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# Antioxidants of 15 onions with white, yellow, and red colors and their relationship with pungency, anthocyanin, and quercetin

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## ABSTRACT

We have investigated the antioxidant activity (AOA) of 15 short-day onions with white, yellow, or red colors and elucidated the relationships between pungency, anthocyanins, quercetin, and the AOA levels. There were substantial variations in both the pungency and total soluble solid content, which showed varying responses by bulb colors. The AOA in white or red onions tended to have low and high levels, respectively. However, there were many exceptions. The AOA levels assessed by the Folin–Ciocalteu (F–C) assay ranged between 440 and 785  $\mu\text{g}/\text{mL}$ . The AOA levels based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay were between 19.1 and 79.8  $\mu\text{g}/\text{mL}$  and displayed 10- to 20-fold differences. There was no correlation between the pungency and the AOA levels. The F–C assay showed no or low correlation with anthocyanin ( $r^2 = 0.07$ ) and quercetin ( $r^2 = 0.59$ ) contents, respectively. The DPPH assay showed correlations with anthocyanin ( $r^2 = 0.65$ ) and quercetin ( $r^2 = 0.76$ ) contents. Therefore, the onions with higher levels of anthocyanin and quercetin would have still higher AOA levels. Samples with alliinase action showed higher AOA levels than the samples without enzyme action. Onion juice without anthocyanin and flavonoids still contained considerable AOA levels. The methanol extract and fresh onion juices showed similar AOA levels by the F–C assay.

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## 1. Introduction

Onions have many health benefits and are one of the major sources of various biologically active phytochemicals, including phenolic acids, flavonoids, cepaenes, thiosulfates, and anthocyanins (Fossen & Andersen, 2003; Slimestad, Fossen, & Vågen, 2007). The quercetin compounds are major flavonoids in onions and are related to skin colors and disease resistance in plants (Trammell & Peterson, 1976). Quercetin has various human health benefits and inhibits cancer cell growth (Chu, Chang, & Hsu, 2000). It also functions as an antioxidant, chelating agent (Torel, Cillard, & Cillard, 1986), and free radical scavenger (Murota & Terao, 2003). The onion ranked highest in quercetin compounds among 29 vegetables and 9 fruits (Galdón, Rodríguez, & Romero, 2008) and has been suggested as a healthy food to prevent coronary heart disease (Hertog, Hollman, & Katan, 1992).

The red onion color is caused by anthocyanins consisting of mainly cyanidin or peonidin glucosides that are acylated with malonic acid or nonacylated (Mazza & Miniati, 1993). There are at least 25 different anthocyanins from red onions. The content of anthocyanin is approximately 10% of the total flavonoid content or 39–240 mg/kg fresh weight (Slimestad et al., 2007). Anthocyanins are also reported to be a source of antioxidant activity (Geetha, Ponmozhi, Saravanakumar, & Suganyadevi, 2011). However, white onions contain less flavonoid content than colored onions (Herrmann, 1976).

The pungent flavor of onions is caused by numerous volatile sulfur compounds produced when alliinase reacts on the flavor precursor compounds (S-alk(en)yl-cysteine-sulfoxides) after the tissues are mechanically damaged (Lancaster & Boland, 1990). One of the sulfur volatiles, thiopropal S-oxide, is a lachrymatory factor uniquely found in onions (Thomas & Parkin, 1994). Pyruvic acid is also produced as a byproduct and has been used to measure the pungency of onions (Randle & Bussard, 1993). The sulfur compounds in onions and garlic have been reported to reduce or prevent many chronic diseases, such as cancer, coronary heart disease, obesity, and hypercholesterolemia (Lanzotti, 2006).

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Reactive oxygen species (ROS) are produced in cells by cellular metabolism and other exogenous environmental agents. The overproduction of ROS can damage cellular biomolecules, such as nucleic acids, proteins, lipids, and enzymes, and can result in several diseases (Prior, Wu, & Schaich, 2005). Antioxidant scavengers of these free radicals are associated with a reduced risk of cancer and cardiovascular diseases. Onions have high levels of antioxidant properties (Stajner & Varga, 2003). Therefore, onions are a food associated with a reduced risk of various human diseases (Sanderson, Mclauchlin, & Williamson, 1999). The total antioxidant capacity is highest in onion peel ethanol extract and plasma quercetin, and the isorhamnetin levels in rats were markedly increased by feeding the onion peel powder and extract (Park, Kim, & Kim, 2007).

There are various ways to measure antioxidant activity (AOA) in plant extracts. The Folin–Ciocalteu (F–C) assay has been widely used due to its convenience of measurement (Prior et al., 2005). Although originally developed to measure phenolic content in protein (Folin & Ciocalteu, 1927), this assay can also be used to estimate AOA because the chemistry is based on the reduction of the F–C reagent (Prior et al., 2005). This reagent reacts with phenolic and many non-phenolic compounds, such as ascorbic acid, reducing sugars, and amino acids (Singleton, Orthofer, & Lamuela-Raventos, 1999; Waterhouse, 2002). Therefore, the chemical nature of a sample may cause significant interference with the total AOA levels. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is also widely used due to its simple and convenient procedure (Prior et al., 2005). The color absorption of the reactant steadily declines over time, and approximately 2 h is recommended to obtain reliable results (Yoo, Lee, & Patil, 2011). Because there are several types of reactions between various reduction compounds in samples and assay reagents, every method measures different reduction compounds. Thus, it is common that two different assays result in quite different responses or patterns (Prior et al., 2005).

