JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2003,** *51,* 3313−3319 **3313**

Stoichiometric and Kinetic Studies of Phenolic Antioxidants from Andean Purple Corn and Red-Fleshed Sweetpotato

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Stoichiometric and kinetic values of phenolics against DPPH (2,2-diphenyl-1-picrylhydrazyl) were determined for Andean purple corn (*Zea mays* L.) and red sweetpotato (*Ipomoea batatas*). Both crops had higher antioxidant capacity and antiradical kinetics than blueberries and higher or similar anthocyanin and phenolic contents. The second-order rate constant (k_2) was 1.56, 1.12, 0.57, and 0.26 (mg antiradical/mL)$^{-1}$ s^{-1} for red sweetpotato, Trolox, purple corn, and blueberry, respectively. On the molar basis of active hydroxyl groups, k_2' showed the same order as for k_2. Corn cob and sweetpotato endodermis contributed the most in phenolic compounds and antioxidant capacity. Both crops studied can be considered as excellent novel sources of natural antioxidants for the functional food and dietary supplement markets.

KEYWORDS: Antioxidant activity; DPPH stoichiometry and kinetics; phenolics; purple corn; red sweetpotato

INTRODUCTION

There is an increasingly growing market for nutraceuticals and functional foods. Products containing nutraceuticals have reached a worldwide estimated value of $65 billion (*1*). In the United States alone, this figure is approximately $24 billion with an expected rise to $35.4 billion by the year 2006. Within the nutraceutical category are antioxidants, essential compounds needed for controlling degenerative oxidation reactions caused by reactive oxygen and nitrogen species in living tissues as well as in the inhibition of lipid peroxidation in foods. These free radical species are associated with aging and related diseases such as cancer and atherosclerosis (*2*). Lipid peroxidation involves deteriorative reactions in foods that occur during processing as well as storage (*3*). In recent years, natural antioxidants have been studied as alternatives to synthetics for inhibiting lipid peroxidation (*3*) and for providing biological systems with protection against harmful free radicals (*4*, *5*).

Plant-derived antioxidants such as vitamin E, vitamin C, and polyphenols are becoming increasingly important dietary factors (*6*−*8*). For example, phenolic compounds, including anthocyanins, have been identified to possess a very high capacity to quench free radicals (*9*−*12*). This has attracted scientists to study commercial fruits and vegetables and indigenous plants for their antioxidant properties. Reports indicate that blueberries (*Vaccinium* spp.) have a higher antioxidant activity than many commercial crops studied (*4*, *13*, *14*).

For hundreds of years, people from the Andean region have utilized native plants and crops to maintain and improve their health. For example, in Peru, people consume a colored infusion made with the purple corn cob, known as "chicha morada", which has been related by folklore to increased health benefits (*15*). In recent years, two Andean crops, purple corn and red sweetpotato, have been studied confirming their potential health benefit properties, which were previously implied by tradition and folklore. Purple corn was shown to inhibit colorectal carcinogenesis in male rats (*16*), while red sweetpotato was shown to be antimutagenic (*17*) as well as a radical scavenger (*18*). The bioactivities of these crops are associated with anthocyanins and other phenolic compounds present.

Most of the studies of natural antioxidants have been conducted to characterize the total antioxidant activity or capacity contained within the tissue, which is associated with the total amount of phytochemicals exerting the activity (such as phenolic compounds) (*4*). However, few researchers have studied the rate of antiradical reaction, which indicates how fast the antioxidants react with the free radicals (*19*, *20*). Reaction kinetics information complements that of total antioxidant capacity (TAC) and is necessary for characterizing a potential antioxidant source.

It is clear that purple corn and red sweetpotato have bioactive properties according to traditional use and scientific evidence; however, level and efficiency of their phenolic antioxidants have not been characterized and compared to known crops with high antioxidant content. We hypothesized that Andean purple corn and red sweetpotato are rich sources of phenolic compounds with high antioxidant properties as compared to other crops. Our approach was to test these properties against blueberries and identify the antioxidant level and kinetics, as well as the distribution of the active compounds within crop tissues.

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10.1021/jf034109c CCC: $25.00
Published on Web 04/18/2003

MATERIALS AND METHODS

Materials. Purple corn (Amazonas Imports, Inc., Sun Valley, CA) and freeze-dried red sweetpotato (Viru, Peru) were imported from Peru. Five fresh blueberry (*Vaccinium* spp.) brands were purchased from a local market (Kroger Co., College Station, TX). All crops were stored at −20 °C before analyses. Chlorogenic acid, Trolox (6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), sodium carbonate, and Folin−Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO). Hydrochloric acid, methanol, and hexane were purchased from EM Science (a division of EM Industries Inc., Gibbstown, NJ).

