

Flavonoids and Antioxidant Capacity of Georgia-Grown Vidalia Onions

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Vidalia onion varieties Nirvana, DPS 1032, Yellow 2025, King-Midas, and SBO 133 grown at Vidalia, Georgia, were analyzed for flavonoid content. A high-performance liquid chromatographic (HPLC) method with photodiode array detection was used for quantification. Compounds were analyzed as aglycons after acid hydrolysis with 1.2 M HCl. Identification of each compound was based on comparison of its retention time and UV spectra with those of pure commercial standards. Three major flavonoids, kaempferol, myricetin, and quercetin, were identified and quantified. Quercetin was the major flavonoid (7.70–46.32 mg/100 g fresh weight, FW) present in all varieties, followed by myricetin (2.77–4.13 mg/100 g FW). Minor quantities of kaempferol (1.10–1.98 mg/100 g FW) were also detected. The total polyphenols and Trolox equivalent antioxidant capacity (TEAC) ranged from 73.33 to 180.84 mg/100 g FW and 0.92–1.56 μ M TEAC/g FW, respectively. A positive but weaker correlation was obtained for total polyphenols versus antioxidant capacity. Nevertheless, a stronger correlation ($r^2 = 0.34$) was obtained between flavonoid content versus antioxidant capacity. The data indicate that Vidalia onions are a rich source of quercetin, and they also contain myricetin and kaempferol.

KEYWORDS: Antioxidant capacity; flavonoids; HPLC diode-array; total polyphenols; Vidalia onion

INTRODUCTION

Flavonoids are widely distributed in the plant kingdom. They are mainly produced as a pigment of many shades, and play an important role in normal growth, development, and defense of plants. At the biochemical level, flavonoids act as enzyme inhibitors, provide defense against ultraviolet radiation, are chelating agents for metals, and act as reducing agents. In addition, flavonoids are also involved in photosensitization and energy transfer, morphogenesis and sex determination, respiration and photosynthesis, plant growth hormones and regulators (1–3), gene expression, and behavior.

Flavonoids are synthesized only in plants. Dietary intake comes only through the food chain, and is obtained from fruits and vegetables, and beverages such as red wine, beer, tea, and coffee. Flavonoids are also found in several medicinal plants and herbal remedies which have been used in folk medicine around the world. They have been reported to exhibit a wide range of biological effects, such as antibacterial, antiviral (4), antiinflammatory, antiallergenic (4, 5), and vasodilatory (4, 6) actions. They are known to inhibit lipid peroxidation (7) and platelet aggregation (8–10) and to affect capillary permeability and fragility (11). Metabolically, flavonoids inhibit the activity of various enzyme systems, including cyclo-oxygenase and lipoxygenase (6, 12), acts as antioxidants, free radical scavengers

(4, 13, 14), and chelators of cations (15). Intake of flavonoids is correlated with low incidence of coronary heart disease (16), and heart disease mortality was inversely associated with flavonoids intake (17).

Growing evidence on the health benefits of flavonoids warrants search for their presence in various fruits and vegetables and their quantification. Flavonoids have been reported in fruits such as apples, berries, grapes, citrus fruits, tea, soybean, onions, and many others. They have been reported in many vegetables and green leaves (18).

Onion is one of the most widely and largely consumed vegetables. There are many varieties of onions such as yellow, white, and red. They are also classified based on their taste, as sweet or nonsweet onions. The importance of onions in cooking comes from their typical taste and flavor. The most popular varieties in the United States are Vidalia (Georgia) and Walla Walla (Washington) onions, which have distinctive flavors and sweetness. Flavonoids were reported in large quantities in white, yellow, and red onions (18, 19). The major flavonoid found in onions is quercetin, present mainly as mono- and diglucosides (20). Because growing Vidalia onions is very popular and commercially important in Georgia, the present study focused on identifying and quantifying their flavonoids content.

MATERIALS AND METHODS

Chemicals. Pure standards of myricetin, quercetin, and kaempferol were purchased from Sigma (St. Louis, MO). 2,2'-Azinobis(3-ethyl-

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benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Fluka (Milwaukee, WI). *tert*-Butylhydroquinone (TBHQ) was obtained from Eastman Chemical Company (Kingsport, TN). Methanol and water (HPLC grade), formic acid, and hydrochloric acid (analytical grade) were purchased from Fisher Scientific (Norcross, GA).

