.4	Prior *et al.* 1998	10.0-42.3	Prior *et al.* 1998
(*Vaccinium corymbosum*)	298-310	Giovanelli and Buratti 2009	92-129	Giovanelli and Buratti 2009	4.6-31.1	Ehlenfeldt and Prior 2001
Chokeberry (*Aronia melanocarpa*)	662.5	Borowska and Szajdek 2003	428	Zheng and Wang 2003	160.2	Zheng and Wang 2003
Cranberry (*Vaccinium macrocarpon*)	120.0-176.5	Wang and Stretch 2001	19.8-65.6	Wang and Stretch 2001	8.2-14.1	Wang and Stretch 2001
Raspberry (*Rubus idaeus*)	192-359	Anttonen and Karjalainen 2005	35.1-49.1	Pantelidis *et al.* 2007	13.1-45.2	Moyer *et al.* 2002
Strawberry (*Fragaria x ananassa*)	317.2-443.4	Skupień and Oszmiański 2004	31.11-35.87	Roussos *et al.* 2009	12.2-17.4	Wang and Lin 2000

[a] mg/100 g fw

[b] µmol Trolox/g fw

2007).

This study analyzes berry fruit species which are most frequently used in juice production by investigating their antioxidant properties and bioactive components responsible for that activity. It also discusses the effect of technological process and storage conditions on selected attributes of fruit and juice.

RAW MATERIAL

Berries – bioactive components and antioxidant properties

Juice is made from fresh berry fruit directly after harvesting, from fruit which has been cold stored for 2–3 weeks and, less frequently, from deep-frozen fruit. Concentrated fruit juice is also used (Rommel *et al.* 1990; Oszmiański 2002). Berry fruit owes its antioxidant activity to the presence of phenolic compounds, including phenolic acids, flavonoids, stilbenes and tannins (González *et al.* 2003; Benvenuti *et al.* 2004; Szajdek and Borowska 2008). Many research results suggest the occurrence of a correlation between the total content of phenolics, including anthocyanins, and antioxidant activity (Wang *et al.* 1996; Kalt *et al.* 1999; Jiao and Wang 2000; Wang and Lin 2000; Ehlenfeldt and Prior 2001; González *et al.* 2003; Bartolomé *et al.* 2004; Taruscio *et al.* 2004). Various authors have demonstrated that antioxidant activity is more correlated with the total content of phenolics than with the level of anthocyanins (Prior *et al.* 1998; Moyer *et al.* 2002; Zheng and Wang 2003).

The quantitative and qualitative profile of bioactive components in fruit and their antioxidant properties are largely determined by species, variety, agricultural conditions, ripeness, duration and conditions of storage (Heinonen *et al.* 1998; Prior *et al.* 1998; Häkkinen *et al.* 1999; de Ancos *et al.* 2000; Häkkinen and Törrönen 2000; Jeppsson 2000; Vinson *et al.* 2001; Wang and Stretch 2001; Wang and Zheng 2001; Connor *et al.* 2002; Haffner *et al.* 2002; Skupień and Oszmiański 2004; Anttonen and Karjalainen 2005; Borowska *et al.* 2005; Cordenunsi *et al.* 2005; Ehala *et al.* 2005; Reyes-Carmona *et al.* 2005; Giovanelli and Buratti 2009; Roussos *et al.* 2009). The fruit of chokeberry, bilberry and blackcurrant are characterized by very high levels of phenolic compounds and antioxidant activity (**Table 1**). It demonstrates the ability of scavenging $\text{DPPH}^{\bullet}$, $\text{ABTS}^{\bullet+}$ and $\text{OH}^{\bullet}$ free radicals and other reactive oxygen species, such as hydrogen peroxide and singlet oxygen (Martín-Aragón *et al.* 1998; Prior *et al.* 1998; Kähkönen *et al.* 1999; Kähkönen *et al.* 2001, Wang and Stretch 2001; Proteggente *et al.* 2002; Wada and Ou 2002; Taruscio *et al.* 2004; Szajdek and Borowska 2008), it inhibits the oxidation of LDL (Meyer *et al.* 1997; Heinonen *et al.* 1998; Meyer *et al.* 1998; Vinson *et al.* 2001; Yan *et al.* 2002), liposomes (Heinonen *et al.* 1998) and prevents the formation of $\text{NO}^{\bullet}$ radicals (Wang and Mazza 2002).