Anthocyanin Content. Total anthocyanin content analysis was adapted from Fuleki and Francis (*21*) measuring absorbance of an extract at pH 1. The sample was homogenized with an ethanolic solvent (0.225 N HCl in 95% ethanol) using an Ultra-Turrax homogenizer (IKA Works, Inc., Wilmington, NC). Tubes were capped and stored for 24 h at 4 °C. Extracts were centrifuged at 29 000*g* for 15 min, and the supernatant was collected. Hexane was added to red sweetpotato extracts to remove any carotenoids present. Spectrophotometric readings at 535 nm were taken subtracting absorbance at 700 nm (due to turbidity). Anthocyanins were expressed as mg cyanidin 3-glucoside equivalent/100 g wet or dry weight, using a molar extinction coefficient of 25 965 M^{-1} cm^{-1} and a molecular weight of 449 g/mol (*22*). For all spectrophotometric readings, a photodiode array spectrophotometer (model 8452A; Hewlett-Packard Co., Waldbronn, Germany) was used.

Total Phenolics. Total soluble phenolic content analysis was adapted from Swain and Hillis (*23*). The sample was homogenized with methanol. Tubes were capped and stored for 20−72 h at 4 °C. Extracts were centrifuged at 29 000*g* for 15 min. A 0.5 mL sample (0.5 mL water for the blank) was taken from the clear supernatant and diluted with 8 mL of Nanopure water. A 0.5 mL aliquot of 0.25 N Folin−Ciocalteu reagent was added and allowed to react for 3 min; then, 1 mL of 1 N Na_2CO_3 was added and allowed to react for 1 h. Spectrophotometric readings at 725 nm were taken. Total phenolics were expressed as mg chlorogenic acid equivalent/100 g wet or dry weight based on a standard curve.

Antiradical Capacity. Antiradical activity or capacity of phenolic compounds was adapted from Brand-Williams and others (*24*). The same methanol extract as for phenolics was used. A total of 150 μL of sample (equivalent methanol volume as control) reacted with 2850 μL of DPPH (98.9 μM in methanol) in a shaker covered with aluminum foil at a temperature of 25 °C. Readings at 515 nm were taken at 15 min and until no significant decrease in absorbance was experienced as compared to the control (around 20 h). The change in absorbance was used, and results were expressed as Trolox equivalents from a standard curve. Additionally, this change in absorbance at steady state was used to determine the stoichiometric number (*n*).

In this study, readings at 15 min were used for calculation of the relative antiradical capacity (RAC), which indicates the antiradical capacity of the sample as compared to Trolox for a specific reaction time (for example, 15 min). Readings at steady state (around 20 h) were used to calculate the TAC, which indicates the total antiradical capacity contained in the sample.

Isolation of Phenolic Compounds with C-18 Resin. For confirming that phenolic compounds in methanol extracts were solely responsible for the reaction with DPPH, phenolic compounds were isolated with C-18 cartridges and reacted with DPPH. Methanol extracts were concentrated to dryness on a Speed Vac Concentrator (model SV0-100H, Savant Instruments, Inc., Hicksville, NY) at 35 °C attached to an aspirator pump. Samples were rediluted with acidified (0.01% HCl) water. Aqueous samples were applied to C-18 Sep-Pak Vac 35 cm^3 cartridges (Waters Association, Milford, MA), previously activated with 20 mL of acidified methanol followed by 30 mL of acidified water. Water soluble compounds, including sugars and acids, were eluted with 30 mL of acidified water, and phenolics were recovered with 15 mL of acidified methanol.

Antiradical Kinetic Assay. Second-order antiradical kinetic determinations were adapted from Espín and others (*19*) using DPPH and methanolic extracts:

$$-\mathrm{d[DPPH]}/\mathrm{d}t = k_2\,[\mathrm{DPPH}]\,[\mathrm{AH}] \quad (1)$$

The second-order rate constant (k_2) was determined by having the antiradical compound [AH] in large excess as compared to the radical compound [DPPH], thus forcing the reaction to behave as first-order:

$$-\mathrm{d[DPPH]}/\mathrm{d}t = k_1\,[\mathrm{DPPH}] \quad (2)$$

where

$$k_1 = k_2\,[\mathrm{AH}] \quad (3)$$

[AH] is considered constant throughout the reaction and can be modified to obtain different k_1 values.