Sample Collection. *Vidalia* onion varieties Nirvana, DPS 1032, Yellow 2025, King-Midas, and SBO 133 were collected from Vidalia, Georgia. The samples were transported to the University facility and frozen at -80°C until analyzed. Analyses of onions were completed within a month of sample collection. The bulb without outer dry skin and stem was analyzed.

Hydrolysis for HPLC Analysis. A 15-g portion of frozen onion sample was ground to a paste with mortar and pestle in the presence of 100 mg of *tert*-butylhydroquinone (TBHQ), 500 mg of washed sand, 5 mL of 6 M HCl, and 20 mL of methanol. The crushed samples were transferred to a reflux flask with further addition of 5 mL of HCl and 20 mL of methanol to final concentration of 1.2 M HCl (21). The flask was wrapped with aluminum foil and flushed with nitrogen for 5 min. The deoxygenated sample was refluxed at 90°C for 2 h to hydrolyze the flavonoid glycosides to aglycons. The hydrolyzed sample was cooled in the dark and filtered through a 0.2-micron syringe nylon filter. A 20- μL aliquot of appropriately diluted sample was injected into HPLC for analysis.

HPLC Analysis. HPLC was performed with a Hewlett-Packard (Avondale, PA) model 1090 Win liquid chromatograph with quaternary pumps and a diode array UV-visible detector (22–24) coupled to a HP ChemStation. A Beckman (Fullerton, CA) Ultrasphere 5 μ , ODS, RP C₁₈ column (250 \times 4.6 mm) was the stationary phase. The solvent system of (A) acidified water (1% formic acid) and (B) acetonitrile, with a flow rate of 1.2 mL/min, was used. The gradient used was the following: 0.00 min, (A) 95.00% and (B) 5.00%; 3.01 min, (A) 100% and (B) 0.00%; and 65 min, (A) 65.00% and (B) 35.00%. UV spectra were recorded from 240 to 600 nm at a rate of 1.00 spectrum/1.28 s and a resolution of 2 nm with a bandwidth of 4 nm and reference wavelength in off mode. HPLC chromatogram was obtained at 360 nm.

Quantitation. Quantification was performed based on external standards of known concentrations. Peak areas were used to quantify the compounds in the sample. Calibration curves of the standards ranging from 0 to 240 ng/mL were used with good linearity and R^2 values exceeding 0.99 (peak areas vs concentration).

Extraction. Frozen sample (5 g) was pasted with mortar and pestle with 25 mL of methanol, flushed with nitrogen for 5 min, and extracted for 1 h at 30°C in a Gyrotory water bath shaker at 200 rpm. The extract was filtered through a 0.2-micron syringe nylon filter and analyzed for total polyphenols and Trolox equivalent antioxidant capacity (TEAC).

Total Polyphenols. Total polyphenols were estimated colorimetrically using the Folin-Ciocalteu method (25). Sample aliquots of 500 μL were added to 500 μL of water, 5 mL of 0.2 N Folin-Ciocalteu reagent, and 4 mL of saturated sodium carbonate solution (75 g/L), and mixed in a cyclomixer. The absorbance was measured at 765 nm with a Shimadzu UV-visible spectrophotometer (Norcross, GA) after incubation for 2 h at room temperature. Quantification was done based on the standard curve generated with 100, 200, 300, and 400 mg/L of gallic acid.

