

# Antioxidant-Rich Foods Retard Lipid Oxidation in Extruded Corn

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## ABSTRACT

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Antioxidant-rich plant materials could provide protection against oxidation in extruded foods and feeds, but their efficacy is not well established. Degermed yellow cornmeal was mixed with 0.02% (w/w) ascorbic acid or quercetin, or with 2% (w/w) spray-dried ginkgo extract, onion powder, potato peels, or wheat bran. The mixtures were processed in a laboratory-scale twin-screw extruder at a feed rate of 227 g/min. Water pump rate was 16 g/min; screw speed was 200 rpm. Mass temperature during extrusion averaged  $\approx 170^\circ\text{C}$ . Samples were cut into small spheres, dried to 5% moisture, then stored in trilaminar bags at  $25^\circ\text{C}$ .

Ground sample headspace was assayed for hexanal and other volatile indicators of oxidation by gas chromatography. Ginkgo and potato peels significantly darkened the extrudates. Total soluble phenolics, as ferulic acid equivalents, were highest in the ginkgo sample. Volatile compounds were lower in several treatments during storage compared with the control. These findings suggest that manufacturers may be able to formulate products with improved shelf-life through addition of antioxidant-rich food materials.

Lipid oxidation can be a concern for extruded foods and feeds. While most extruded foods have low lipid content, lipid oxidation can be a problem in foods and feeds with higher lipid levels or unsaturated lipids. Although grains and other common ingredients may contain only 3% lipids, they are susceptible to oxidation leading to off-flavors and odors due to small amounts of unsaturated fatty acids. Factors that favor oxidation in extruded foods specifically include low moisture content, increased surface area due to expansion, and higher levels of iron, a catalyst for oxidation, caused by the wearing of the screw and barrel during extrusion (Artz and Rao 1994). Extrusion lowered levels of endogenous antioxidant tocopherols and carotenoids in grass peas (Grela et al 1999), thus addition of antioxidants to raw materials before extrusion could slow oxidation during storage and help to offset losses of existing antioxidants.

Phenolic compounds act as free radical terminators, chelators of metal catalysts, and singlet oxygen quenchers. Synthetic antioxidants may be steam-distilled during extrusion and some consumers prefer “natural” products. Antioxidant-rich plant materials could provide protection against oxidation, but their efficacy is not well established. Cinnamic acid and vanillin were better antioxidants than BHT in extruded corn snacks that were fried postextrusion (Camire and Dougherty 1998). Simulated ready-to-eat breakfast cereals consisting of 60% rolled oats, 30% oat flour, and 10% sucrose were formulated to contain 200 ppm of BHA, vanillin, and caffeic, cinnamic, ferulic, gallic, *p*-coumaric, or vanillic acids, then were twin-screw extruded and stored at  $35^\circ\text{C}$  for six months (Camire et al 1999). No difference in antioxidant activity was found among the treatments compared with a control with no added phenolic compounds. In a related study, 1,000 ppm of benzoin, catechin, chlorogenic acid, ferulic acid, and quercetin were added to oatmeal (Viscidi et al 2004). Benzoin and quercetin inhibited the production of hexanal. Benzoin and ferulic acid each reduced peroxide values and conjugated dienes in cereals stored for four weeks. Quercetin fortification also caused lower peroxide values to be detected, especially at the 1,500-ppm level. A trained sensory panel perceived less painty flavor in cereals fortified with ferulic acid and with all concentrations of quercetin.

The purpose of this study was to examine the effects of antioxidant-rich plant material in extruded yellow cornmeal. Incorporation of phenolic-rich materials as antioxidants may have implications for functional food development because whole foods may not need GRAS affirmation that might be required for purified chemical compounds. Although estimates of daily consumption of phenolic compounds and flavonoids are scarce, this study also demonstrates the feasibility of fortifying a food with additional phenolic compounds to increase dietary consumption of these natural antioxidants.

## MATERIALS AND METHODS

### Materials

Degermed yellow corn meal was purchased from Conagra Corn Processing, Atkinson, KS. Quercetin was purchased from Sigma Chemical Co., St. Louis, MO. Steamed potato peels were provided by Basic American Foods, Blackfoot, ID. Wheat bran was purchased from AACC International, St. Paul, MN. Ascorbic acid was purchased from Jungbunzlauer, Newton Center, MA. A spray-dried *Ginkgo biloba* extract was provided by DRACO Natural Products, San Jose, CA, and was standardized at a 15:1 concentration. Minced onion powder (Hannaford Bros. Co., Scarborough, ME) was purchased locally and ground to pass a 2-mm screen.