We have been developing low pungency, high quercetin containing red onion cultivars in our breeding program to provide more health benefits, including antioxidants. However, the relationship between antioxidant activities vs. onion pungency and color has not yet been characterized. In this study, we investigated the antioxidant activity of 15 short-day onions with white, yellow, and red bulb colors using the F–C and DPPH assays to elucidate the relationships between pungency, anthocyanin, and quercetin contents and the AOA levels.

## 2. Materials and methods

### 2.1. Chemicals

All chemicals and solvents used in this study were purchased from Sigma (St. Louis, MO., USA) or Fisher Scientific (Pittsburg, PA, USA).

### 2.2. Plant materials

Fifteen short-day onion genotypes with different colors (2 white, 10 yellow, 3 red) shown in Fig. 1 were used. The onions were planted in mid-October and harvested in late-April from the field of the Texas A&M University Research and Extension Center at Weslaco (26°15'N, 97°98'W) in the Rio Grande Valley of South Texas, USA. The onions were managed according to the standard culture practice. The fields were clay or loam soil with pH ranging from 7.4 to 8.2. The plots were 102 cm wide and 150 cm long, and the onions were planted in two rows 12 cm apart in the row. When the plants matured and the foliage was down, the onions were harvested and transported to the laboratory for analysis.

### 2.3. Pyruvic acid and total soluble solids measurements

The procedure described by Hamilton, Yoo, and Pike (1998) was followed. The neck and base of the onions were cut approximately 1-cm from the surface and the dry skin was removed. The bulb was cut in half vertically, and a half was sliced into ~2 cm square pieces and blended in a home mixer for ~2 min (without adding water). The completely macerated puree was incubated for 30 min at room temperature (24 °C) and filtered through Whatman no. 2 filter paper to collect juice. The juice was stored in a 15-mL plastic tube and preserved in a –20 °C freezer until pyruvic acid analysis.

The pyruvic acid content of the onion juice was measured by the automated method (Lee, Yoo, Jifon, & Patil, 2009a, 2009b). A 10- $\mu$ L fresh juice sample was injected into a dinitrophenylhydrazine (DNPH) stream at 0.7 mL/min (25 mg DNPH and 25 mL H<sub>3</sub>PO<sub>4</sub> in 1 L water) with an Alltech 570 autosampler. The mixture was heated at 70 °C and mixed with 0.5 mol/L NaOH solution, at a rate of 1.4 mL/min. The signal was detected with a UV/Visible detector set to 485 nm. A sodium pyruvate standard ranging from 0 to 10  $\mu$ mol/mL was used to construct standard curves. The TSS content in the onion juice was measured with a hand refractometer and expressed as °Brix.

### 2.4. F–C assay

The F–C assay was modified in a 10:1 scale reduction (Wang et al., 2003) to accommodate a 15-mL-tube (Yoo, Lee, Leskovaar, & Patil, 2012). For this assay 50  $\mu$ L of onion juice was mixed with 4.75 mL of F–C solution (10-fold diluted with water) for 5 min. Then, one mL of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) (20 g/100 mL) was added, and the solution was mixed well before incubating for 2 h at room temperature (24 °C). The absorbance of the mixture was measured with a spectrophotometer at 760 nm. The total phenolic content was calculated using the standard curve of gallic acid in a range between 16.1 and 500  $\mu$ g/mL with 2-fold dilution series. The result was expressed as gallic acid equivalent (GAE) per mL juice.

### 2.5. DPPH assay

The AOA for DPPH radicals was determined by a spectrophotometric method as described by Brand-Williams, Cuvelier, and Berset (1995), with some modifications. The reaction solution was prepared by dissolving 24 mg of DPPH in 100 mL of ethanol solution (80 mL ethanol in 100 mL water). Then, 20  $\mu$ L of onion juice was reacted with 3500  $\mu$ L of DPPH solution for 2 h in the dark. The absorbance was measured at 515 nm. The AOA was calculated using the standard curve of gallic acid in a range between 16.1 and 500  $\mu$ g/mL with a 2-fold dilution series. The result was expressed as  $\mu$ g of gallic acid equivalents (GAE) per mL juice.

### 2.6. Anthocyanin measurement

The onion juices of the 3 red genotypes prepared in the pyruvic acid measurement were used (N = 30). Two hundred microliters of the juice was mixed with 3 mL of 1 mol/L HCl solution, and the absorbance was measured at 530 nm. The concentration was calculated by using the molar extinction coefficient of cyanidin-3-glucoside of 29,600. The results are expressed as  $\mu$ g of cyanidin-3-glucoside equivalents per mL juice (Lee, Yoo, & Patil, 2011). We also conducted a correlation analysis between anthocyanin content and AOA.

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