Changes in Antioxidant Effects and Their Relationship to Phytonutrients in Fruits of Sea Buckthorn (*Hippophae rhamnoides* L.) during Maturation

Xiangqun Gao,* Maria Ohlander, Niklas Jeppsson, Lars Björk, and Viktor Trajkovski

Phytochemical Center, Balsgård, Swedish University of Agricultural Sciences, Fjalkestadsv. 123-1, S-291 94 Kristianstad, Sweden

Different fractions of sea buckthorn fruits were investigated for antioxidant activity and its relationship to different phytonutrients. Capacity to scavenge radicals of the crude extract, like the phenolic and ascorbate extracts, decreased significantly with increased maturation. The changes were strongly correlated with the content of total phenolics and ascorbic acid. Antioxidant capacity of the lipophilic extract increased significantly and corresponded to the increase in total carotenoids. The phenolic fractions made a major contribution to the total antioxidant capacity due to the high content of total phenolics. The lipophilic fractions were most effective if the comparison was based on the ratio between antioxidant capacity and content of antioxidants. The crude extract of fruits showed the highest inhibitory effect in both 2,2-azobis(2,4-dimethylvaleronitrile) (AMVN) and ascorbate−iron induced lipid peroxidations. The aqueous and ascorbate-free extracts showed higher inhibition in the AMVN assay, but lower inhibition in ascorbate-iron induced peroxidation, than the lipophilic extract.

Keywords: *Sea buckthorn; Hippophae rhamnoides; antioxidant effects; phytonutrients; maturation*

INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides* L.; Elaeagnaceae) is a temperate bush and is native to Europe and Asia (Rousi, 1971). It was used as a medicinal plant in Tibet as early as 900 A.D. (Lu, 1992). Since the 1950s, many medicinal preparations of sea buckthorn, from both wild and cultivated sources, have been clinically used to treat radiation damage, burns, oral inflammation, and gastric ulcers in China and the former Soviet Republics (Lebedeva et al., 1989; Jiang et al., 1989; Fu et al., 1993).

In addition to the medicinal use, the berries of sea buckthorn are able to be processed to make juice and jam, or to be used for flavoring of dairy products because of the unique taste of sea buckthorn berries. The strong association between increased fruit and vegetable intake and cancer and heart disease prevention has been explained by the content of antioxidant phytonutrients (Halliwell, 1997). Besides the commonly mentioned antioxidants (vitamin C, tocopherols, and carotenoids) flavonoids can also act as cancer preventing nutrients (Halliwell, 1997) and contribute significantly to the antioxidative activity of the diet (Rice-Evans et al., 1997).

Recently, a number of studies have investigated the relationship between antioxidant activity and phytonutrients from different fruits (Plumb et al., 1996; Heinonen et al., 1998; Prior et al., 1998). However, all of the studies concentrated on the phenolic substances in hydrophilic oxidation models. Compared to the fruits studied, the berries of sea buckthorn have a much higher content of ascorbic acid (100−700 mg/100 g), tocopherols (1−10 mg/100 g), and carotenoids (3−15 mg/100 g) (Yao and Tigerstedt, 1992; Lu, 1992; Fu et al., 1993). These components cannot be ignored in any investigation of sea buckthorn.

Previously, sea buckthorn has been studied on the basis of its nutrient content (Rousi, 1971; Yao and Tigerstedt, 1992). There is little information available about the relationship between antioxidant effects and the different active compounds in the berries of sea buckthorn.

It is well-known that the content of phytonutrients depends on both genetic and environmental factors. The content and alteration of bioactive compounds need to be considered not only in the plant breeding process but also in the evaluation of cultivation techniques as well as in the choice of harvest date.

The purpose of the present study was to investigate the change in antioxidant effects and different phytonutrients during maturation.

MATERIALS AND METHODS

Plant Material. Berries of three cultivars of *Hippophae rhamnoides*, Botanitjetskaja, Trofimovskaja, and Aromatnaja, were collected from an experimental field of our department in southern Sweden (56′07″N, 14′10″E). Fruit samples were collected on 6th, 11th, 14th, 18th, 21st, and 25th of August 1997 and stored at −20 °C until analysis. The sampling period was estimated to be a reasonable time range for commercial harvesting; before the 6th of August the berries were unpalatable and after the 25th of August the berries started to be difficult to harvest due to loss in firmness.

All chemicals were of the highest quality available and were purchased from Sigma (USA) and Merck (Germany).