### Sample Preparation

Duplicate batches of degermed yellow cornmeal were mixed with 2% (w/w) of one of the following treatments: ginkgo extract, steamed potato peels, or wheat bran. Ascorbic acid and quercetin were mixed at 0.02% (w/w) to give a concentration of 200 ppm of the antioxidant in the mix. The control contained no additives. The duplicate batches and the control were extruded in a Werner-Pfleiderer ZSK-30 (Coperion Werner & Pfleiderer, Ramsey, NJ) twin-screw extruder. All of the treatments were extruded without the addition of water. The screw speed was 200 rpm. The temperature profile of the barrel from the inlet to the die was 58, 95, 102, 125, 134, and  $166^\circ\text{C}$ . The length and diameter of each screw were 963 and 30 mm, respectively. The high shear screw configuration used in the experiments consisted of a total of 504 mm of  $42^\circ$  forward conveying elements; 56 mm of  $28^\circ$  conveying elements; 14 mm of  $28^\circ$  narrow pitch conveying element; 260 mm of  $20^\circ$  conveying elements; 7 mm of  $41^\circ$  Igel; 14 mm of 45/5 kneading block elements at 671 mm; 20 mm of 45/5 kneading block elements at 739, 813, and 883 mm; 14 mm of 45/5 left-handed kneading block elements at 685 and 759 mm; and 20 mm of  $20^\circ$  reverse elements. A single  $9.7 \times 4$ -mm round die was used. Temperatures, torque, and pressure were recorded from the extruder instrument panel. Specific mechanical energy (SME) was calculated according to the equation described by Frame (1994).

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Extrudates were cut with a single blade cutter at 450 rpm. The collets were collected onto metal screen trays then dried to 5% moisture in a convection oven (DRO Series G, Hobart Corp., Troy, OH) at 121°C. When cooled to room temperature, the samples were sealed in tri-laminate bags (Cadill Products, Paris, IL) and stored at 25°C.

### Physical Analyses

Cereals were lightly packed into a 1,250 mL beaker, weighed, and bulk density recorded as g/cm<sup>3</sup>. Six samples were analyzed per extrusion run. For diametric expansion, 20 pieces from each extrusion run were randomly selected and measured with a caliper (Monostat Mecamin type 69X1L, KWB, Switzerland). The expansion ratio was calculated as the cross-sectional diameter of an extrudate divided by the diameter of the die opening.

Ground collets were analyzed for Hunter *L\*a\*b\** color with a color meter (Labscan, Hunter Associate Laboratory, Reston, VA) with a 6-cm optical aperture. In each treatment, the reflectance measurement was obtained from the average of three readings; duplicate samples were evaluated.

### Moisture

Moisture was determined using Approved Method 44-15A (AACC International 2000). Samples were ground to pass through a 2-mm screen in a Thomas-Wiley laboratory mill (model 4, Arthur H. Thomas Co., Philadelphia, PA) and dried for 16 hr at 105°C.

### Soluble Phenolics

Phenolics were measured after 13 weeks of storage to determine residual phenolic compounds. Samples (10 g) were ground through a 2-mm screen in a Thomas-Wiley laboratory mill (model 4). Samples (400 mg) were extracted over 2 hr with 5 mL of 80% methanol containing 1% HCl, with constant stirring at room temperature. Samples were centrifuged (TJ-6R, TA 10 rotor, Beckman Instruments, Palo Alto CA) at 1,000 × *g* for 10 min. The supernatant was decanted into vials. The pellet was re-extracted under the same conditions. Supernatants were combined. Extract

(200 µL) was mixed with 1.5 mL of Folin-Ciocalteu (Sigma, St. Louis, MO) reagent (diluted 1:10 with distilled water) and allowed to stand at room temperature for 5 min, then 1.5 mL of sodium bicarbonate (T.J. Baker Chemical Co., Phillipsburg, NJ) solution (6 g/100 mL) was added (Velioglu et al 1998). After 90 min at room temperature, the absorbance at 725 nm was read (Spectronic 20D+ Spectronic Instruments, Rochester, NY). A standard curve based on ferulic acid (Sigma) standards of 0.0, 0.1, 1.0, 10, and 100 µg was used to determine the regression equation. Fresh standards were run each day.