Antioxidant Capacity Assay. Antioxidant capacity assay was performed on the Shimadzu UV-visible spectrophotometer in a kinetic mode based on the method of Re et al. (26). Briefly, ABTS⁺ radical cation was produced by reacting 7 mM 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2.45 mM potassium persulfate after incubation at room temperature in dark for 16 h. The ABTS⁺ solution was diluted with ethanol to an absorbance of 0.70 ± 0.1 at 734 nm. The filtered sample was diluted with ethanol so as to give 20–80% inhibition of the blank absorbance with 20 μL of sample. ABTS⁺ solution (980 μL ; absorbance of 0.70 ± 0.1) was read at 734 nm for a minute; exactly after 1 min, 20 μL of the sample was added and mixed thoroughly. Absorbance was continuously taken at every 6 s up to 7 min. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-

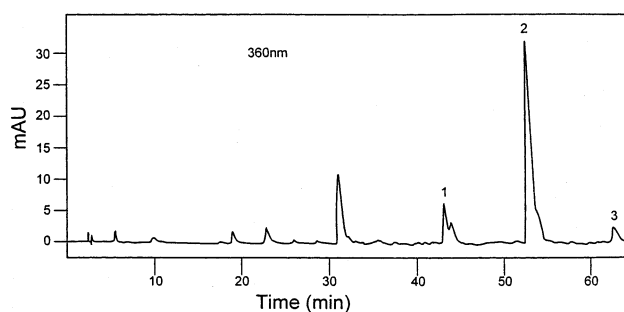


Figure 1. HPLC Chromatogram of onion sample Nirvana. Peaks: 1, kaempferol; 2, quercetin; and 3, myricetin.

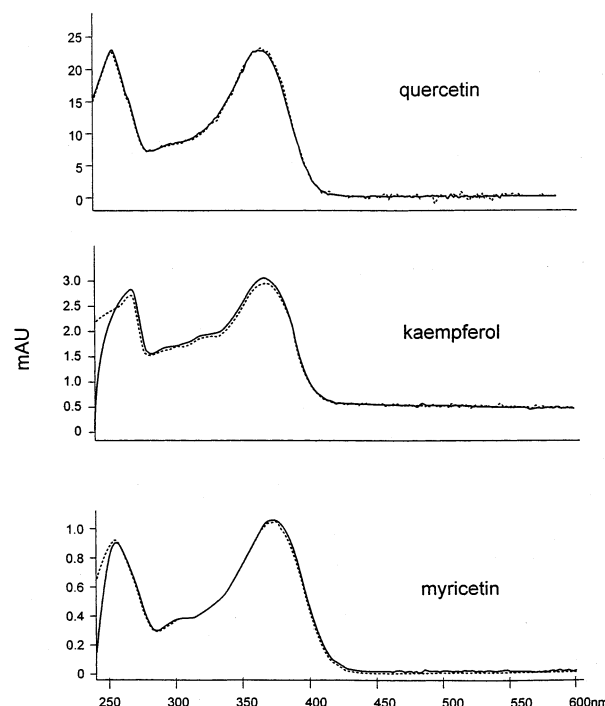


Figure 2. UV-visible spectra of sample (solid line) and standard (dotted line) of quercetin, kaempferol, and myricetin.

2-carboxylic acid, a vitamin E analogue) standards of final concentration 0–15 μM in ethanol were prepared and assayed under the same conditions. Trolox equivalent antioxidant capacity of sample was calculated based on the inhibition exerted by standard Trolox solution at 6 min.

Dry Weight Determination. Approximately 10 g of each sample was dried in an oven at 105°C for 16 h. The weight difference was calculated and expressed as g/100 g FW.

Statistics. The Statistical Analysis Systems software (27) was used to analyze data. Data are expressed as average of triplicate experiments. Duncan's multiple range test for variable was used to determine significant differences. Significance was determined at $p < 0.05$.

RESULTS AND DISCUSSION

The HPLC chromatogram of the Nirvana onion variety is shown in **Figure 1**. Three flavonoids were separated and identified in the following order: kaempferol, quercetin, and myricetin, with the retention times of 43.26, 52.82, and 62.74 min, respectively. The UV-visible spectra (240–600 nm) of all three flavonoids were recorded and compared to those of corresponding standards as shown in **Figure 2**. The spectra match factors for all three compounds were above 980/1000 as performed with ChemStation software.