Sample Preparation. Ten grams of berries from each collection were randomly selected and extracted according to the scheme depicted in Figure 1. Triple extractions were prepared from each source of sea buckthorn for the assays. The concentration of extracts used in the assays was 20 mg of

* To whom correspondence should be addressed. Fax: 46 46 755 30. E-mail: xiangqun.gao@hvf.slu.se.

10.1021/jf991072g CCC: $19.00 © 2000 American Chemical Society
Published on Web 04/14/2000

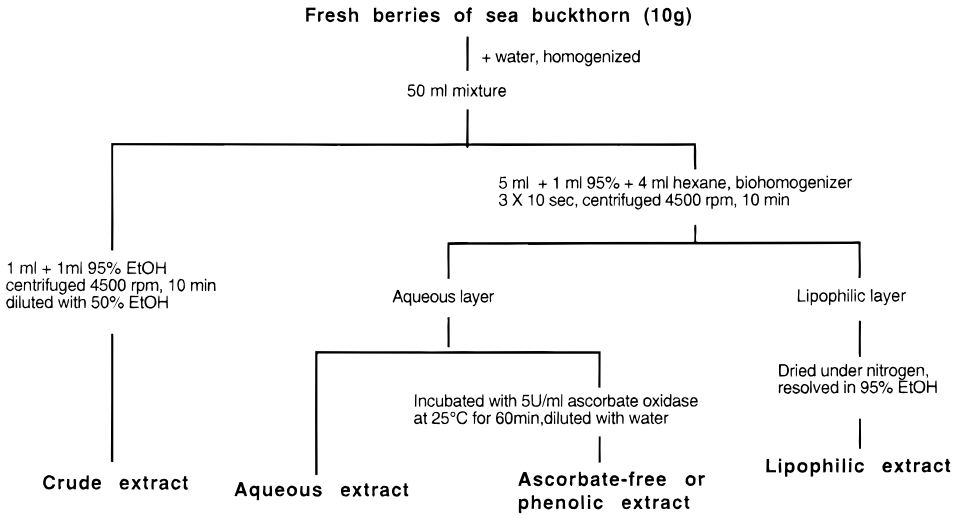

Figure 1. Scheme of the sample preparation of different extracts from sea buckthorn berries.

fresh berries/mL for the free-radical scavenging assay, determination of ascorbate, total phenolics, and carotenoids; 5 mg of fresh berries/mL for AMVN-induced lipid peroxidation; 2 mg of fresh berries/mL for ascorbate−iron induced lipid peroxidation.

Free-Radical Scavenging Assay. ABTS stock solution was prepared by mixing 5 mL of 7 mM 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) with 88 μL of 140 mM $K_2S_2O_8$. The stock solution was diluted with 95% EtOH to give an absorbance at 734 nm of 0.7 ± 0.05 (Pellegrini et al., 1999). Extracts of sea buckthorn (100 μL of 20 mg/mL) were mixed with 1 mL of ABTS reagent and the absorbance was measured at 734 nm after 30 min incubation at room temperature. The absorbance difference between aqueous and phenolic (ascorbate-free) extracts corresponded to the antioxidant capacity of ascorbic acid. Trolox was used as a standard and the free-radical scavenging capacity was expressed as μmol/g trolox equivalent antioxidant capacity (TEAC).

Ascorbate−Iron Induced Lipid Peroxidation. The assay was performed as described by Aruoma et al. (1997). Essentially, bovine brain extract (Sigma, B-1627, 50 mg) was mixed with 10 mL of phosphate-buffered saline (PBS, pH 7.4), and sonicated in an ice bath until a milk-like suspension was obtained containing phospholipid liposomes. The liposomes (0.2 mL) were added with 0.5 mL of PBS buffer, 0.1 mL of 1 mM $FeC1_3$, and 0.1 mL of sea buckthorn extract (2 mg/mL) in a test tube. The peroxidation was initiated by adding 0.1 mL of 1 mM ascorbate. The mixture was incubated at 37 °C for 60 min. After incubation, 50 μL of 2% butylated hydroxytoluene (BHT) in 95% EtOH was added to each tube followed by 1 mL of 2.8% (w/v) trichloroacetic acid (TCA) and 1 mL of 1% (w/v) 2-thiobarbituric acid (TBA). The solutions were heated in a water bath at 80 °C for 20 min. The TBA-MDA chromagen was extracted into 2 mL of 1-butanol, and the extent of peroxidation was measured in the organic layer using the absorbance at 532 nm.