Table 2 Total phenols content, L-ascorbic acid content and antioxidant capacity in blackcurrant juices of different cultivars (Dietrich *et al.* 2004)

Cultivar	Total phenols[a]	L-ascorbic acid[a]	Antioxidant capacity TEAC[b]
Ometa	5713	660	27.9
Rosenthals	5627	1020	27.2
Ben Lomond	5940	1180	33.2
Titania	3950	830	24.6
Ben Nevis	5939	1305	26.8
Black Dawn	5968	1043	37.7
Tsema	7758	1267	33.3
Ben Tirran	8719	1345	39.0
Ben More	7417	961	35.6

[a] mg/L

[b] mmol Trolox/L

Varietal features are also believed to have a strong impact on the antioxidant properties of berry fruit. The above has been validated by Dietrich *et al.* (2004) who investigated the process of juice extraction from various blackcurrant varieties (**Table 2**).

The results of numerous studies indicate that phenolic extracts of berry fruit are marked by higher levels of antioxidant activity than many pure phenolics or vitamins, which is indicative of synergistic interactions between antioxidants (Vinson *et al.* 2001). According to Liao and Yin (2000), Vinson *et al.* (2001), the protective effect of ascorbic acid and α-tocopherol on lipoproteins increases with the addition of catechin, epicatechin or caffeic acid. Clinical research has also shown that owing to their bioavailability and effectiveness, the antioxidants present in natural products such as juice and fruit deliver greater health benefits than pharmaceutical dietary supplements (Wang *et al.* 1996). Different mechanisms are responsible for the antioxidant activity of the bioactive components of berry fruit, subject to their structure (Cao *et al.* 1997; Wang *et al.* 1997; Heim *et al.* 2002; Kähkönen and Heinonen 2003). Flavonoids inhibit lipid oxidation, they chelate metals and scavenge active oxygen species *in vitro* and *in vivo* (Heim *et al.* 2002). Anthocyanins, a flavonoid subgroup, inhibit the oxidation of human LDL and liposomes, and they show free radical scavenging capacity *in vitro* (Satué-Gracia *et al.* 1997; Wang *et al.* 1997; Kähkönen and Heinonen 2003). They prevent the oxidation of ascorbic acid *in vitro* (Sarma *et al.* 1997). In chokeberries, the dominant anthocyanin is cyanidin-3-galactoside (57%), showing the highest antioxidant capacity among all anthocyanins. The dominant anthocyanin in strawberry fruit is pelargonidin-3-glucoside (82%), generally believed to be a relatively weak antioxidant (Bridle and García-Viguera 1997; Espín *et al.* 2000; Wang and

Lin 2000). According to Cao *et al.* (1997), the antioxidant capacity determined in the ORAC test is 1.54 for pelargonidin and 2.24 for cyanidin. Cranberries contain large amounts of peonidin-3-galactoside, and blackcurrant fruits – of delphinidin-3-rutinoside (Andersen 1989; Slimestad and Solheim 2002). According to the results of *in vitro* tests, hydroxycinnamic acid and proanthocyanins are also capable of inhibition LDL oxidation (Meyer *et al.* 1998; Porter *et al.* 2001). Ellagic acid accounts for 35-40% phenols in strawberries (Häkkinen and Törrönen 2000; Hannum 2004). High levels of tannins are found in chokeberry (5513.5 mM/kg) and blackthorn fruit (3614.0 mM/kg) (Oszmiański and Moutounet 1995). Oszmiański and Wojdyło (2005) have demonstrated that proanthocyanins account for 66% of total polyphenols in chokeberry, while anthocyanins have a 25% share. Resveratrol also displays potent antioxidant activity, and it is found in large quantities in grapes (6500 ng/g dm). Other berry fruits, like bilberry, cranberry, deerberry, highbush blueberry, lowbush blueberry, rabbiteye blueberry and sparkleberry, contain from 7 to about 5900 ng resveratrol per g of dry sample (Rimando *et al.* 2004).