Therefore, DPPH was depleted from the medium under pseudo-first-order conditions following the equation:

$$[\mathrm{DPPH}] = [\mathrm{DPPH}]_0\,\mathrm{e}^{-k_1 t} \quad (4)$$

where [DPPH] is the radical concentration at any time (t), $[\mathrm{DPPH}]_0$ is the radical concentration at time zero, and k_1 is the pseudo-first-order rate constant. This constant (k_1) is linearly dependent on the concentration of the antiradical, and from the slope of these plots, k_2 is determined (*19*).

Kinetic studies were conducted by measuring the disappearance of DPPH at 515 nm under pseudo-first-order conditions at a temperature of 25 °C. DPPH solution in methanol was freshly prepared for each experiment (<1 day old). Determinations of k_1 were conducted in triplicate using five different extract concentrations per sample. Fitting of the experimental data to obtain k_1 values was carried out by using an exponential decay (single, two parameter) equation generated by Sigma Plot 2.01 (*25*).

An aliquot of sample extract ranging from 80 to 200 μL was placed on a cuvette inside the cuvette holder. To this, 2800−2920 μL of DPPH was added with enough pressure so as to achieve a homogeneous solution (<1 s). Spectrophotometric readings began immediately after DPPH addition. The specific pseudo-first-order antiradical kinetic conditions of reaction between DPPH and samples were as follows: 95 μM DPPH and red sweetpotato extract (0.073−0.097 mg phenolics/mL); 79 μM DPPH and purple corn extract (0.229−0.286 mg phenolics/mL); 86 μM DPPH and blueberry extract (0.124−0.155 mg phenolics/mL); and 96 μM DPPH and Trolox (0.052−0.087 mg/mL).

Crop Section Analysis. Purple corn and red sweetpotato were divided into different sections to determine the level of active compounds in different structural parts. In purple corn, the main sections analyzed were cob and kernels; the cob was subdivided into epidermis and endodermis, and the kernels were subdivided into pericarp and seed. Red sweetpotato was only divided into epidermis and endodermis. Total anthocyanin and phenolic contents and RAC were determined on whole tissues and their sections. TAC and antiradical kinetics (AK) were conducted on whole tissues.

Analysis of Variance (ANOVA). One way ANOVA was performed using the SAS Statistical Analysis System v8.1 (SAS Institute Inc., Cary, NC). Means were compared with Duncan's multiple range test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Anthocyanin and Total Phenolic Contents of Andean Crops. Several studies with anthocyanins and other phenolics have shown that these have multiple functional roles, for example, as antioxidants (*9*, *20*), antimutagens (*17*, *26*), anticarcinogens (*16*, *27*), and natural colorants (*19*). When screening for new antioxidant sources, there is a need for information on the concentration of the active compounds present in the tissue. Thus, anthocyanin and total phenolic contents were determined for the crops studied.

Our results indicate that anthocyanin and total phenolic contents for whole purple corn were 1642 mg/100 g and 1756 mg/100 g, respectively (**Table 1**). The most abundant anthocyanin in purple corn is cyanidin 3-glucoside (*28*, *29*). Pelargonidin and peonidin glucosides, with their respective malonyl

Table 1. Anthocyanin and Total Phenolic Content of Purple Corn and Red Sweetpotato as Compared to Blueberries

crop	dry matter (%)	anthocyanin content[f] wet basis	anthocyanin content[f] dry basis	phenolic content[g] wet basis	phenolic content[g] dry basis
purple corn	92.3	$1642^{a} \pm 92$	$1779^{b} \pm 99$	$1756^{a} \pm 64$	$1903^{d} \pm 69$
sweetpotato	29.3	$182^{d} \pm 2$	$618^{c} \pm 7$	$945^{b} \pm 82$	$3220^{c} \pm 279$
blueberry 1	14.9	$138^{d} \pm 20$	$925^{c} \pm 134$	$292^{e} \pm 32$	$1956^{d} \pm 211$
blueberry 2	16.0	$385^{b} \pm 54$	$2404^{a} \pm 336$	$672^{c} \pm 41$	$4202^{a} \pm 253$
blueberry 3	16.4	$271^{c} \pm 52$	$1654^{b} \pm 319$	$520^{d} \pm 30$	$3170^{c} \pm 185$
blueberry 4	16.8	$296^{c} \pm 12$	$1761^{b} \pm 72$	$606^{cd} \pm 9$	$3608^{b} \pm 52$
blueberry 5	16.5	$276^{c} \pm 25$	$1675^{b} \pm 149$	$574^{d} \pm 35$	$3480^{bc} \pm 212$