AMVN-Induced Lipid Peroxidation. AMVN-initiated lipid peroxidation was performed as descrided by Terao et al. (1992). Methyl linoleate solution (100 μL of 2 mg/mL in 80% EtOH, 0.2 mM EDTA) was mixed with 50 μL of extract (5 mg/mL in 50% EtOH), and then 50 μL of 10 mM 2,2-azobis (2,4-dimethyvaleronitrile) (AMVN) in 95% EtOH was added to initiate peroxidation. After 60 min incubation at 37 °C, the mixture was combined with 1.5 mL of FOX2 reagent (containing 250 μM ammonium ferrous sulfate, 100 μM xylenol orange, 25 mM H_2SO_4, and 4 mM BHT in 90% MeOH; Nourooz-Zadel et al., 1994), and the content of hydroperoxides was measured at 560 nm.

Determination of Ascorbic Acid. Equal volumes (100 μL) of aqueous and phenolic extracts (20 mg/mL) were mixed with 0.9 mL of ferric-TPTZ reagent (prepared by mixing 300 mM acetate buffer, pH 3.6; 10 mM of 2,4,6-tripyridyl-*s*-triazine in 40 mM HCI; and 20 nmM $FeCI_3$ in the ratio of 10:1:1; Benzie & Strain, 1999) and the absorbance measured at 593 nm. The absorbance difference between aqueous and phenolic extracts was used as an indicator of the content of ascorbic acid. L-Ascorbic acid was used as a standard.

Determination of Total Phenolics. Phenolic extract (100 μL of 20 mg/mL) was mixed with 0.2 mL Folin−Ciocalteu reagent (Sigma), 2 mL of H_2O, and 1 mL of 15% Na_2CO_3, and the absorbance was measured at 765 nm after 2 h incubation at room temperature. Gallic acid was used as a standard and the total phenolics were expressed as mg/100 g gallic acid equivalents (GAE).

Determination of Total Carotenoids. A 1 mL aliquot from the lipophilic layer of the homogenized mixture (0.1 g/ml) was added to 0.5 mL of 5% NaCl, vortexed for 30 s, and centrifuged for 10 min at 4500 rpm. The supernatant (100 μL) was diluted with 0.9 mL of hexane and measured at 460 nm. β-Carotene was used as a standard and total carotenoids were expressed as mg/100 g β-carotene equivalents.

Statistical analysis of the results was carried out with SYSTAT v. 5.2.1 for Macintosh. Linear regression was used to analyze the change in antioxidants and antioxidative activity during maturation. The slope of change was expressed as coefficient of regression and significance was calculated by the p value.

RESULTS

Free-Radical Scavenging and Content of Phytonutrients. The free-radical scavenging capacity of ascorbic acid fractions decreased significantly during maturation in all three cultivars. The capacity decreased from 6.60 to 5.18 μmol TEAC/g for Botanitjetskaja, 7.02 to 4.16 μmol TEAC/g for Trofimovskaja, and 6.69 to 4.54 μmol TEAC/g for Aromatnaja. An analysis of linear regression showed that the overall change in free-radical scavenging capacity of the three cultivars was also significant (Table 1).

Corresponding to the change in the capacity to scavenge free radicals, the ascorbic acid content showed a significant reduction. Ascorbate declined from 110.3 to 93.1 mg/100 g for Botanitjetskaja (−15.5%), 105.2 to 65.2 mg/100 g for Trofimovskaja (−38.0%), and 106.2 to 69.3 mg/100 g for Aromatnaja (−34.7%).

The capacity to scavenge free radicals of the ascorbic acid fraction and the content of ascorbic acid was highly correlated, $r = 0.91$ ($p < 0.01$) in Botanitjetskaja, 0.99 ($p < 0.01$) in Trofimovskaja and 0.89 ($p < 0.01$) in Aromatnaja. The overall correlation coefficient for all three cultivars was 0.91, $p < 0.01$(Table 1).

Table 1. Capacity To Scavenge ABTS Radical Cation and Contents of Phytonutrients of Sea Buckthorn Berries