Vitamin C also plays a role in forming the antioxidant properties of berry fruit (Wang *et al.* 1996; Knapik-Czajka 1998; Benvenuti *et al.* 2004). Recent research has shown that ascorbate and dehydroascorbate effectively prevent LDL oxidation (Knapik-Czajka 1998). Ascorbic acid neutralizes free radicals and atomic oxygen in an aqueous environment. Yet according to some authors (Wang *et al.* 1996; Deighton *et al.* 2000), owing to the high content of polyphenols in berry fruit, the share of vitamin C in total antioxidant activity, measured by the ORAC method, does not exceed 15%. These findings are supported by the work of González *et al.* (2003) which shows a weak correlation between $DPPH^{\bullet}$ radical scavenging activity and ascorbic acid levels.

Research results indicate that the levels of bioactive components and antioxidant capacity differ in fruit of the same variety which has been grown in various climatic regions and in different years (Wang and Zheng 2001; Reyes-Carmona *et al.* 2005). Researchers have postulated that genotype is the most important factor. Their findings suggest a very strong correlation between antioxidant activity, measured by the ORAC and FRAP methods, and total concentrations of phenolic compounds and anthocyanins for blackberry genotypes. Post-harvest storage conditions prior to fruit processing strongly affect polyphenol changes and the antioxidant properties of fruit. According to a study of ten cranberry cultivars, carried out by Wang and Stretch (2001), a storage period of 3 months at a temperature of 0–20°C supports polyphenol synthesis and stimulates antioxidant activity. Fruit stored under the above conditions was marked by the highest antioxidant capacity (ORAC) which was highly correlated with polyphenol and anthocyanin concentrations. Similar results were reported by Connor *et al.* (2002) for stored blueberry cultivars. The above authors noted that phenolic compound concentrations and antioxidant activity levels, determined by the FRAP method, are strongly dependent on the cultivar. Ayala-Zavala *et al.* (2004) have observed that the post-harvest storage of strawberries over a period of 13 days at a temperature of 10 or 5°C increased the fruit's antioxidant capacity, measured in ORAC units, and polyphenol concentrations in comparison with fruit stored at 0°C. The freeze-storage of fruit, on the other hand, induces changes that lead to a reduction in free radical scavenging capacity (de Ancos *et al.* 2000). The above authors reported a 4–26% reduction in activity levels in raspberry fruit stored for one year at a temperature of –20°C, subject to the studied cultivar. González *et al.* (2003) observed a strong correlation between free radical scavenging capacity and anthocyanin content and total phenolic content (r=0.85 and 0.83 respectively) in four Spanish raspberry cultivars stored for 12 months at a temperature of –24°C, but no correlation was found between this parameter and ellagic acid concentration and vitamin C content.