[a–e] Means within a column with the same superscript letter are not significantly different ($\alpha = 0.05$). [f] Concentration expressed in mg cyanidin 3-glucoside/100 g. [g] Phenolic concentration expressed in mg chlorogenic acid/100 g.

derivatives, have also been identified (*28*). Cyanidin 3-galactoside has been found in low amounts in a Peruvian dark-seeded corn, where it may be acylated with *p*-coumaric acid (*30*). Other phenolic compounds still need to be identified. For Andean red sweetpotato, we observed that anthocyanin and total phenolic contents were 182 mg/100 g and 945 mg/100 g, respectively. The major anthocyanins in red sweetpotato are acylated cyanidin and peonidin glucoside derivatives (*31*, *32*), while chlorogenic acid is the major polyphenolic present (*18*).

Blueberries were used as reference in this study since they have the highest antioxidant activity and phenolic content among many fruits and vegetables tested previously (*4*, *13*, *14*). The anthocyanin and total phenolic content of blueberries analyzed ranged from 138 to 385 mg/100 g and 292 to 672 mg/100 g, respectively. A similar range has been previously reported (*4*). The range observed could be due to different blueberry varieties and/or blueberries with different maturity levels since different sources were used. Studies have shown that phenolic content and antioxidant activity increase with increasing blueberry maturity (*4*). Most prevalent blueberry anthocyanins are nonacylated glycosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin, while chlorogenic acid is the greatest contributor of total polyphenolics (*33*).

In general, the anthocyanin concentration of the crops studied would be as follows: purple corn > blueberries > sweetpotato. On a dry basis, the order would be similar, with purple corn = blueberries > sweetpotato. For total phenolic content, the order would be as follows: purple corn > sweetpotato > blueberries. However, on dry basis, the order is inverted to blueberries ≥ sweetpotato > purple corn. According to our results, Andean purple corn and red sweetpotato can be considered as rich sources of phenolic compounds comparable to blueberries with the additional advantage of giving potentially higher yields per hectare and with lower production costs.

TAC. For antiradical assays, DPPH was used as the free radical source, since it simulates reactive oxygen and nitrogen species affecting biological systems (*34*). In addition, free radical scavenging is generally the accepted mechanism for antioxidants inhibiting lipid oxidation (*24*).

TAC was evaluated to determine whether the high level of phenolic compounds present in purple corn and red sweetpotato tissues will in turn yield high antioxidant capacity. TAC based on crop weight reflects the total antiradical capacity of the analyzed crop expressed as Trolox equivalents after all antiradicals present in the sample extracts have reacted with DPPH radicals. The synthetic antioxidant Trolox was used to obtain a standard curve where 1 mol of Trolox reacted with 2 mol of DPPH, which is in agreement with that previously reported with DPPH and ABTS radicals (*35*).

Table 2. TAC of Purple Corn and Red Sweetpotato as Compared to Blueberry

	TAC[d] wet basis	TAC[d] dry basis	TAC[d] phenolic basis[e]
sweetpotato	$12\,409^{b} \pm 1024$	$42\,303^{a} \pm 3492$	$1149^{b} \pm 95$
purple corn	$21\,351^{a} \pm 121$	$23\,132^{c} \pm 131$	$1380^{a} \pm 10$
blueberry 5	$5646^{c} \pm 389$	$35\,232^{b} \pm 2427$	$988^{b} \pm 109$

[a–c] Means within a column with the same superscript letter are not significantly different ($\alpha = 0.05$). [d] TAC in μg Trolox equiv/g. [e] TAC in μg Trolox equiv/mg chlorogenic acid equiv present in the reacting extract.

In our study, the TAC values obtained are compared with blueberry since this fruit is considered to have one of the highest TAC values (*4*). According to our results, purple corn and red sweetpotato are 3.8 and 2.2 times higher in TAC, respectively, than blueberry (**Table 2**). On a dry basis, the order changes to sweetpotato > blueberry > purple corn. These TAC trends observed are similar to the trends observed for total phenolic content (**Table 1**), suggesting that phenolic compounds are responsible for the antiradical capacity.

When TAC is expressed on a phenolic basis, information is obtained on the effectiveness of the phenolics present in each crop on neutralizing the radicals present. A higher Trolox equivalence would mean a higher antioxidant capacity of the phenolics present. Thus, purple corn and red sweetpotato appear to have phenolic compounds with a capacity to stabilize a greater number of free radicals as compared to phenolics from blueberries (**Table 2**).