	Trolox equiv	antioxidant capacity (TEAC) (μmol/g)			contents (mg/100 g)		
	crude	ascorbic	phenolic	lipophilic	ascorbate	GAE	carotenoids
			Botanitjetskaja				
6th Aug	23.18	6.60	17.70	1.29	110.3	177.4	1.0
11th Aug	22.13	6.17	14.93	1.29	101.2	148.5	3.5
14th Aug	22.51	5.97	15.26	1.25	95.8	168.0	4.5
18th Aug	20.34	5.69	13.43	1.97	95.2	151.0	6.1
21st Aug	22.04	5.52	16.45	1.68	95.0	164.3	5.5
25th Aug	19.83	5.18	13.11	1.79	93.1	156.6	6.5
coeff	−0.548	−0.267	−0.578	0.125	−3.006	−2.103	1.235
p	0.063	0.000	0.193	0.079	0.022	0.486	0.018
A/C ratio		0.059	0.094	0.342			
			Trofimovskaja				
6th Aug	24.26	7.02	15.68	1.47	105.2	209.8	5.1
11th Aug	24.29	6.78	12.50	1.85	104.9	156.7	7.3
14th Aug	24.48	4.92	11.17	2.36	76.9	176.3	8.2
18th Aug	23.76	4.94	9.76	1.98	77.2	115.9	10.1
21st Aug	24.20	4.53	11.48	3.68	76.9	117.1	11.5
25th Aug	23.36	4.16	11.18	5.35	65.2	114.8	8.0
coeff	−0.157	−0.601	−0.771	0.70	−8.106	−18.691	0.829
p	0.118	0.007	0.112	0.017	0.012	0.019	0.124
A/C ratio		0.064	0.081	0.332			
			Aromatnaja				
6th Aug	25.16	6.69	18.69	2.51	106.2	244.1	8.2
11th Aug	24.49	6.01	16.51	2.55	105.7	230.8	10.5
14th Aug	24.17	5.44	15.24	3.01	98.1	225.5	9.8
18th Aug	23.76	5.21	14.20	4.44	97.7	196.7	10.6
21st Aug	24.51	4.66	15.17	4.62	77.4	201.2	12.4
25th Aug	23.10	4.54	12.99	5.55	69.3	187.9	13.3
coeff	−0.304	−0.429	−0.959	0.653	−7.709	−11.389	0.914
p	0.054	0.001	0.011	0.002	0.006	0.002	0.006
A/C ratio		0.059	0.072	0.350			
overall coeff	−0.336	−0.432	−0.769	0.493	−6.273	−10.728	0.915
p	0.095	0.000	0.016	0.007	0.000	0.041	0.039
A/C ratio		0.061	0.081	0.342			

[a] The assays were performed as described in Materials and Methods. Values are the means of triplicate measurements based on sample concentration of 20 mg/mL. Data expressed as per gram of fresh weight. [b] GAE = total phenolics (gallic acid equivalents). [c] Coeff = coefficient of linear regression for the changes of phytonutrients. [d] Overall coeff = coefficient of overall changes in all three cultivars. [e] A/C ratio = ratio between antioxidant capacity of extracts and content of antioxidants.

Only one cultivar, Aromatnaja, showed a significant decrease of TEAC values of the phenolic extracts (18.69 to 12.99 μmol TEAC/g, coeff = −0.959, $p = 0.011$). However, the overall change in the three cultivars was significant (coeff = −0.769, $p = 0.016$) (Table 1).

Although the total phenolics or gallic acid equivalents (GAE) reduced from 177.4 to 156.6 mg/100 g for Botanitjetskaja (−11.7%), the decrease was not significant (coeff = −2.103, $p = 0.486$). The other two cultivars showed significant reduction in total phenolics, 209.8 to 114.8 mg/100 g for Trofimovskaja (−45.3%), and 244.1 to 187.9 mg/100 g for Aromatnaja (−23.0%).

Similar to the changes in ascorbate, the antioxidant capacity of the phenolic fraction was correlated with the content of total phenolics. The correlation was high in all three cultivars, $r = 0.79$ in Botanitjetskaja, 0.81 in Trofimovskaja, and 0.93 in Aromatnaja. The overall correlation coefficient for all three cultivars was 0.73, $p < 0.01$.

In contrast to the phenolic and ascorbate extracts, the lipophilic extracts of all three cultivars showed a significant increase in scavenging capacity during ripening. The TEAC values of lipophilic extracts changed from 1.29 to 1.79 μmol/g for Botanitjetskaja (+39.4%), 1.47 to 5.35 μmol/g for Trofimovskaja (+ 265.0%), and 2.51 to 5.55 μmol/g for Aromatnaja (+121.1%) (Table 1).

Corresponding to the changes in antioxidant capacity of lipophilic extracts, total carotenoids increased significantly in two cultivars (Table 1). The content of carotenoids changed from 1.0 to 6.5 mg/100 g for Botanitjetskaja and 8.2 to 13.3 mg/100 g for Aromatnaja. The cultivar from Trofimovskaja showed a less significant change, 5.1 to 8.0 mg/100 g (coeff = 0.829, $p = 0.124$).

The correlation between antioxidant activity and content of total carotenoids varied from $r = 0.78$ ($p < 0.05$) in Botanitjetskaja, 0.87 ($p < 0.01$) in Aromatnaja, to $r = 0.36$ ($p > 0.05$) in Trofimovskaja.