### Headspace Analysis of Volatiles

The procedures used were adapted from Frankel et al (1989) and Frankel (1993). Cereals (8 g) ground to pass a U.S. 30 sieve were weighed into a 22-mL vial. A gas chromatograph (Hewlett Packard GC 6890 with Chem Station, Agilent Technologies, Palo Alto, CA) fitted with a RESTEK Stabilwax (Bellefonte, PA) 30 m, 0.32 mm, i.d., 1.0 µm df column was used. The initial oven temperature was 50°C, initial time was 2 min, and the ramp temperature was 5°C/min to 65°C. A split injector (split ratio 100:1) was used and heated to 180°C. The carrier gas was helium. A FID detector set to 180°C was used. A headspace analyzer (model 7050, Tekmar-Dohrmann, Cincinnati, OH) was connected to the gas chromatograph. The vials were 22 mL and sealed with a teflon cap. A 1-mL sample loop was used at 65°C. The platen temperature was 100°C with an equilibration time of 10 min.

Hexanal standards were prepared by making a 500 µM hexanal stock solution (Sigma) (50.0 mg hexanal/L of distilled water). The stock solution was diluted with distilled water to give concentrations of 50, 25, 12.5 and 6.25 µM. Standards (5 mL) were placed in 22-mL vials and sealed with teflon caps. The peak area of the standards was used to calculate a regression equation (v. 9, Systat, Evanston, IL). Fresh standards were prepared for each analysis run. The retention times for pentane (1.476 min), octanal (2.016), nonanal (2.716 min), pentanal (6.135 min), and hexanal (9.900 min) were determined by adding 50 mg of each compound to a vial and analyzing as described.

**TABLE I**  
Physical Properties in Extruded Cornmeal Containing Antioxidant-Rich Additives<sup>a</sup>

Treatment	% Expansion (n = 40)	Bulk Density (kg/m <sup>3</sup> ) (n = 12)
Control	434 ± 26b	52.1 ± 1.0c
Ascorbic acid	446 ± 24c	50.9 ± 1.2c
Ginkgo	451 ± 23d	43.8 ± 1.7a
Onion	438 ± 25bc	49.2 ± 1.3b
Quercetin	445 ± 24cd	49.3 ± 1.9b
Potato peel	436 ± 22b	47.9 ± 1.3b
Wheat bran	395 ± 24a	51.4 ± 1.1c

<sup>a</sup> Values followed by the same number in the same column are not significantly different (*P* < 0.05, Fisher's LSD test).

**TABLE II**  
Hunter Color of Extruded Cornmeal Containing Antioxidant-Rich Additives<sup>a</sup>

Treatment	<i>L</i>	<i>a</i>	<i>b</i>
Control	74.04 ± 0.85cd	4.94 ± 0.38b	31.38 ± 1.48b
Ascorbic acid	74.53 ± 1.05d	6.59 ± 0.26d	34.96 ± 0.33d
Ginkgo	61.29 ± 0.50a	6.85 ± 0.17e	25.23 ± 0.18a
Onion	75.07 ± 0.46d	5.85 ± 0.26c	35.05 ± 0.31d
Potato peel	70.55 ± 0.46b	5.08 ± 0.25b	30.99 ± 0.24b
Quercetin	73.54 ± 5.81c	5.81 ± 0.23c	34.79 ± 0.30d
Wheat bran	74.40 ± 0.62d	4.55 ± 0.28a	32.78 ± 0.30c

<sup>a</sup> Mean of six values. Values followed by the same letter within a column are not significantly different (*P* < 0.05, Fisher's LSD test). Hunter values: *L* 0 = black, 100 = white; +*a* = red, -*a* = green; +*b* = yellow, -*b* = blue.

**TABLE III**  
Total Soluble Phenolics (as ferulic acid equivalents, mg/kg) in Extruded Cornmeal Containing Antioxidant-Rich Additives After 13 Weeks in Storage<sup>a</sup>

Treatment	Total Phenolics (n = 6)
Control	449 ± 20a
Ascorbic acid	505 ± 152a
Ginkgo	822 ± 106b
Onion	468 ± 82a
Potato peel	506 ± 26a
Quercetin	447 ± 31a
Wheat bran	462 ± 63a

<sup>a</sup> Values followed by the same letter within a column are not significantly different (*P* < 0.05, Fisher's LSD test).