EFFECT OF THE TECHNOLOGICAL PROCESS OF JUICE PRODUCTION ON CHANGES IN BIOACTIVE COMPONENTS AND ANTIOXIDANT ACTIVITY

Fruit crushing

According to many authors, fruit crushing before juice pressing can have an adverse effect on phenolic acid levels and the fruit's antioxidant properties (Kader *et al.* 1997; Skrede *et al.* 2000; Wilska-Jeszka 2002). Polyphenol oxidase enzymes found in the cytoplasm have a destructive effect by catalyzing the oxidation of phenolic compounds and facilitating the access of oxygen to polyphenols released from cellular structures. Anthocyanins, found in the rind of most shrub berry fruit species, are highly susceptible to oxidation (Wrolstad *et al.* 1994; Versari *et al.* 1997; Meyer 2002; Mikkelsen and Poll 2002; Lee and Wrolstad 2004). Strawberries are one of the few exceptions where anthocyanins are found in the flesh of the fruit, but anthocyanin levels are four times higher in external tissue layers (Gil *et al.* 1997). At the cellular level, anthocyanins are found in vacuoles in the form of different-sized granules, while cell walls contain no anthocyanins. Mechanical or heat-induced damage to the cellular structure leads to the pigmentation of all tissues (Wilska-Jeszka 2002). Anthocyanins are not directly oxidized by polyphenol oxidase. They are oxidized indirectly by quinones synthesized from other phenolic acids. Research results have demonstrated that oxidation is stimulated by, among others, the presence of chlorogenic acid. Those adverse changes may increase the oxidative potential of the environment and lead to the formation of antioxidant-derived pro-oxidants. The most effective method of inhibiting the adverse changes catalyzed by native enzymes is through their inactivation by mash heating (Kader *et al.* 1997, 1999; Skrede *et al.* 2000; Wilska-Jeszka 2002; Grajek 2003).

Mash maceration

In the juice production process, mash maceration before pressing plays an important role in formation of the antioxidant capacity of berry juice (Oszmiański and Sożyński 1989; Czyżowska and Pogorzelski 2002; Bagger-Jørgensen and Meyer 2004; Dietrich *et al.* 2004; Landbo and Meyer 2004; Buchert *et al.* 2005; Szajdek *et al.* 2006). Mash maceration increases the yield of juice pressing, it improves the extractability of bioactive components and enhances the product's antioxidant properties (Helbig 2001; Urlaub 2002; Muñoz *et al.* 2004; Koponen *et al.* 2008; Wang *et al.* 2009). Fruit mash is subjected to thermal and enzymatic processing or a combined processing method involving the application of both heat and enzymes (Oszmiański 2002; Landbo and Meyer 2004; Buchert *et al.* 2005; Szajdek *et al.* 2006, Borowska *et al.* 2009). Heat processing leads to the denaturation of cell membrane proteins and causes their structural loosening, which facilitates the extraction of pigments from the skin to the juice. It removes air from the mash, thus preventing the oxidation of bioactive components, it destroys surface microflora and inactivates enzymes. Thermal processing is believed to deliver the best results at a temperature of 80-90°C. Enzymatic processing and the selective activity of enzymes, mainly pectinolytic enzymes, leads to polysaccharide degradation and decreases the system's viscosity. This, in turn, supports the exudation of juice with dissolved bioactive components. The findings of Buchert *et al.* (2005) indicate a positive linear correlation between the degree of polysaccharide degradation and the quantity of polyphenols released from bilberry and blackcurrant mash to fruit juice.

As noted by Landbo and Meyer (2004), the use of preparations with different enzymatic composition leads to a selective release of bioactive compounds which is responsible for blackcurrant juice's selective capacity of inhibiting LDL oxidation (**Table 3**). Bagger-Jørgensen and Meyer (2004) observed that differences in the extractability of phe-

Table 3 Total phenols yield, anthocyanins yield, ascorbic acid content and antioxidant activity in blackcurrant juices (Landbo and Meyer 2004).

Maceration enzyme	**Total phenols**[a] **Yield**	**Anthocyanins**[a] **Yield**	**Ascorbic acid**[b]	**Antioxidant activity at**[c] **7 µM (min)**	**Antioxidant activity at**[c] **10 µM (min)**
Macer8™ [FJ]	4547	2036	1120	84	>276
Pectinex Superpress	4358	1917	1200	51	>276
Pectinex BE	4344	2188	1188	49	>276
Pectinex Ultra SP-L	4441	2082	1328	42	245
Rapidase BE Super	4392	1966	1120	20	189
Rohapect B5L	4480	2129	1168	25	126
Rapidase EX Color	4394	2038	1372	43	234
Klerzyme Color	4480	1982	1220	22	134
Rapidase Vino Super	4468	2029	1160	34	166
Vinozyme G	4521	2113	1316	55	181