Crude extracts of sea buckthorn contained both hydrophilic and lipophilic antioxidants. The capacity to scavenge free radicals was attributed to all of the antioxidative components. Since the contents of ascorbate and phenolics were decreasing and the content of lipophilic antioxidants was increasing during maturation, the antioxidant capacity of the crude extracts showed a reduction with low significance. The TEAC values reduced from 23.18 to 19.83 μmol/g in Botanitjetskaja (coeff = −0.548, $p = 0.063$) and 25.16 to 23.1 μmol/g of Aromatnaja (coeff = −0.959, $p = 0.054$). No significant change was observed in Trofimovskaja.

The TEAC of the crude extract was significantly correlated to the TEAC of phenolic extracts and ascorbic acid in two cultivars, $r = 0.90$ ($p < 0.01$) for the phenolics, $r = 0.92$ ($p < 0.01$) for the ascorbate in Botanitjetskaja, and $r = 0.94$ ($p < 0.01$) for the phenolics, $r = 0.76$ ($p < 0.05$) for the ascorbate in Aromatnaja. However, the correlation was much lower in Trofimovskaja, $r = 0.40$ ($p > 0.05$) for the phenolics and $r = 0.53$ ($p > 0.05$) for the ascorbate.

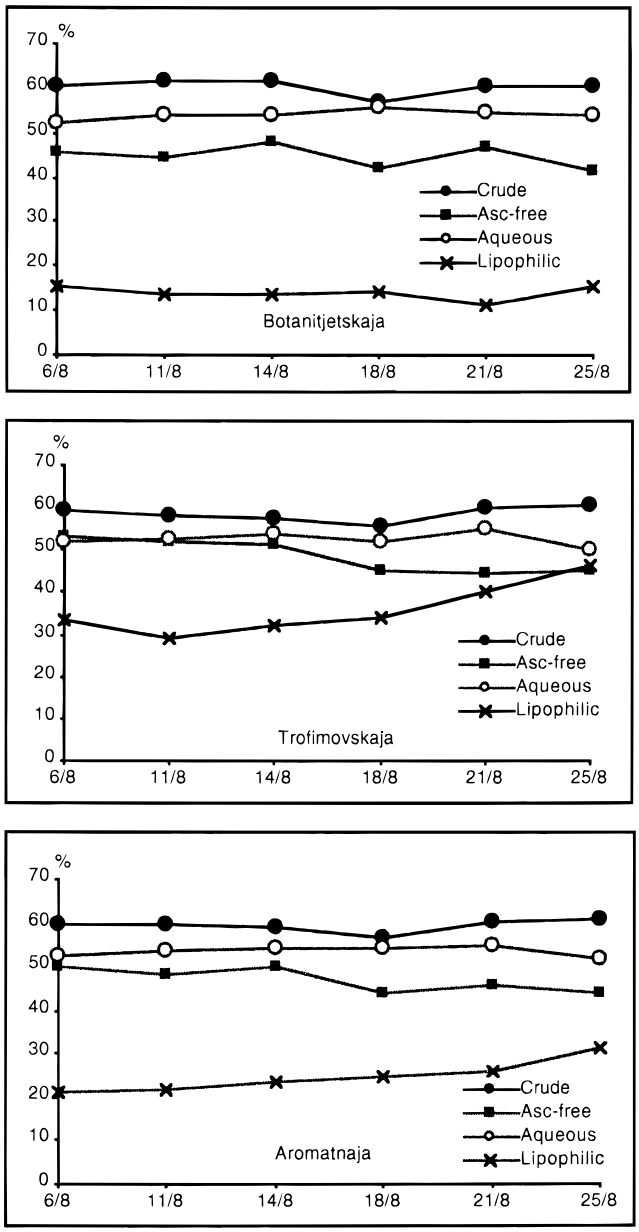

Figure 2. Inhibition of lipid peroxidation induced by AMVN.