**TABLE IV**  
% Inhibition of DPPH Radical by Extruded Products Containing Antioxidant-Rich Materials<sup>a</sup>

Sample	% Inhibition (n = 6)
Control	30.2 ± 3.5a
Ascorbic acid	31.4 ± 4.5ab
Ginkgo	33.4 ± 2.9ab
Onion	31.1 ± 2.0ab
Potato peel	33.5 ± 2.7ab
Quercetin	35.0 ± 4.4b
Wheat bran	30.1 ± 3.3a

<sup>a</sup> Values followed by the same letter within a column are not significantly different (*P* < 0.05, Fisher's LSD test).

TABLE V  
Headspace Volatiles ( $\mu\text{M}$ ) in Extruded Samples ( $n = 6$ ) Stored at 25°C for Three Weeks<sup>a</sup>

Sample	Decanal	Hexanal	Nonanal	Octanal	Total
Control	2.7 ± 1.8a	5.2 ± 1.3b	1.5 ± 0.1ab	10.3 ± 0.4c	31.8 ± 2.3ab
Ascorbic acid	5.9 ± 1.0c	1.8 ± 0.3a	1.2 ± 0.3a	7.3 ± 1.4b	26.8 ± 5.1ab
Ginkgo	4.4 ± 0.2b	1.8 ± 0.1a	1.2 ± 0.2a	8.2 ± 0.3b	28.5 ± 1.8ab
Potato peel	4.2 ± 2.1b	1.9 ± 0.3a	2.1 ± 0.7b	7.0 ± 2.6b	33.4 ± 7.6ab
Onion	5.9 ± 2.2c	1.4 ± 0.4a	1.5 ± 0.5ab	10.2 ± 2.9c	43.8 ± 14.5b
Quercetin	6.3 ± 0.8c	1.4 ± 0.2a	1.5 ± 0.3ab	7.5 ± 1.0b	28.5 ± 5.3ab
Wheat bran	2.8 ± 0.6a	1.6 ± 0.5a	0.9 ± 0.2a	3.8 ± 0.9a	18.9 ± 5.9a

<sup>a</sup> Values followed by the same letter within a column are not significantly different ( $P < 0.05$ , Fisher's LSD test).

TABLE VI  
Headspace Volatiles ( $\mu\text{M}$ ) In Extruded Samples ( $n = 6$ ) Stored at 25°C for Six Weeks<sup>a</sup>

Sample	Decanal	Hexanal	Nonanal	Octanal	Total
Control	4.6 ± 0.9bc	1.9 ± 0.6ab	1.6 ± 0.4a	9.0 ± 2.1b	32.6 ± 10.6bc
Ascorbic acid	4.3 ± 1.9b	1.3 ± 0.3a	1.4 ± 0.2a	6.2 ± 2.3a	26.3 ± 9.2ab
Ginkgo	3.1 ± 1.2a	2.4 ± 2.0b	1.6 ± 0.5a	5.5 ± 1.7a	24.7 ± 6.6a
Potato peel	4.6 ± 1.6b	1.9 ± 0.5ab	1.4 ± 0.4a	6.8 ± 1.8a	30.9 ± 4.2a
Onion	4.1 ± 0.7ab	1.4 ± 0.5a	1.5 ± 0.5a	6.0 ± 1.4a	27.1 ± 4.0a
Quercetin	5.9 ± 2.1c	1.6 ± 0.5ab	1.9 ± 0.7a	8.6 ± 4.3b	41.8 ± 14.9c
Wheat bran	4.9 ± 1.0bc	1.5 ± 0.5ab	1.3 ± 0.2a	8.2 ± 1.0b	29.4 ± 4.8a

<sup>a</sup> Values followed by the same letter within a column are not significantly different ( $P < 0.05$ , Fisher's LSD test).