Phenolic compounds of each product were isolated using C-18 cartridges, and no significant difference ($p > 0.05$) was found between the TAC (phenolic basis) of methanol extracts as compared to the TAC of the C-18-purified phenolic compounds from the studied products. These results confirm that phenolic compounds are responsible for the antioxidant properties of the sample methanol extracts.

Stoichiometric Number and AK. *Stoichiometric Number (n).* To determine the number of free radicals stabilized per unit of phenolic compounds present in each crop, stoichiometric analyses were conducted. The reaction of DPPH with a hydrogen-donating antioxidant can be represented by

$$\mathrm{DPPH}\bullet + \mathrm{AH} \rightarrow \mathrm{DPPH{-}H} + \mathrm{A}\bullet \qquad (5)$$

On the basis that one DPPH molecule reacts with one active hydrogen, we can determine the number of active phenolic hydroxyl groups in 1 g of total phenolics for each crop. This allows us to determine the stoichiometric number of radicals (*n*) trapped by each antioxidant. According to Shi and Niki (*20*), *n* can be obtained using the following relationship:

$$n = \Delta A/(\epsilon\, l\, C_{\mathrm{x}}) \qquad (6)$$

where ΔA is the absorbance difference of DPPH at 515 nm between initial and end reaction, ϵ is the molar extinction coefficient of DPPH (11 126 M^{-1} cm^{-1}), l is the cell length, and C_x is the concentration of AH. C_x for pure substances is expressed in mol/L and for total phenolics in g/L.

According to our results, the *n* value for Trolox indicates that it contains 2 mol of active hydroxyl groups per mol to reduce DPPH (**Table 3**). The *n* value for purple corn phenolics was determined to be 1.1×10^{-2} mol/g, indicating that 1 g of phenolics could react with 1.1×10^{-2} mol DPPH or that there are 1.1×10^{-2} mol or 6.62×10^{21} molecules of active hydroxyl groups in 1 g of phenolics. Assuming that the majority of purple

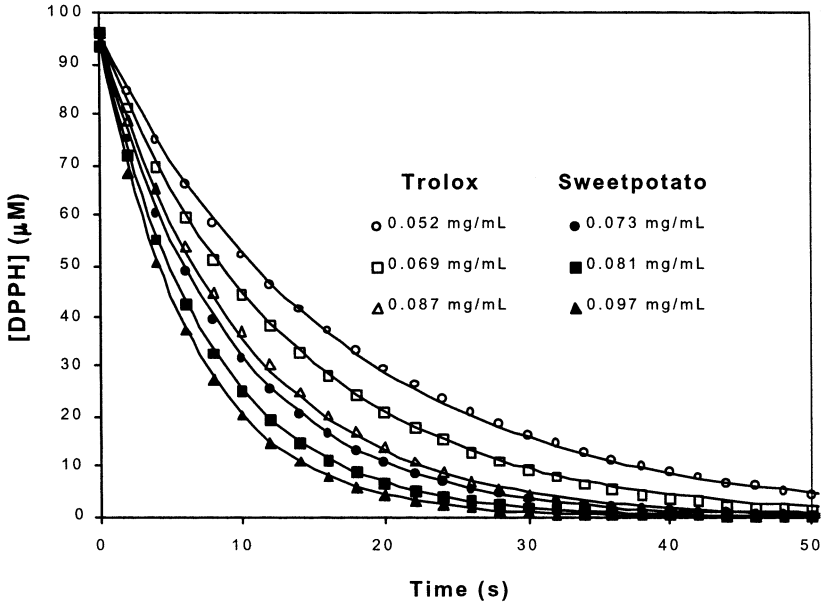

Figure 1. Spectrophotometric recordings of the disappearance of DPPH in the presence of different Trolox and phenolic red sweetpotato concentrations obeying pseudo-first-order conditions. Symbols represent experimental data and solid lines fitting of the experimental data to eq 4.

Table 3. Stoichiometric Number (*n*) and AK of Purple Corn and Red Sweetpotato Extracts as Compared to Blueberry Extracts and Trolox

	n		k_2		k_2'
	mol/g[e]	mol/mol[f]	$(g/L)^{-1}s^{-1}$	$M^{-1}s^{-1}$	$M^{-1}s^{-1}$
sweetpotato	$8.9^{b} \times 10^{-3}$		1.56^{a}		$1.75^{a} \times 10^{2}$
purple corn	$1.1^{a} \times 10^{-2}$		0.57^{c}		$0.53^{c} \times 10^{2}$
blueberry 5	$7.7^{c} \times 10^{-3}$		0.26^{d}		$0.34^{d} \times 10^{2}$
Trolox	$8.0^{bc} \times 10^{-3}$	2.0	1.12^{b}	2.8×10^{2}	$1.40^{b} \times 10^{2}$

[a–d] Means within a column with the same superscript letter are not significantly different ($\alpha = 0.05$). [e] mol active hydroxyl groups (OH)/g chlorogenic acid equiv or Trolox. [f] mol active hydroxyl groups (OH)/mol Trolox.