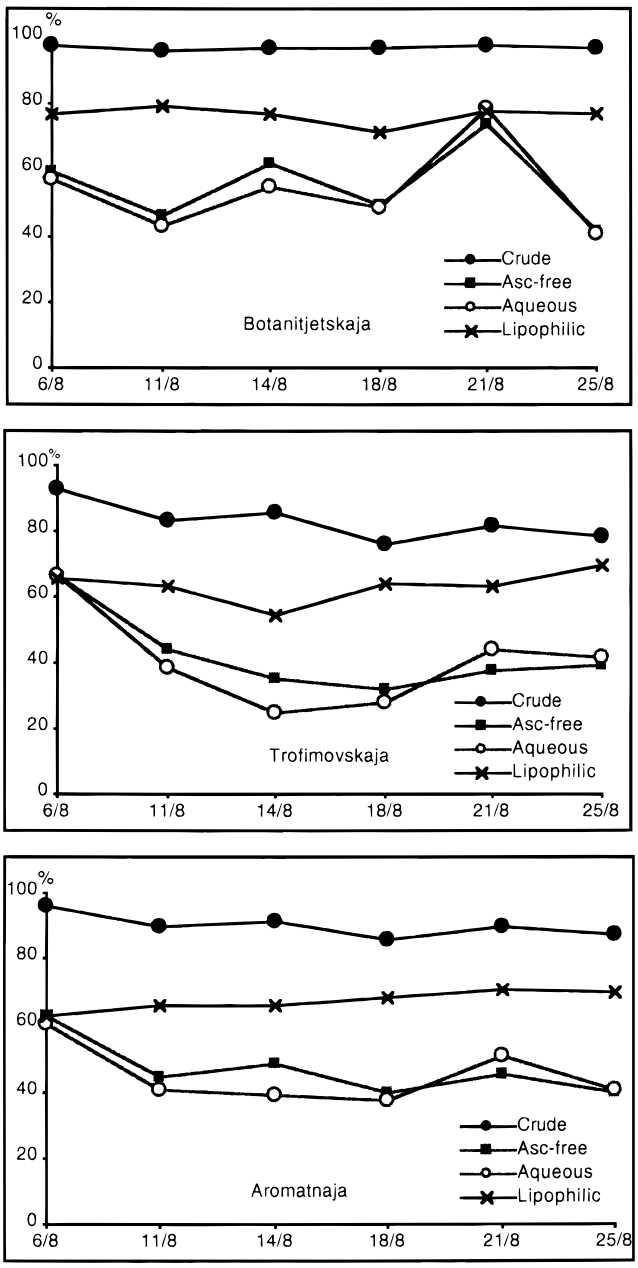

Figure 3. Inhibition of lipid peroxidation by ascorbate−iron.

Inhibition of AMVN-Induced Lipid Peroxidation. At the concentration of extract (5 mg/mL) used in the assay, the crude extract of three cultivars exhibited powerful inhibitory effects on peroxidation. The inhibition ranged from 57.3 to 61.5% for Botanitjetskaja, 56.0 to 61.1% for Trofimovskaja, and 56.6 to 60.8% for Aromatnaja (Figure 2). All of the aqueous extracts (containing phenolics and ascorbate) showed more than 50% inhibition and were more inhibitory than the ascorbate-free extracts, which varied between 40 and 50% inhibition. Corresponding to the change in total phenolies (GAE), a correlation was observed for the inhibitory effect of ascorbate-free extracts, $r = 0.81$ ($p < 0.01$) for Botanitjetskaja, 0.89 ($p < 0.01$) for Trofimovskaja, and 0.92 ($p < 0.01$) for Aromatnaja (Figure 2). However, no correlation between inhibitory effects and the content of ascorbate was observed.

Similar to the ABTS scavenging assay, the lipophilic extracts from two of the three cultivars, Trofimovskaja and Aromatnaja, showed increased inhibition against AMVN-induced lipid peroxidation although the inhibition was still low, due to the low content of lipophilic antioxidant, compared to the aqueous and phenolic extracts (Figure 2).

Inhibition of Ascorbate−Ferric Ion Induced Lipid Peroxidation. All crude extracts showed very high antioxidant activity. The inhibitory effects against metal ion induced lipid peroxidation at the concentration of 2 mg/mL were up to 96.7−98.8% for Botanitjetskaja, 79.0−93.2% for Trofimovskaja and 88.1−96.0% for Aromatnaja (Figure 3). At concentrations of 10−20 μM GAE, the aqueous and ascorbate-free extracts exerted a 30−70% of inhibitory effect. Although there was a high content of ascorbate (7−15 μM in the assay) in the aqueous extracts, the endogenous ascorbate did not interfere with the assay and there was no significant difference between the aqueous and ascorbate-free extracts (Figure 3).

In contrast to the assay of free-radical scavenging and AMVN-induced lipid peroxidation, the lipophilic extracts showed a greater inhibition than the aqueous and

phenolic extracts against metal ion induced lipid peroxidation. The inhibitory effects of lipophilic extracts ranged from 71.7 to 79.6% for Botanitietskaja, 54.5 to 66.8% for Trofimovskaja, and 63.3 to 7 1.1% for Aromatnaja (Figure 3).