### Antioxidant Activity

Cereals (5 g) were ground to pass through a U.S. 30 sieve, then a subsample (0.5 g) was extracted with methanol (4 mL) for 1 hr at room temperature with constant stirring. Samples were centrifuged (TJ-6R, TA 10 rotor, Beckman Instruments, Palo Alto, CA) at  $1,000 \times g$  for 10 min. The supernatant was decanted into 4-mL vials. The pellets were re-extracted under the same conditions. Supernatants were combined. The percent inhibition assay, as described by Miller et al (2000), was performed using DPPH (Aldrich, Milwaukee, WI).

### Statistical Analyses

All data were analyzed by the General Linear Mode Hypothesis program (Systat 9.0). Most analyses were analyzed with the treatment (source of antioxidant or control) as the independent factor. For the headspace data, each treatment at each week was compared with the other treatments at the same time period. Fisher's LSD test was used to separate means at  $P < 0.05$ . Mean values for physical properties, phenolics, and antioxidant activity were correlated using Pearson's method, and Bonferroni probabilities were calculated for the correlations using Systat.

## RESULTS

### Extrusion

Extrusion conditions did not change significantly with feed ingredients. Cook temperature was 142°C; mass temperature was 170°C, as read from the control panel on the extruder. Torque was 69% and die pressure was 1.61 MPa. The SME of the onion sample was significantly less that of the control (data not shown).

### Physical Properties

The samples containing ginkgo extract had greater diametric expansion than all other samples (Table I). Wheat bran had the lowest percent expansion. The bulk density of the ginkgo samples was significantly lower than the other treatments (Table I). Potato peel, quercetin, and onion extrudate bulk density were significantly lower than those for wheat bran, ascorbic acid, and the control. Ginkgo samples were darkest (Table II), followed by potato peels. The highest Hunter *a* values (more red) and lowest Hunter *b* readings (more blue) were also found for the ginkgo samples. Consumer research is needed to determine whether these color

differences are unacceptable. Changes in physical properties are expected when the base grain is replaced by other ingredients. Substitution of rice with the Chinese medical herb *Euryale ferox* Salisb. to increase antioxidant activity in extruded breakfast cereals increased lightness (Hunter *L*) and bulk density (Lin et al 2003).

### Soluble Phenolics

Phenolics of the extrudates containing ginkgo extract were significantly higher than all other treatments (Table III); there were no significant differences between any other treatments. However, because these measurements were made 13 weeks after extrusion, there may have been losses during storage. The ginkgo extract was highly concentrated and thus contributed more phenolics than did the other additives.

The method we employed to measure phenolic compounds does not include phenolic acids bound to plant cell walls, and thus does not provide a complete accounting of all phenolics in the samples. Adom and Liu (2002) found that corn had higher levels of phenolic compounds than did wheat, oats, and rice, and that 85% of the phenolics in corn existed in the bound form. While bound phenolic acids may contribute to grain health benefits, it is not clear whether these bound compounds could serve as antioxidants in foods. Zieliński et al (2001) reported that free and esterified phenolic acids increased in wheat, barley, rye, and oats after extrusion. Extrusion decreased soluble phenolics in oats to which individual compounds had been added (Viscidi et al 2004), but antioxidant activity was dose-dependent.

### Antioxidant Activity

Samples containing quercetin were the most effective antioxidants using the DPPH inhibition assay (Table IV). Phenolic content was not correlated with antioxidant activity. Possibly compounds that are not detected or extracted during the phenolics procedure contribute to antioxidant activity.

### Headspace Volatiles

Volatile aldehydes were measured in the headspace of vials containing freshly ground extrudates to provide another estimate of lipid oxidation control. Only wheat bran had a decanal value as low as that for the control (Table V). The control sample had higher headspace hexanal values than any other sample at three weeks of storage. Nonanal values were similar, with only small

**TABLE VII**  
**Headspace Volatiles ( $\mu\text{M}$ ) In Extruded Samples ( $n = 6$ ) Stored at 25°C for 11 Weeks<sup>a</sup>**

