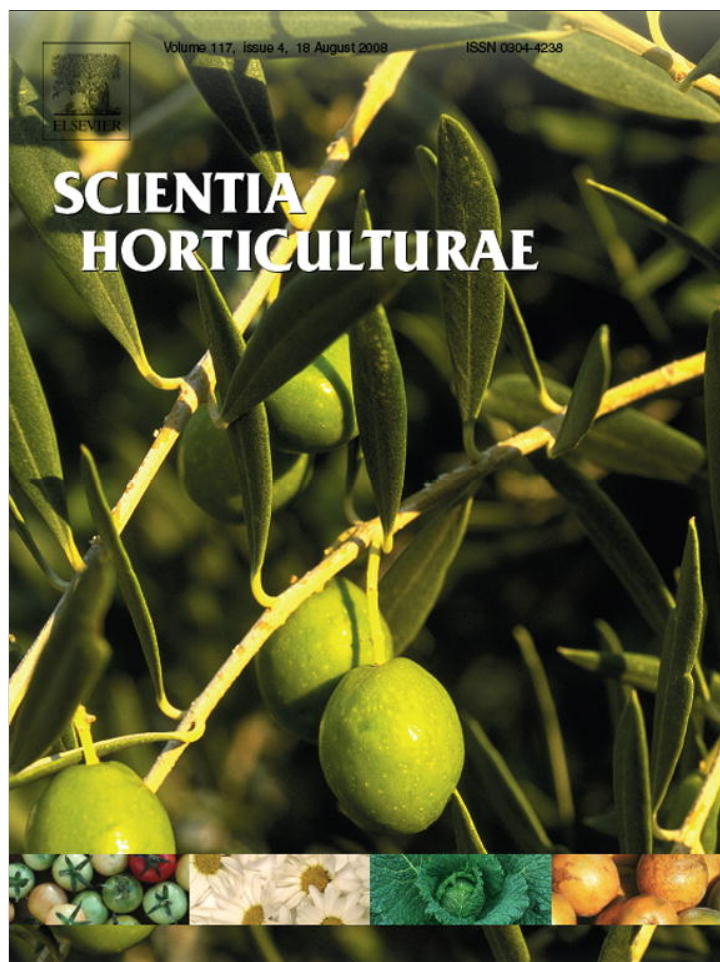


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Phytochemical accumulation and antioxidant capacity at four maturity stages of cranberry fruit

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ABSTRACT

Specific components of cranberry fruit are being associated with human health attributes, such as maintenance of urinary tract health and antioxidant status. Some of the chemical properties and antioxidant capacity of cranberry (*Vaccinium macrocarpon* Ait. cv. Pilgrim) fruits were investigated at light green, blush, light red and dark red maturation stages. Fruit total phenolics, total monomeric anthocyanins, soluble solids, titratable acidity and individual organic acids were examined. Antioxidant capacity of fruits were determined by both the ferric reducing antioxidant power (FRAP) and trolox equivalent antioxidant capacity (TEAC) assays. The fruit color was measured using a portable chromameter. A converse relationship was found between total phenolics and anthocyanin as fruits mature. Total phenolic concentration was declined from 7990 to 4745 mg GAE/kg fw, while total monomeric anthocyanin content was increased from 0.8 to 111.0 mg/kg fw from green to dark red stage. Brix was increased from 6 to 9.3% as well. The main organic acid was citric acid determined by the HPLC method. The antioxidant capacity of cranberries decreased to light red stage; when a fruit accumulates more anthocyanin the activity increased again in both FRAP and TEAC methods. Averaged antioxidant capacity measured was 12.61 and 17.48 mmol TE/kg fw by FRAP and TEAC methods.

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1. Introduction

The cultivated cranberry, *Vaccinium macrocarpon* Ait., is a member of the *Ericaceae* family, evergreen, creeping shrubs native to cool temperate, acidic soils and peat wetlands of the north-eastern US and southern Canada (Eck, 1990; Roper and Vorsa, 1997). US and Canada is produce more than 90% of total world production. Latvia, Belarus, Azarbaijan and Ukraine are other producer countries from Europe (FAO, 2004). Due to low soil PH requirement its production areas are limited in Europe. The Black Sea region of northern Turkey has cool climates and acidic soils. Lately, cranberry plantations have been established as a result of alternative fruit crops search for this region.