Table 1. Flavonoids, Total Polyphenols, and Trolox Equivalent Antioxidant Capacity (TEAC) of *Vidalia* Onions^a

onion variety	dry weight (g/100 g FW)	flavonoids (mg/100 g FW)				total polyphenols (mg/100 g FW)	TEAC (μ M/g FW)
		kaempferol	quercetin	myricetin	total flavonoids		
Nirvana	9.95 \pm 0.00	1.83 \pm 0.03 ^{ab}	16.62 \pm 1.81 ^c	3.26 \pm 0.03 ^c	21.71 \pm 1.87	73.33 \pm 1.14 ^e	1.13 \pm 0.04 ^c
DPS 1032	10.10 \pm 0.00	1.98 \pm 0.21 ^a	46.32 \pm 1.44 ^a	4.13 \pm 0.26 ^a	52.43 \pm 1.91	131.47 \pm 1.33 ^b	1.56 \pm 0.03 ^a
Yellow 2025	10.00 \pm 0.00	1.89 \pm 0.04 ^{ab}	43.36 \pm 1.09 ^b	3.73 \pm 0.03 ^b	48.19 \pm 1.13	180.84 \pm 3.53 ^a	1.04 \pm 0.05 ^d
King-Midas	10.00 \pm 0.00	1.54 \pm 0.08 ^c	8.57 \pm 0.06 ^d	3.20 \pm 0.06 ^c	13.31 \pm 0.2	95.91 \pm 3.27 ^c	1.19 \pm 0.02 ^b
SBO 133	10.00 \pm 0.00	1.74 \pm 0.04 ^{bc}	7.70 \pm 0.34 ^d	2.77 \pm 0.00 ^d	12.21 \pm 0.38	78.30 \pm 2.75 ^d	0.92 \pm 0.07 ^c

^a Values are average and standard errors of triplicates. FW = Fresh weight. Values with the same letter in each column are not significantly different at $p < 0.05$ as determined by Duncan's multiple range test.

Table 1 shows the dry weights of the onion varieties. The average value was approximately 10.0 g/100 g FW. The distribution of quercetin, myricetin, and kaempferol in five varieties of onions is also shown in **Table 1**. Quercetin is the dominant flavonoid present in all varieties. Among five varieties, DPS 1032 had significantly ($p < 0.05$) the highest concentration of quercetin (46.32 mg/100 g, fresh weight, FW), which is 6 times higher than that of SBO 133 (7.70 mg/100 g FW) and 5.4 times higher than that of King-Midas (8.57 mg/100 g FW). Nirvana had approximately three times less quercetin (16.62 mg/100 g FW) than DPS 1032. Quercetin content of Yellow 2025 (43.36 mg/100 g) was second highest to DPS 1032. King-Midas and SBO 133 had the least quercetin and are not significantly different from each other ($p < 0.05$). The values are in good agreement with previous reports of 378 mg/kg FW (28), 385 mg/kg FW (16), 409.5 mg/kg (18), 410 mg/kg (21), 520.3 mg/kg (29), and 518 mg/kg FW (30). Highest reported values of quercetin were 1187 (31), 942.8 (32), and 933.5 mg/kg (33), and the lowest reported values were 65 (19) and 170 mg/kg (34). Quercetin occurs in free, as well as in conjugated, form with carbohydrates, mainly as glucosides. The presence of 20 other minor forms of quercetin has also been reported (20). The two major glucosides of quercetin present in onions are quercetin 3,4'-diglucoside and quercetin 4'-glucoside (29), and these represent approximately 80% of the total flavonol content of onion (20).

Kaempferol was identified and quantified in all five varieties and ranged from 1.54 to 1.98 mg/100 g FW, which was slightly higher than previously reported values of 3–7 mg/kg (19). Kaempferol content of Nirvana, DPS 1032, and Yellow 2025 were not significantly different from each other, and King-Midas had the least. Kaempferol has been reported in onions in minor quantities in comparison to quercetin as kaempferol 3- and 4'-glucoside (34). Onions grown in the United States were reported to have kaempferol at 0.68 g/kg in the outer dry skin and 3–7 mg/kg in outer and inner skins of bulb (19), whereas onions grown in the United Kingdom did not have any detectable quantities of kaempferol (18). These variations may be due to many factors including variety, climatic conditions, and maturity.

Myricetin was detected and quantified in all varieties. DPS 1032 had the highest concentration (4.13 mg/100 g FW) and SBO 133 had the lowest concentration (2.77 mg/100 g FW). Myricetin content of Nirvana and King-Midas were not significantly different ($p < 0.05$) with values of 3.26 and 3.20 mg/100 g FW, respectively. The presence of myricetin in green onion tops has also been reported (36).