[a] mg/kg berry mash

[b] mg/L

[c] Antioxidant activities of black currant juices toward in vitro human LDL oxidation; antioxidant activities are given as the average net prolongation times of the induction time for the conjugated diene hydroperoxide formation

nolic compounds in the same fruit species treated with different enzymatic preparations could also be due to various types of side activity induced by those preparations. The above authors (2004) also demonstrated that phenolic concentrations in fruit juice are also affected by the degree of fruit crushing, preparation dosage, temperature and time of mash maceration. The above has been validated by Landbo and Meyer (2004) who reported the presence of polyphenols in blackcurrant juice within a wide range of 1340-3220 mg/L, subject to mash maceration conditions. The work of Skrede *et al.* (2000) also points to significant differences in the extractability of various compounds treated with different enzymatic preparations. A high level of anthocyanin extractability from chokeberry mash was reported by Oszmiański and Wojdyło (2005) as a result of combined thermal and enzymatic processing with the use of Rapidase Super BE. According to Oszmiański and Sożyński (1989), the anthocyanin content of juice produced from frozen fruit was around 1.6-fold lower than that of fresh fruit juice. The above could be due to native enzymes' destructive effect on anthocyanins in fruit which was not thermally processed prior to freezing. The above results validate the earlier findings of Rosa and Krugły (1987).

Thermal processing is recommended for fruit with a low pectin content, such as bilberry, while enzymatic processing delivers optimal results in pectin-abundant fruit, such as blackcurrant and blackberry (Oszmiański 2002; Landbo and Meyer 2004; Szajdek et al. 2006). Yet in some cases, the activity of the accompanying enzymes may destroy bioactive substances, lower their antioxidant capacity and lead to undesirable changes in color and flavor (Wrolstad *et al.* 1994; Wightman and Wrolstad 1996; Versari *et al.* 1997; Buchert *et al.* 2005) The presence of β-galactosidase or β-glucosidase in some preparations may lead to anthocyanin hydrolysis and the formation of aglycones which are easily converted to brown-colored polymers or colorless compounds (Wrolstad *et al.* 1994; Versari *et al.* 1997). Maceration time is an important consideration. According to Wightman and Wrolstad (1996), strawberry mash maceration with the use of commercial enzymatic preparations for more than 2 hours lowered the anthocyanin content of juice. The above authors claim that this reduction was due to the fact that the applied preparations contained β-glucosidase which has an antagonistic effect on cyanidin-3-glucoside. Similar observations were made by Versari *et al.* (1997) as regards raspberry juice. When maceration time was extended to 4 and 6 hours with the use of Pectinex BE 3-L, Rohapect B1L, Rohament MAX and Pectinex 3XL preparations, an estimated 20% reduction in anthocyanin levels was reported in comparison with control. The presence of β-glucosidase, β-galactosidase and β-arabinosidase in enzymatic preparations was also noted by Buchert *et al.* (2005). Anthocyanin hydrolysis can be avoided by strictly adhering to preparation dosages and reaction times recommended by the manufacturers (Wightman and Wrolstad 1996). According to Skrede *et al.* (2000), the fruit's endogenous enzymes also contribute to anthocyanin destruction. The above has been validated by Lee *et al.* (2002) and Rossi *et al.* (2003) who steam-blanched highbush blueberry fruit before crushing. This processing method inactivated endogenous polyphenol oxidase, and the anthocyanin concentrations of the resulting juice were approximately two-fold higher. According to Płocharski and Markowski (2003), the maceration of blackcurrant mash with a combination of Pectinex BE XXL and Pectinex BE Color preparations (1:1) contributed to the extractability of anthocyanins, while previous thermal processing treatment did not produce such results.

The choice of the right enzymatic preparation is difficult, especially in view of the wide selection and availability of such products on the market. Recent years have witnessed the introduction of new generation pectinases produced by genetically modified microorganisms and showing one or two types of enzymatic activity. Those pectinases comprise mainly pectin lyase or pectinesterase plus polygalacturonase without side activity. Single, pure enzymes extracted from modified strains are sometimes added to enhance traditional pectinases (Grassin *et al.* 2005). Enzymatic mash maceration is generally more widely recognized than thermal processing.