DISCUSSION

The first conclusion to be drawn from the present study is that total antioxidant activity or capacity to scavenge ABTS radical cations decreases with increased maturity of sea buckthorn fruits, and the decrease of this capacity is due to the decreased content of phenolics and ascorbate. The increased content of lipophilic antioxidants does not have a great deal of influence on the change in total antioxidant capacity due to the low content of the lipophilic antioxidants.

The antioxidant capacity of sea buckthorn berries is mainly attributed to the phenolic substances, which are present in the highest amount and exert the greatest inhibitory effect. The major contribution of phenolics can be confirmed by the high correlation between the TEAC of crude extracts and the TEAC of phenolic extracts, and the high correlation between the TEAC of crude extracts and the content of total phenolics (GAE) (Table 1). It can be observed that the TEAC of crude extracts and phenolic extracts decreases with the same pattern, for example, an interruption by a peak on 21st Aug. The nonlinear change of phenolics is probably due to climate factors, or some physiological factors; for instance, Zhang et al.(1990) reported that endogenous phytohormones showed two peak values in sea buckthorn fruits, 105−109 days and 128−132 days after anthesis.

There is always a dispute about whether ascorbate is a pro-oxidant or an antioxidant (Podmore et al., 1998; Poulsen et al., 1998). The results of our study agree with previous studies; ascorbate plays an antioxidant role in the ABTS scavenging assay (Rice-Evans & Miller 1994), and in AMVN-induced lipid peroxidation (Niki et al. 1984). An interesting observation is that the endogenous ascorbate from aqueous extracts shows no pro-oxidant effect in the ascorbate−iron induced lipid peroxidation, in spite of the considerable concentration of ascorbate used in the assay (7−15 μM). The pro-oxidant effect of ascorbate is probably outweighed by the antioxidant activity of phenolics, or the iron-binding effect of phenolics plays a major role in the assay since all extracts are much more effective in the metal ion induced peroxidation (89−90%) (Figure 3) than in the AMVN induced lipid peroxidation (60−70%) (Figure 2).

Lipophilic phytonutrients are often ignored in many investigations. An important observation from the present work is that the lipophilic components are more effective than phenolic and ascorbic components in the antioxidant assays if we compare the antioxidant capacity of different fractions and the content of different antioxidants. The lipophilic fractions showed the highest A/C ratio (ratio between the means of TEAC and content of antioxidants), from 0.342 and 0.332 to 0.350 in the three cultivars examined. The phenolic fractions had the lowest A/C ratios, 0.059, 0.064, and 0.059, in the three cultivars (Table 1).

Lipophilic fractions exerted more powerful inhibitory effects than the phenolic and aqueous extracts in the metal ion induced lipid peroxidation (Figure 3). One possible explanation of this result is a partition effect. In the ascorbate−iron induced peroxidation, all components are in an aqueous suspension. The lipophilic antioxidants enter the liposomal bilayer more readily than the other compounds. The lipophilic antioxidants are more concentrated in the lipophilic phase of the suspension and the phenolic and aqueous extracts exist only in the hydrophilic phase of the suspension. Therefore, the lipophilic antioxidants can protect the lipid more effectively. When the AMVN assay is performed, all components are dissolved in EtOH and therefore spread throughout the lipophilic assay solution. The antioxidant capacity of different fractions depends on the total antioxidant concentration, and the lipophilic antioxidants take no advantage of the partition effect.

It should be noted that the composition of lipophilic extracts of sea buckthorn is very complex. Fruit oil of sea buckthorn contains a large number of bioactive substances (Schapiro, 1989); for instance, more than 100 compounds were identified by Wang et al. (1989) from the unsaponifiable fraction of fruit oil, which is thought to be responsible for the biological activity of sea buckthorn oil. The carotenoids measured in the present study were only used as a marker of the lipophilic bioactive compounds in sea buckthorn fruits.

ACKNOWLEDGMENT

This study was financed by a grant from the Swedish Research Council for Forestry and Agriculture. We thank Dr. A. P. Handel and Dr. A. Rehman for their valuable comments and Dr. T. Miura for the kind offer of AMVN.

LITERATURE CITED

Aruoma, O. I.; Spencer, J.; Warren, D.; Jenner, P.; Butler, J.; Halliwell, B. Characterization of food antioxidants, illustrated using commercial garlic and ginger preparations. *Food Chem*. **1997**, *60*, 149−156.

Benzie, I.; Strain, J. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol*. **1999**, *299*, 15−27.

Fu, Q.; Yang, Q.; Yang, G. Analysis of alpha-tocopherol contents in sea-buckthorn oil by reversed phase-high performance liquid chromatography. *J. Xi'an Med. Univ*. **1993**, *14*, 181−183.