The percent distribution of each flavonoid is shown in **Figure 3**. Quercetin is the most abundant flavonoid present, and ranged from 63.06 to 89.97%, which is in good agreement with values previously reported (20). Myricetin ranged from 7.74 to 24.04%, which is found to be the second largest flavonoid. Kaempferol is present in the range of 2.28–14.25%.

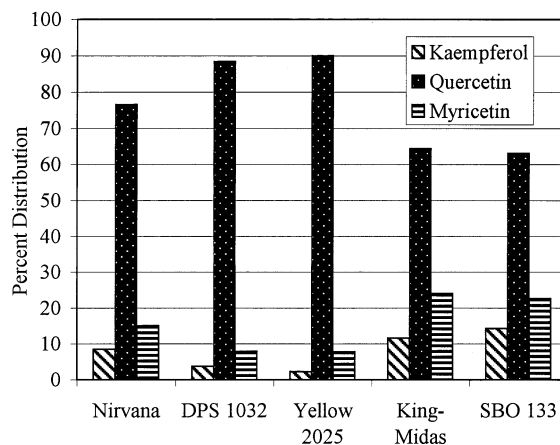


Figure 3. Percentage distribution of individual flavonoids. Quercetin, kaempferol, and myricetin together represent 100%.

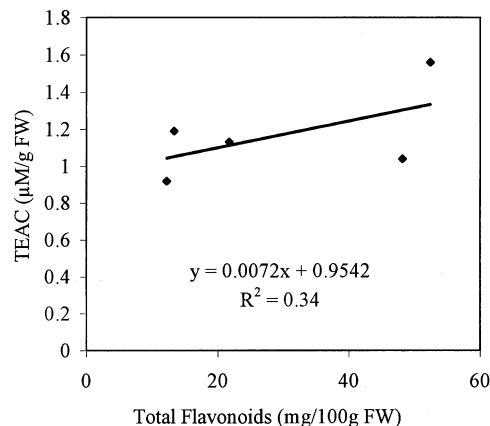


Figure 4. Correlation curve between total flavonoids and Trolox equivalent antioxidant capacity.

Further analyses (**Table 1**) were performed to estimate the total polyphenols and Trolox equivalent antioxidant capacities (TEAC) of the samples. The total polyphenols content of Yellow 2025 was the highest (180.84 mg/100 g FW) among all varieties and Nirvana had the lowest (73.33 mg/100 g FW). It should be noted that the total polyphenol contents of all varieties were significantly different from each other ($p < 0.05$). The percentages of flavonoids in total polyphenols were calculated. DPS 1032 had the highest flavonoid 39.87% of total polyphenols. The rest of the samples, Nirvana, Yellow 2025, SBO 133, and King-Midas had 29.60, 26.64, 15.59, and 13.87%, respectively.

A high antioxidant capacity of 1.56 μ M TEAC/g FW was observed for DPS 1032 despite its lower content of total polyphenols (131.47 g/100 g) than that of Yellow 2025. However, the total flavonoids content was higher for DPS 1032 (52.43 mg/100 g FW) than for Yellow 2025 (48.19 mg/100 g FW). As with total polyphenols, the antioxidant capacities of

the onion varieties differed from each other ($p < 0.05$). To further understand the relationships among antioxidant capacity and total polyphenols and flavonoids, the correlation coefficients were calculated. The relationship between total polyphenols and antioxidant capacity was positive but weaker ($r^2 = 0.04$, graph not shown). This is in agreement with a previous report of a correlation between total polyphenols and antioxidant capacity ($r^2 = 0.85$) in berries (37). Nevertheless, the correlation between total flavonoids and TEAC seems to be much better ($r^2 = 0.34$) as shown in **Figure 4**. This could be because quercetin, myricetin and kaempferol are well-known antioxidants.

In conclusion, quercetin was the major flavonoid (89.97%) in Georgia-grown *Vidalia* onions, followed by minor quantities of myricetin and kaempferol. The presence of measurable quantities of myricetin is a unique and an interesting feature of these onions.

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