Despite numerous research attempts to optimize the production of fruit juice showing high antioxidant properties, large quantities of bioactive substances remain in press residues (Landbo and Meyer 2001; Meyer 2005; Oszmiański and Wojdyło 2005). Owing to its high anthocyanin concentrations, fruit pomace is used in the production of dyes and pharmaceuticals (Bridle and Timberlake 1997; Meyer 2002; Muñoz *et al.* 2004).

Juice preservation

Conventional juice preservation methods, such as pasteurization, guarantee product safety and a long shelf-life, but they lead to changes in the product's sensory properties, bioactive substance content and antioxidant activity (**Table 4**). The above is largely due to the profile of phenolic compounds and to the presence of other substances, such as sugars, ascorbic acid, oxygen and metals. The conditions of particular operations, such as mash maceration prior to juice pressing, are also an important consideration (Oszmiański 2002; Dietrich *et al.* 2004, Oszmiański and Wojdyło 2005; Szajdek *et al.* 2006). The findings of Dietrich *et al.* (2004) indicate that the pasteurization of blackcurrant juice (85°C, 30 s) lowers its antioxidant capacity (TEAC) by 11%. The reduction in phenolic and ascorbic acid concentrations reached 20 and 10%, respectively. The author noted that during pasteurization, phenolic acids may contribute to the formation of new structures with antioxidant properties. This process may also result in the development of Maillard reaction products which enhance the antioxidant capacity of fruit juice. Walkowiak–Tomczak (2007) observed a correlation between the pH of black currant juice and changes in

Table 4 Total phenols content, anthocyanins content and DPPH radical scavenging activity in berry fruit juices (Szajdek *et al.* 2006).

Juices	**Total phenols**[a]		**Anthocyanins**[a]		**$DPPH^{\bullet}$ scavenging activity**[b]	
	Nonpasteurized	**Pasteurized**	**Nonpasteurized**	**Pasteurized**	**Nonpasteurized**	**Pasteurized**
Bilberry	1237	852	694	416	6.54	6.33
Black currant	2624	3011	1327	882	19.84	18.47
Chokeberry	1926	2052	1457	951	22.37	20.21
Cranberry	1040	1061	137.7	87.2	10.73	10.35
Strawberry	504	368	255.2	81.2	4.06	4.02

[a] mg/L

[b] μmol Trolox/mL juice

anthocyanins and TEAC during pasteurization. According to the author, the optimum pH level is 3. The work of Szajdek *et al.* (2006) gives supporting evidence to changes in concentration levels of bioactive compounds and a decrease in $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical scavenging activity in the juice of chokeberry, bilberry, blackcurrant, strawberry and cranberry fruit as a result of pasteurization (**Table 4**). The authors also noted that the degree of the said changes varied subject to fruit species and the method of mash maceration prior to juice pressing. The least stable anthocyanins were found in strawberry and cranberry juice (their concentrations decreased 2- to 4-fold after pasteurization). Pasteurized strawberry juice was also characterized by the lowest $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical scavenging capacity. According to Skrede *et al.* (2000), delphinidin glycoside was the most thermolabile substance, cyanidin and petunidin derivatives were more resistant to heat, while malvidin glycoside was marked by the highest stability. The hydrolysis of the anthocyanidin–sugar bond opens the ring and leads to the formation of chalcone or α-diketone, a colorless or light yellow compound. Alternatively, the process of oxidative polymerization is responsible for the brown coloring of products. Factors that speed up those adverse changes during pasteurization include metal ions (Cu^{2+}) and ascorbic acid which may form colorless copigments with anthocyanins (Stasiak *et al.* 1998). According to Oszmiański and Sożyński (1989), a significant loss of anthocyanins may follow from the depectinization of the fruit mash which leads to aglycone formation. Aglycones are less stable and are more likely to undergo undesirable change.