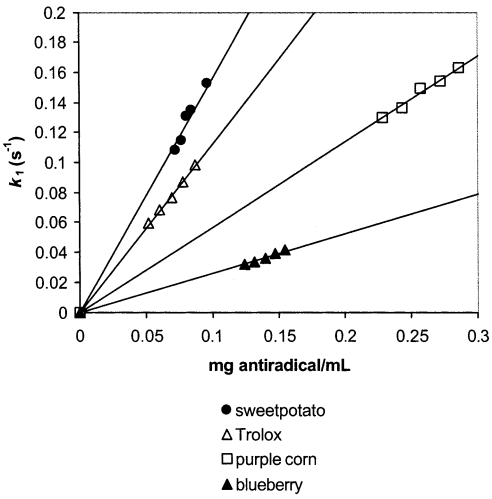

Figure 2. Pseudo-first-order rate constant (k_1) dependence on antiradical concentration. Solid lines show the linear regression fitting of the data (eq 3).

corn phenolics are cyanidin 3-glucoside, *n* can be expressed as mol active hydroxyl groups per mol of cyanidin 3-glucoside giving $n = 4.94$. This would indicate that cyanidin 3-glucoside has five DPPH reactive hydroxyl groups in its molecule. Rice-Evans and others (*36*) reported a stoichiometric number *n* of 5 for the aglycone cyanidin and *n* of 6 for pelargonidin against ABTS radicals, indicating that glycosylation of anthocyanins in the 3-position diminishes the antioxidant activity, giving a lower *n* value. They also showed that acylation of the molecule provides more active hydroxyl groups increasing *n* (*36*). Thus, the higher *n* value that we obtained could be due to acylation of anthocyanins or the presence of a mixture of phenolic compounds with higher *n* values. To obtain a precise *n* value, fractionation and identification of the phenolic mixture using high-performance liquid chromatography and mass spectroscopy are recommended.

In summary, *n* values from **Table 3** show that 1 g of phenolics from purple corn and red sweetpotato have a larger number of active hydroxyl groups as compared to phenolics from blueberries, confirming information presented in **Table 2**, showing that phenolics from both Andean crops have a larger capacity to react with DPPH radicals.

AK. AK was evaluated to determine the reaction rate (efficiency) of purple corn and red sweetpotato phenolics against free radicals (DPPH). Reaction kinetics indicate how much an antioxidant reduces the rate of oxidation (*20*). Kinetics information can be used in food systems to design strategies to inhibit lipid, flavor, and color oxidation and preserve the quality of foods. It can also be used to design strategies to reduce oxidative stress in vivo, where antioxidants will scavenge, quench, or interact with superoxide, hydroxyl, peroxyl radicals, and nitric oxide produced from cell or biochemical reaction systems (*20*).

Our results indicate that when Trolox or sample extracts were added to the DPPH solution, DPPH radicals were depleted under pseudo-first-order conditions (eq 4), where [DPPH] $\ll$ [AH], as shown in **Figure 1**. Similar curves were obtained for purple corn and blueberry (data not shown). When fitting experimental data to eq 4 or its linearized form, the regression coefficients were around 0.997 and 0.999 for all samples studied. According to our results, the calculated pseudo-first-order constant (k_1) depended on antiradical concentration (**Figure 2**).

Second-order rate constants (k_2) were obtained by linear regression fittings of k_1 and antiradical concentration, following eq 3 (**Figure 2**). The k_2 values were expressed based on the phenolic weight present in the extracts, since we showed that phenolics were responsible for the reaction with DPPH. Other authors (*20*) have expressed k_2 based on extract dry weight;