Cranberry contains high levels of phytochemicals which have health promoting properties (Leahy et al., 2002; Yan et al., 2002; He and Liu, 2006; Neto, 2007a,b). Some of these phytochemicals, which act as antioxidants, are increasingly being shown to help to

optimize human health by neutralizing harmful free radicals in the body. These antioxidants reduce oxidative damage by cells that can lead to cancer, heart disease, and other degenerative diseases. For example anthocyanin, compounds that give cranberries their red color are powerful antioxidants (Prior, 2003; Zafra-Stone et al., 2007). Laboratory studies have shown that cranberry extract reduces oxidation of LDL-cholesterol which may be important in maintaining a healthy heart (Chu and Liu, 2005). In addition, current research suggests that cranberries contain proanthocyanidins that can prevent the adhesion of certain bacteria, including *E. coli* which is associated with urinary tract infections (Howell, 2002; Griffiths, 2003). These anti-adhesion properties may explain why cranberry is used to prevent urinary tract infections, stomach ulcers and gum disease.

The antioxidant properties of cranberries are documented in the literature and cranberries are ranked one of the highest antioxidant activities among many other fruits (Sun et al., 2002). Effects of cultivar and storage temperatures on the antioxidant capacity of cranberries were also investigated (Wang and Stretch, 2001). Vvedenskaya and Vorsa (2004) studied the change of flavonoid composition over fruit development and maturation in

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cranberries. Most of the current literature is focused on antioxidant properties of mature red cranberries. However, recently immature white cranberries are also being used in juice industry. The aim of this study was to determine phytochemical accumulation and antioxidant capacity of cranberries at four different maturity stages. FRAP and TEAC assays suggested by Ozgen et al. (2006) were used to determine antioxidant capacity of cranberries. In addition, this is the first study from Turkey to report commercial cranberry production.

2. Materials and methods

2.1. Plant material

Cranberry (cv. Pilgrim) fruits were harvested from a commercial site established in Hayrat, Trabzon, Turkey. Fruits were hand harvested approximately in 15 days intervals to get four different maturity stages (light green, blush, light red and dark red) starting the beginning of July to end of August 2007. Fruits were transferred to laboratory for physical and phytochemical analysis. The fruit color was measured using a Minolta portable chromameter (Minolta, Model CR-400) which provided CIE L^* , a^* and b^* values. Chroma and h° values were also calculated according to McGuire, 1992. Then, about 100 g fruits with three replications for each maturity stage were frozen at -20°C . Cranberry fruits from each maturity stages were thawed at room temperature and then homogenized in a food processor. Slurries were used to determine total soluble solid (TSS) contents by refractometry (Atago, Pal-1) and titratable acidity (TA) using standard methodology.

2.2. Analytical procedures

2.2.1. Determination of total phenolic (TP)

TP content was measured according to Singleton and Rossi (1965) procedure. Fruit slurries were extracted with buffer containing acetone, water, and acetic acid (70:29.5:0.5, v/v/v) for 1 h in darkness. Each of the four maturity stages was replicated three times. Then, extract, Folin-Ciocalteu's phenol reagent and water was added and it was incubated for 8 min followed by adding 7% sodium carbonate. After 2 h, the absorbance was measured by an automated UV–vis spectrophotometer (Model T60U, PG Instruments) at 750 nm. Gallic acid was used as standard. The results were expressed as mg gallic acid equivalent in kg fresh weight basis (GAE/kg fw).

2.2.2. Total monomeric anthocyanins (TMA)

TMA were estimated by a pH differential method (Wrolstad, 1976; Giusti et al., 1999) using a UV–vis spectrophotometer (model T60U, PG Instruments). Absorbance was measured at 535 and 700 nm in pH 1.0 and 4.5 buffers using $A = (A_{535} - A_{700})_{\text{pH}1.0} - (A_{535} - A_{700})_{\text{pH}4.5}$ with a molar extinction coefficient of 30,900. Results were expressed as mg of cyanidin-3-galactoside equivalent in kg fresh weight basis.

2.2.3. The total antioxidant activity (TAA)

TAA was estimated by two standard procedures FRAP and TEAC assays as suggested by Ozgen et al. (2006).

2.2.3.1. The ferric reducing ability of plasma (FRAP). FRAP was determined according to the method of Benzie and Strain, 1996. Assay was conducted using three aqueous stock solutions containing 0.1 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ [2,4,6-tris(2-pyridyl)-1,3,5-triazine] acidified with concentrated hydrochloric acid (1000:3.3, v/v/v), and 20 mmol/L ferric chloride. These solutions were prepared and stored in darkness under

refrigeration. Stock solutions were combined (10:1:1 v/v/v) to get the FRAP reagent just prior to analysis. For each assay laboratory duplicate, 2.97 mL of FRAP reagent and 30 μL of sample extract were mixed. After 10 min, the absorbance of the reaction mixture was determined at 593 nm on a UV–vis spectrophotometer (Model T60U, PG Instruments).

2.2.3.2. Trolox equivalent antioxidant capacity (TEAC). For the standard TEAC assay, ABTS was dissolved in acetate buffer and prepared with potassium persulfate as described in Ozgen et al. (2006). The mixture was diluted in acidic medium of 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.700 ± 0.01 at 734 nm for longer stability (Ozgen et al., 2006). For the spectrophotometric assay, 3 mL of the ABTS⁺ solution and 20 μL of fruit extract were mixed and incubated in 10 min and the absorbance was determined at 734 nm by a UV–vis spectrophotometer (Model T60U, PG Instruments).