Recent years have witnessed the advent of modern juice processing methods which preserve the attributes of the raw material, among them non-thermal methods such as high pressure processing (HPP), pulsed electric fields (PEF), high-intensity pulsed electric fields (HIPEF) and high-frequently ultrasound (2000 cycles/s). The HPP method is used on an industrial scale in Japan and the USA. The European Commission has included products preserved by HPP in the novel foods group (Oszmiański 2008; Odriozola-Serrano *et al.* 2009). There is scant published research that looks into the effect of those methods on antioxidant capacity. The findings of Odriozola-Serrano *et al.* (2009) indicate that HIPEF-treated strawberry juice maintained higher amounts of phenolic acids (ellagic and *p*-coumaric acid) and total anthocyanins than thermally treated juices. Regarding the antioxidant capacity, similar $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ values were obtained so that differences among pasteurized juices were not significant. HIPEF processing may be as effective as thermal treatment not only to achieve safe and stable juices, but also to obtain juices with a high content of antioxidant compounds.

JUICE STORAGE

Bioactive components undergo changes during juice storage, and this process alters the products antioxidant capacity. The stability and direction of changes in phenolic compounds are determined primarily by their qualitative and quantitative profile in juice as well as by pH, temperature, period of storage, light access, and other factors (Garzón and Wrolstad 2002; Rein and Heinonen 2004; Walkowiak-Tomczak 2007). The least stable substances are strawberry anthocyanins, in particular the predominant pelargonidin 3-glucoside (around 77% share) (Garzón and Wrolstad 2002; Proteggente *et al.* 2002). Rein and Heinonen (2004) found that the stability of anthocyanins in stored strawberry juice can be improved through the addition of phenolic acids. These authors demonstrated that sinapic acid has a beneficial influence on anthocyanin stability in strawberry juice, while sinapic and ferulic acids improve anthocyanin stability in raspberry juice. Sinapic and ferulic acids formed new intramolecular copigmentation molecules with strawberry and raspberry anthocyanins, which were detected as novel peaks in the HPLC chromatograms. Presumably, the new molecules are a result of covalent bonding between an anthocyanin and a phenolic acid since they endure the acidic HPLC conditions. Rosmarinic acid obviously stabilized lingonberry and cranberry anthocyanins via intermolecular copigmentation reactions since their color was stabilized and the diminishing of anthocyanins was reduced, but no new anthocyanin compounds were detected with HPLC. According to Piljac-Žegarac *et al.* (2009), changes in the polyphenol content of six dark fruit juices, determined with the use of the Folin-Ciocalteu reagent, were negligible after 29 days of cold storage (4°C). The authors noted much more pronounced changes in vitamin C concentrations and $DPPH^{\bullet}$ radical scavenging capacity. The highest decrease in this activity was reported in cherry and strawberry juice. According to Walkowiak-Tomczak (2007), an increase in the storage temperature of chokeberry juice from 10 to 30°C over a period of 30 days decreased the $ABTS^{\bullet+}$ radical scavenging capacity by 7 and 35%, respectively. The author also observed that the pH of juice significantly contributed to changes in antioxidant capacity, and the optimum pH level was 3. Juice with lower acidity was characterized by a lower level of anthocyanin stability and a greater reduction in antioxidant activity.

CONCLUSIONS

Berry fruit juices are characterized by strong antioxidant properties and constitute a rich source of natural antioxidants, among which particular attention should be paid to phenolic compounds. Antioxidant capacity is also affected by vitamin C content, though to a lesser degree. According to the findings of numerous authors, antioxidant capacity may be increased before juice pressing, at the stage of fruit mash maceration with the use of "new generation" enzymatic preparations obtained from genetically modified microorganisms. Modern approaches to juice preservation, such as HPP, PEF, HIPEF and high-frequency ultrasound treatment, also seem promising.

Many international studies have led to the conclusion that the health benefits of fruit juices, including their antioxidant properties, may by improved in the course of the optimization process comprising, in particular, proper selection of fruit varieties and control over the parameters of unit operations during processing, as well as over preservation and storage conditions.

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