Table 4. Anthocyanin and Total Phenolic Content of Purple Corn and Red Sweetpotato Sections

crop	section	anthocyanin content[h]	phenolic content[i]	% of total crop weight	% anthocyanin contribution	% phenolic contribution
purple corn	whole cob	$3752^{b} \pm 478$	$4395^{b} \pm 84$	17.9	54.6	59.3
	cob epidermis	$8230^{a} \pm 373$	$9350^{a} \pm 247$	4.2	34.3	33.9
	cob endodermis	$1495^{d} \pm 42$	$2156^{d} \pm 8$	13.7	20.3	25.4
	whole kernel	$681^{e} \pm 10$	$657^{f} \pm 33$	82.1	45.4	40.7
	kernel pericarp	$3105^{c} \pm 374$	$3233^{c} \pm 175$	15.2	45.0	35.7
	kernel seed	$6.4^{f} \pm 0.5$	$102^{g} \pm 26$	66.9	0.4	5.0
red sweetpotato	epidermis	$361^{a} \pm 27$	$2654^{a} \pm 78$	4.8	9.1	10.8
	endodermis	$182^{b} \pm 2$	$1101^{b} \pm 32$	95.2	90.9	89.2

[a−g] Means within a column and crop with the same superscript letter are not significantly different ($\alpha = 0.05$). [h] Anthocyanin concentration expressed in mg cyaninidin-3-glucoside/100 g wet basis. [i] Phenolic concentration expressed in mg chlorogenic acid/100 g wet basis.

however, not all compounds present in an extract have antiradical activities; thus, we suggest that these values be expressed based on the weight of the active compounds. The k_2 for red sweetpotato extracts was the highest, indicating the highest AK. The order of AK, according to k_2 values, was red sweetpotato > Trolox > purple corn > blueberry.

These results would indicate that phenolics from red sweetpotato and purple corn have faster reaction kinetics than phenolics from blueberries. Phenolics from red sweetpotato also react faster to stabilize DPPH radicals as compared to Trolox, which is considered to be a powerful antioxidant (*37*). Comparison with food antioxidants previously reported in the literature (*19*) shows that k_2 for red sweetpotato phenolics is slightly lower than that for vitamin E (1.87 ± 0.10) but higher than those for BHA (0.42 ± 0.02) and BHT (0.05 ± 0.003).

When we introduce the stoichiometric factor n in the antiradical kinetic analysis, eq 1 can be rewritten as (*20*)

$$-\mathrm{d[DPPH]}/\mathrm{d}t = k_2\,[\mathrm{DPPH}]\,[\mathrm{AH}] = n\,k_2'\,[\mathrm{DPPH}]\,[\mathrm{AH}] \quad (7)$$

where k_2' is the rate constant of a single active hydroxyl group in the hydrogen-donating compound and is defined as k_2/n.

Values of k_2' for our samples give information on the average reactive potential of the active hydroxyl groups and allow fair comparison between sample extracts and pure compounds on a molecular basis. Results were as follows: red sweetpotato > Trolox > purple corn > blueberry (**Table 3**). This suggests that hydroxyl groups from red sweetpotato phenolics are arranged in a certain configuration that allow a better interaction with DPPH radicals than hydroxyl groups present in Trolox and in the other sample phenolic extracts studied.

Relationship between TAC and AK. TAC values of different tissues expressed on a wet and a dry basis are related to the total reactive capacity present in the tissues. These values will greatly depend on the concentration of phenolic compounds present. However, TAC values expressed on a phenolic basis are related to n, giving the total reactive potential of phenolic antioxidants present in the tissue. These values are independent of phenolic concentration and reflect the capacity of the type of phenolic compounds present to stabilize free radicals. TAC (phenolic basis) and n values do not necessarily correlate with the antioxidant kinetic constants (k_2 and k_2'). For example, purple corn has the highest TAC (phenolic basis) and n values but lower k_2 and k_2' values. On the other hand, red sweetpotato has both high TAC and high kinetic constants (**Tables 2** and **3**). In summary, n is related to the total reactive potential of the active hydroxyl groups present, while kinetic rates indicate the rate at which those active hydroxyl groups react with free radicals.

According to this, we propose that the reaction kinetics information obtained should complement that of TAC. Stoichiometric studies allow the comparison of antioxidant activity between samples on a molecular level. All of these parameters should be used together as standard procedures for determining the capacity and efficiency of natural antioxidants from different plant sources.

Distribution of Phenolic Compounds in Different Structural Parts. Information of the distribution of active compounds within plant tissues is important for maximizing the use of their beneficial physiological functions (*17*). This information can also facilitate the design of appropriate extraction processes or improve existing ones. For example, in commercial juice processing of blueberries, more than 42% of anthocyanins and more than 15% of phenolics still remain in the skin, which contains the majority of the phenolic compounds present. With the use of appropriate SO_2 and heat treatments, the recovery of anthocyanins may increase in the final juice (*33*).