2.2.3.3. Extraction of organic acids. Fruit slurries (5 g) were diluted with meta-phosphoric acid (2.5%) solution for individual organic acid analysis. The homogenate was centrifuged at 6000 rpm for 5 min. Supernatants were filtered through a 0.45- μm membrane filter (Iwaki Glass) before HPLC analysis, and the mobile phase solvents were degassed before use.

2.2.3.4. Chromatographic conditions. The HPLC analyses were carried out using a PerkinElmer HPLC system with Totalchrom navigator 6.2.1 software, a pump and UV detector (PerkinElmer, Series-200) (Waltham, MA, USA). Separation and determination of organic acids modified by Shui and Leong (2002) were used. The separation was carried out on a SGE wakosil C18RS 5 μm column (250 mm \times 4.6 mm i.d.). Detection was performed at 215 nm. Optimum efficiency of separation was obtained using pH 2.5 sulfuric acid solution (solvent A), and methanol (solvent B). Other parameters adopted were as follows: injection volume, 20 μL ; column temperature, 30 C; detection wavelength, 215 nm.

2.3. Statistical analysis

Data were analyzed using SAS software and procedures (SAS, Cary, NC, USA). Means and standard deviations were calculated using PROC TABULATE. Analysis of variance tables were constructed using PROC GLM procedure. The means were separated using the Tukey's Studentized Range (HSD) Test at 0.05 significance level.

3. Results and discussion

As expected with natural ripening of cranberries as a result of chlorophyll breakdown and anthocyanin accumulation (Palta and Stang, 1983) a , b and h° values dramatically changed (Table 1) which determines the relationship between fruit color and maturation for each stage. P values showed that all variables were significantly different among the fruit maturation stages. The mean separations indicated that all stages were statistically different except light red and dark red stages of a and Chroma. L values, designating the lightness of the color, gradually decreased as fruit matures. These results are in agreement with expectations as the fruits accumulate more wax with maturation (Ozgen et al., 2002). The results of b values indicating color change from yellow to blue were similar to those of L . Similar patterns were also observed for h° . a values indicate color turn from green to red. The mean separations pointed out that the stages could be grouped into three sections: green, blush, light and dark red. Although the mean of a values for dark red was higher than that of light red, this

Table 1
Means and significances for color parameters of cranberry fruits sampled from for maturation stages

Stage	L	a	b	Chroma	Hue
Green	68.9 a	−13.3 c	32.5 a	35.1 a	112.2 a
Blush	58.7 b	3.9 b	24.2 b	25.0 b	80.4 b
Light red	42.4 c	24.7 a	17.4 c	30.3 ab	35.6 c
Dark red	27.1 d	28.4 a	10.7 d	30.8 ab	20.4 d
Mean	49.3	10.9	21.2	30.2	62.1
Significance					
P value	**	**	**	**	**
HSD _{0,05}	6.5	7.4	3.5	5.3	79.8

Means in each column not followed by the same letter are significantly different at $P < 0.05$ according to the Tukey's Studentized Range (HSD) Test; **Significant at 0.01.

difference was not supported statistically. Taken together, these results indicated that the sampling of fruit stages were adequate.

The berry size progressively increased during the fruit maturation (Table 2). Similarly, the Brix values increased from 6.0 to 9.3% (Table 2). Both variables were significantly different.

Total phenolic and total anthocyanin contents of each maturation stages were statistically different (Table 2). However, the variables had converse relationship. During maturation, from green to dark red stage, the total phenolic content was decreased from 7990 to 4745 mg GAE/kg fw, while total monomeric anthocyanin content was increased from 0.8 to 111.0 mg/kg fw. Total phenolic content of cranberries found in mature red berries by Sun et al. (2002) was about 5200 mg GAE/kg fw, one of the highest content among the fruits. This is inline with the data presented and it is not surprising to observe the decline in the amount of total phenolic and rise in anthocyanin content as a typical maturation process. However, the difference of total phenolic was almost 70% between green and dark red stage. On the other hand, most of the anthocyanin was accumulated in the dark red stage.

The most abundant organic acid was citric (73%) followed by malic and ascorbic acid. Overall citric and malic acid concentration increased; ascorbic acid concentration decreased as fruit matures (Table 2). All contents were statistically significant; however, they had different trends; malic acid had the highest content in mature stages, light red and dark red. However, citric acid contents were highest in blush and dark red stages. Conversely, ascorbic acid concentrations were highest in green stages. When total acidity was considered an overall increase pattern was detected from green to dark red stages from 1.50 to 2.04 g/100 mL.