Sample	Decanal	Hexanal	Nonanal	Octanal	Total
Control	4.9 $\pm$ 0.8bc	1.4 $\pm$ 0.6a	1.4 $\pm$ 0.3a	7.7 $\pm$ 1.3c	24.4 $\pm$ 3.23b
Ascorbic acid	6.1 $\pm$ 0.7cd	2.4 $\pm$ 0.2b	1.6 $\pm$ 0.2ab	7.0 $\pm$ 0.9c	27.2 $\pm$ 2.7bc
Ginkgo	3.8 $\pm$ 0.9b	1.9 $\pm$ 0.6a	1.5 $\pm$ 0.5a	7.3 $\pm$ 1.8c	26.2 $\pm$ 6.7bc
Potato peel	2.4 $\pm$ 1.4a	1.4 $\pm$ 0.5a	1.8 $\pm$ 0.7ab	5.2 $\pm$ 1.5b	24.8 $\pm$ 8.6b
Onion	6.6 $\pm$ 2.1d	1.8 $\pm$ 0.4ab	2.3 $\pm$ 0.7b	11.1 $\pm$ 3.3d	47.8 $\pm$ 13.7d
Quercetin	6.5 $\pm$ 1.2cd	1.7 $\pm$ 0.1ab	2.1 $\pm$ 0.3ab	7.2 $\pm$ 0.9c	32.2 $\pm$ 4.6c
Wheat bran	1.9 $\pm$ 0.6a	2.5 $\pm$ 1.0b	1.1 $\pm$ 0.1a	2.9 $\pm$ 0.9a	15.8 $\pm$ 6.8a

<sup>a</sup> Values followed by the same letter within a column are not significantly different ( $P < 0.05$ , Fisher's LSD test).

**TABLE VIII**  
**Pearson Correlation Matrix for Physical and Chemical Properties of Extruded Cornmeal Containing Antioxidant-Rich Additives**

Property	Expansion	Bulk Density	Hunter <i>L</i>	Hunter <i>a</i>	Hunter <i>b</i>	Soluble Phenolics	Antioxidant Activity
Expansion	1.000						
Bulk density	-0.507	1.000					
Hunter <i>L</i>	-0.446	0.863	1.000				
Hunter <i>a</i>	0.721	-0.668	-0.508	1.000			
Hunter <i>b</i>	-0.267	0.642	0.929 <sup>a</sup>	-0.230	1.000		
Soluble phenolics	0.445	-0.842	-0.962 <sup>a</sup>	0.648	-0.867	1.000	
Antioxidant activity	0.518	-0.690	-0.452	0.405	-0.193	0.314	1.000

<sup>a</sup> Values have a Bonferroni probability  $\leq 0.05$ .

difference among samples. At three weeks, wheat bran had the significantly lowest octanal values. All other samples, except onion, had lower octanal concentrations compared with the control. Total volatile range was 18.9–43.9  $\mu\text{M}$ , with few differences among samples. By six weeks, ginkgo had lower decanal levels than did the control (Table VI). All samples were similar to the control for hexanal and nonanal. Ascorbic acid, ginkgo, potato peel, and onion had lower octanal concentrations compared with the control, while only ginkgo, potato peel, and onion had significantly lower total volatiles than did the control with no added antioxidants. At 11 weeks, the control sample had more decanal than did the potato peel and wheat bran samples (Table VII); hexanal and nonanal values for most samples were similar or higher than that for the control. Onion and wheat bran had lower octanal values than did the control, but only wheat bran had lower total volatiles. A significantly lower value than the control was found only for octanal by 15 weeks of storage (data not shown). The control sample had a mean value (4.0) double that for wheat bran (1.9). Headspace analysis of volatile compounds may not be the most practical method for assessing lipid oxidation in foods to which antioxidant-rich materials have been added. For example, onions may naturally contain decanal (Nielsen and Poll 2004).

## DISCUSSION

Some phenolic compounds do survive the extrusion process and contribute to antioxidant activity in the final product. Optimization of extrusion conditions for retention of these compounds is needed. The extrusion process itself may have created new antioxidants. Oatmeal cookies containing extruded potato peels had lower peroxide values than Arora and Camire (1994). This antioxidant effect could be due to the formation of Maillard reaction compounds. Extruded crispbread with 5% glucose added had slightly lower peak viscosity compared with similar bread to which 0.02% BHT was added pre-extrusion (Yokota et al 1987). Sensory quality must also be considered because color and flavor could be changed by the antioxidants. Hunter *L* value was positively correlated with Hunter *b*, but negatively correlated with soluble phenolics (Table VIII). Until quercetin and other flavonoids are granted GRAS status in the United States, food compounds rich in these

compounds could prove to be a practical source of natural antioxidants.

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