Purple Corn Sections. The distribution of anthocyanins in whole cob and kernel sections was about 54.6 and 45.4%, respectively (**Table 4**). In these major structures, cob epidermis and endodermis had 34.3 and 20.3% of the total anthocyanins, respectively, while the distribution in kernel pericarp and kernel seed was 45% and less than 1%, respectively. Total phenolics showed similar distribution in the different corn structural parts studied (**Table 4**). The high anthocyanin and phenolic accumulation found in the corn cob section does not agree with the trend observed in nature where phenolic compounds tend to accumulate in external plant tissue surfaces for protection (*38*). It is possible that the cob serves as a storage system for later redistributing the phenolic compounds at different locations within the plant upon need.

For extractions targeting pigment yield, information on the volume and weight of specific crop sections would be useful. Results indicate that the cob weight is only 18% of the whole corn, while the volume represents about 45% of the whole corn. The large cob volume, due to a highly porous structure, and the easy removal of kernels make the cob a potential source of phenolics for extraction processes. For example, anthocyanins can be easily removed from the cob by immersing the cob in acidified water using organic acids. This anthocyanin extract is called "chicha morada", a popular drink consumed by Peruvian people and an important source of antioxidants in their diet.

Anthocyanins in different purple corn sections have been previously identified. Nakatani and others (*29*) found that most of the anthocyanins present in the cob and kernel sections were cyanidin 3-glucoside. In another study, this anthocyanin was also found to be the main one present in purple corn cob with contributions of pelargonidin-3-glucoside, peonidin-3-glucoside, and their respective malonyl derivatives (*28*).

Antiradical capacity tests expressed as RAC were conducted to identify purple corn sections with high antioxidant capacity. RAC results in decreasing order were cob epidermis > whole cob > kernel pericarp > whole corn > cob endodermis > whole kernel > kernel seed. A positive correlation ($r^2 = 0.9878$) was obtained when plotting RAC against total phenolic content for different purple corn sections. Similar results were obtained when plotting RAC against anthocyanins ($r^2 = 0.9868$). A coefficient of determination close to 1 and a high percentage of phenolics as anthocyanins suggest that the main compounds exhibiting RAC in purple corn are anthocyanins. RAC is a good index of RAC obtained in a short period of time and can be used for screening and ranking of several samples simultaneously. However, RAC does not indicate the total antiradical capacity of the samples. Work with different crops high in anthocyanins has shown similar correlations suggesting a direct antiradical capacity of these phenolic compounds (*4*, *11*). Cyanidin 3-glucoside, the main anthocyanin in purple corn (*28*, *29*), was shown to be the most active compound tested against carbon tetrachloride-induced lipoperoxidation (*39*). In another study, cyanidin 3-glucoside was shown to have the highest antioxidant activity among different anthocyanins using the ORAC method (*10*).

Red Sweetpotato Sections. In red sweetpotato, the epidermis showed a higher concentration of anthocyanins and total phenolics as compared to the endodermis. However, the epidermis represents only 4.8% of the total weight, making the flesh (endodermis) the larger contributor of most of the anthocyanins and phenolics present per root (**Table 4**). Anthocyanins were distributed in the epidermis and endodermis by about 9.1 and 90.9%, respectively, while total phenolics by 10.8 and 89.2%, respectively (wet basis). Although phenolic compounds are mostly in the flesh, the peel or epidermis can still be considered a rich source of phenolic compounds. Similarly to corn cob, the accumulation of phenolics in sweetpotato flesh may also serve as a storage system for these compounds.

When measuring RAC, epidermis had almost three times higher activity than endodermis. The higher RAC in the epidermis section is due to the higher phenolic concentration by weight (**Table 4**). Thus, the peel could still be utilized as a concentrated source of phenolics for the nutraceutical market due to its high antiradical capacity.

In previous studies, anthocyanins in colored sweetpotato were shown to have antimutagenic activity, which was higher in the epidermis as compared to the endodermis (*17*). The high RAC observed for the epidermis section in our study would most likely be related to the antimutagenic properties described earlier.

This study has shown that Andean purple corn and red sweetpotato are crops that have high anthocyanin and total phenolic contents. Their higher phenolic antioxidant capacity and AK, as compared to blueberries, indicate that these crops have the potential to be considered as important novel natural antioxidant sources for the functional food and dietary supplement markets.

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Received for review February 3, 2003. Revised manuscript received March 26, 2003. Accepted March 28, 2003.

JF034109C