Halliwell, B. Antioxidants and human disease: a general introduction. *Nutr. Rev*. **1997**, *55*, 44−52.

Heinonen, M.; Meyer, A.; Frankel, E. Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *J. Agric. Food Chem*. **1998**, *46*, 4107−4112.

Jiang, Z.; Qian, D.; Chou, S. An experimental study of *Hippophae rhamnoides* seed oil against gastric ulcer. *Proc. Int. Symp. Sea Buckthorn, Xi'an, China* **1989**, 401−402.

Lebedeva, L.; Rachmov. I.; Kchaidarov, K. Screening investigation of the antiinflammation activity of sea buckthorn oil. *Proc. Int. Symp. Sea Buckthorn, Xi'an, China* **1989**, 398−399.

Lu, R. *Seabuckthorn: A multipurpose plant species for fragile mountains*; ICIMOD Publication Unit: Katmandu, Nepal, 1992.

Niki, E.; Saito, T.; Kawakami, A.; Kamiya, Y. Inhibition of oxidation of methyl linoleate in solution by Vitamin E and Vitamin, C. *J. Biol. Chem*. **1984**, *259*, 4177−4182.

Nourooz-Zadel, J.; Tajaddini-Sarmadi, J.; Wolff, S. Measurement of plasma hydroperoxide concentrations by the ferrous oxidation-xylenol orange assay in conjunction with triphenylphophine. *Anal. Biochem*. **1994**, *220*, 403−409.

Pellegrini, N.; Re, R.; Yang, M.; Rice-Evans, C. Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying 2,2′-azinobis(3-ethyeneben-

zothiazoline-6-sulfonic acid) radical cation decolorization assay. *Methods Enzymol.* **1999**, *299*, 379−389.

Plumb, G.; Chambers, S.; Lambert, N.; Bartolome, E.; Heaney, R.; Wanigatunga, S.; Arouma, O.; Halliwell, B.; Miller, N.; Williamson, G. Antioxidant actions of fruit, herb and spice extracts. *J. Food Lipids* **1996**, *3*, 171−188.

Podmore, I.; Griffiths, H.; Herbert, K.; Mistry, N.; Mistry, P.; Lunec, J. Vitamin C exhibits pro-oxidant properties. *Nature* **1998**, *392*, 559.

Poulsen, H.; Weimann, A.; Salonen, J.; Nyyssonen, K.; Loft, S.; Cadet, J.; Douki, T.; Ravanat, J. Does vitamin C have a pro-oxidant effect? *Nature* **1998**, *395*, 231−2.

Prior, R.; Cao, G.; Martin, A.; Sofic, E.; McEwen, J.; O'Brien, C.; Lischner, N.; Ehlenfeldt, M.; Kalt, W.; Krewer, G.; Mainland, C. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of Vaccinium species. *J. Agric. Food Chem.* **1998**, *46*, 6, 2686−2693.

Rice-Evans, A.; Miller, N. Total antioxidant status in plasma and body fluids. *Methods Enzymol.* **1994**, *234*, 279−293.

Rice-Evans, A.; Miller, N.; Paganga, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* **1997**, *2*, 152−159.

Rousi, A. The genus *Hippophae* L. A taxonomic study. *Ann. Bot. Fennici* **1971**, *8*, 177−227.

Schapiro, D. C. Biochemical studies on some hopeful forms and species of sea buckthorn in USSR. *Proc. Int. Symp. Sea Buckthorn, Xi'an, China* **1989**, 64−66.

Terao, J.; Nagao, A.; Park, D. K.; Lim, B. P. Lipid hydroperoxide assay for antioxidant activity of carotenoids. *Methods Enzymol.* **1992**, *213*, 454−460.

Wang, H.; Ge, H.; Zhi, J. The components of unsaponifiable matters in sea buckthorn fruit and seed oil. *Proc. Int. Symp. Sea Buckthorn, Xi'an, China* **1989**, 81−90.

Yao, Y.; Tigerstedt, P. Variation of vitamin C concentration and character correlation between and within natural sea buckthorn (*Hippophae rhamnoides* L.) populations. *Acta Agric. Scand.* **1992**, *42*, 12−17.

Zhang, W.; Zhang, H.; Deng, X.; Yao, H.; Du, C.; Li, Y. Studies on the active changes of GA-3 and cytokinins during development and ripening in sea buckthorn fruits. *Acta Bot. Sinica* **1990**, *32*, 611−615.

Received for review September 29, 1999. Revised manuscript received February 25, 2000. Accepted February 25, 2000.

JF991072G