The results of antioxidant capacities (Fig. 1) showed that FRAP results were 38% higher than TEAC. However, the determination

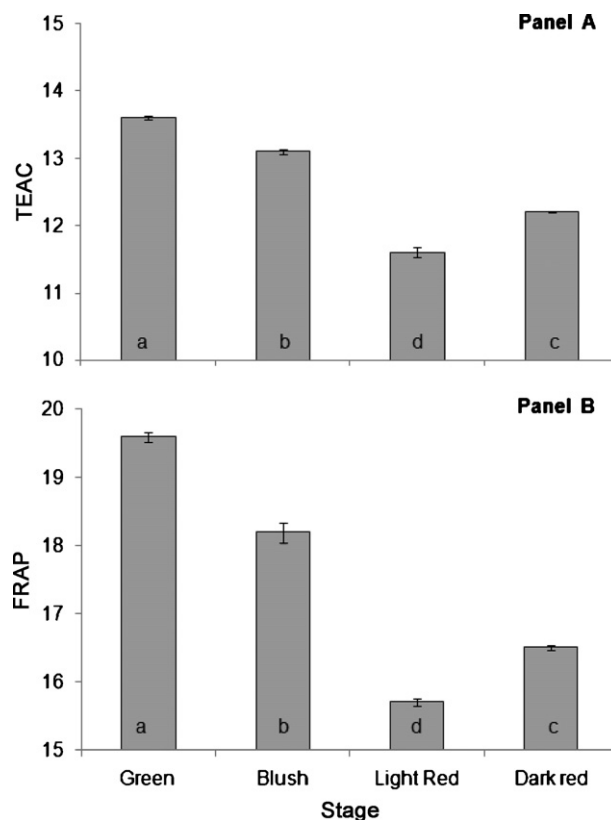


Fig. 1. Total antioxidant capacities of the cranberry fruits sampled from four maturation stages. TEAC values (Panel A) were determined by the method of Ozgen et al. (2006) and FRAP values (Panel B) were determined by the method of Benzie and Strain (1996). The values are expressed as mmol of trolox equivalents/kg fw. The means were calculated from three replicates and the bars indicate standard deviations. The means were separated by Tukey's Studentized Range (HSD) Test.

methods were significantly correlated ($r^2 = 0.98$; $P < 0.001$). Similar trends were present in both methods where the stages had significant difference. The highest antioxidant capacities were recovered from green stage followed by blush. The lowest antioxidant capacities were determined at light red. After the light red, the antioxidant capacities increased again; however, this increase did not reach level of blush stage. The antioxidant activity in cranberries is mostly due to phenolic compounds and anthocyanin-anthocyanidins (Sun et al., 2002). In both FRAP and TEAC methods antioxidant capacity starts to increase when the fruit accumulates more anthocyanins. Averaged antioxidant

Table 2
Mean and significances for several pomological and chemical parameters of cranberry fruits sampled from four maturation stages

Stage	Berry size (g/100 berries)	Brix (%)	Total phenolics content (TP) ^a	Total anthocyanin content (TMA) ^b	Organic acids content (g/100 mL)			
					Malic	Ascorbic	Citric	Total
Green	67.3 c	6.0 d	7990 a	0.8 c	0.22 b	0.21 a	1.07 c	1.50 c
Blush	87.6 b	6.8 c	6853 b	6.6 c	0.18 b	0.13 b	1.39 a	1.71 b
Light red	135.3 a	8.7 b	4901 c	50.2 b	0.53 a	0.06 d	1.22 b	1.81 b
Dark red	141.8 a	9.3 a	4745 c	111.0 a	0.54 a	0.07 c	1.43 a	2.04 a
Mean	108	7.7	6122	42.2	0.37	0.12	1.28	1.76
Significance								
P value	**	**	**	**	**	**	**	**
HSD _{0,05}	11.8	0.4	218	7.6	0.04	0.01	0.09	0.14

Means in each column not followed by the same letter are significantly different at $P < 0.05$ according to the Tukey's Studentized Range (HSD) Test.

^a TP were estimated by the Folin-Ciocalteu assay of Singleton and Rossi (1965). Values are expressed in mg gallic acid equivalents (GAE)/kg fw.

^b TMA were determined by the pH-differential method of Giusti et al. (1999). Values are expressed as mg cyanidin 3-galactoside equivalents/kg fw.

capacity measured was 12.61 and 17.48 mmol TE/kg fw by FRAP and TEAC methods.

4. Conclusions

The results clearly demonstrated that some of the chemical properties and antioxidant capacities of cranberry fruits are affected by maturation stages. Significant variability was found for overall total phenolic, anthocyanin, organic acids and antioxidant capacity of four maturation stages. Total phenolic and anthocyanins content affected the antioxidant capacity. Green stage of cranberry fruits had the highest antioxidant capacity. However, more studies are needed to explore specific details of this trend.

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