Comparison of Phenolic Content and Antioxidant Capacity of Red and Yellow Onions

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Abstract

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The total polyphenol (TP), flavonoid, proanthocyanidin (PAC) content, and antioxidant capacity of both onion varieties (red and yellow) were compared. The content of TP, flavonoids, and PAC was determined by Folin-Ciocalteu colorimetric method, $AlCl_3$, and by DMAC colorimetric method, respectively. The results showed that the contents of TP and flavonoids decreased from the outer to the inner layers in both onions, but there was no significant difference in PAC content. The outer layers had the highest antioxidant activity of extracts followed by a continuous decrease towards the inner layers in both varieties. The contents of phenolic acids and flavonoids were quantified by HPLC. Gallic acid, ferulic acid, and quercetin, as the main compounds in polyphenols, were detected in each layer of both onions. The red variety showed better antioxidant activity than yellow onion according to the linoleic acid system and DPPH assay. The higher contents of TP and flavonoids were associated with higher antioxidant activity.

Keywords: *Allium cepa* L.; antioxidant activity; polyphenols; flavonoids

Onion (*Allium cepa* L.) is one of the most consumed vegetable planted widely across the world. Many reports have indicated that onions have a wide range of beneficial properties for human health, such as anti-cholesterolaemic (Yin & Cheng 1998), anti-mutagenic (Singh *et al.* 2009), and antioxidant capacity (Lu *et al.* 2011; Pérez-Gregorio *et al.* 2011). Recently, an increasing attention has been paid to the antioxidant content of onion because epidemiological studies have indicated that regular consumption of onions is associated with a reduced risk of neurodegenerative disorders, many forms of cancer, and cataract formation (Roldán *et al.* 2008).

Antioxidants can scavenge radicals by three major mechanisms: hydrogen atom transfer, electron transfer, and combination of both these transfers (Prior *et al.* 2005). The antioxidant capacity of onion was widely studied by both *in vivo* and *in vitro* methods. The common *in vitro* methods of antioxidant capacity are as follows: DPPH (1,1-diphenyl-2-picrylhydrazyl) assay (Roldán *et al.* 2008), ferric reducing antioxidant powder (Re *et al.* 1999), oxygen radical absorbance capacity (ORAC) (Zill-E-Huma *et al.* 2011), and ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate)] assay (Santas *et al.* 2008). The *in vivo* methods are based on animal models (mice, rabbit) or cells models (Caco-2, Raw 264.7) to measure the antioxidant activity, such as SOD (superoxide dismutase), LOX (lipoxygenase), MDA (malondialdehyde). However, *in vitro* methods are commonly used to evaluate the antioxidant activity of onion.

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Polyphenols, anthocyanins, flavonoids, quercetin, and their glycosides have been reported in onions (Rhodes & Price 1996). Polyphenols are natural substances in plants that are antioxidants with the potential to protect the body from some diseases. Previous studies showed that the main phenolics found in onion are quercetin, gallic acid, ferulic acid, and their glycosides (Singh *et al.* 2009; Pérez-Gregorio *et al.* 2010). Flavonoids are a class of phenolic compounds which impart bitter and astringent flavours to fruits and vegetables. Proanthocyanidins (PAC) are a class of flavonoids composed of flavan-3-ol monomeric units, these monomeric units can be constituted of two types of double linkage (A- and B-type) between the flavan monomeric units (Matsuo *et al.* 2010). Specifically, onion has been characterised for its flavonol quercetin and quercetin derivatives (Roldán-Marín *et al.* 2009).

Within the vegetable family, the composition and quantity of the phenolics vary significantly according to different intrinsic and extrinsic factors, such as plant genetics and cultivar, soil and growing conditions, maturity state and harvest conditions (Jaffery *et al.* 2003). The onion bulbs consist of multilayer tissues. Therefore, there may be differences in the aging of each layer tissue. The total polyphenol (TP), antioxidant capacity, specific phenolic compositions in each layer of onion varieties may also be different. But, the relevant information is scanty.

The aim of this research is to identify and quantify the phenolic compounds and antioxidant capacity of both common onion varieties in China, yellow and red onions. The phenolic compositions of each layer were analysed by HPLC-DAD method. The antioxidant capacity of extracts was analysed by two spectrophotometric methods (e.g. linoleic acid system and DPPH assay), and the correlation between the extract content and the antioxidant capacity was also determined in this study.

MATERIAL AND METHODS

Material. Red and yellow onions (*Allium cepa* L.) were purchased from the local market in Shandong Province (China). Onion bulbs exhibited a diameter of 80–90 mm. The dried outer layer of onions was removed. The entire onion was separated into six layers from the outer to the inner layer according to its own characteristics, and named layer 1, 2, 3, 4, 5, and 6, respectively. Then, the materials from each layer were chopped, blended and finally freeze-dried. The freeze-dried materials were stored in polythene bags and placed in a drier at 4°C.

Chemicals and reagents. All chemical reagents, methanol, acetic acid, potassium chloride, sodium carbonate, acetonitrile, linoleic acid, Tween-20, ammonium thiocyanate, ferrous chloride, HCl and DPPH were bought from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Vanillin, Folin-Ciocalteu phenol reagent (2 mol/l), 4-dimethylaminocinnamaldehyde (DMAC) and standard samples, gallic acid, ferulic acid, epicatechin, quercetin, and procyanidin B2, cyanidin-3-glucoside and rutin were purchased from Sigma-Aldrich Corp. The standard samples, quercetin-3,4'-diglucoside, quercetin-4'-glucoside, quercetin-3'-glucoside were purchased from Shanghai Tauto Biotechnology Co. Ltd. (Shanghai, China).

Extraction methods. About 500 mg of dried onion powder was added to 25 ml of methanol/water (80:20, v/v). The mixture was sonicated for 1 h at room temperature, and the supernatants were collected after centrifugation at 4000 *g* for 10 minutes. Extraction was performed twice, the extracts were combined for further analysis.

Determination of total polyphenol (TP) content. TP contents in the extracts were determined by the Folin-Ciocalteu colorimetric method (Slinkard & Singleton 1977). Absorbance of each sample was measured at 765 nm. Values were determined from a calibration curve prepared with gallic acid (ranging from 10 to 500 mg/l final concentration, $R^2 = 0.999$). TP content was expressed as gallic acid equivalent (GAE) in mg/g dry weight (DW) of samples).

Determination of flavonoid content. Flavonoid contents were determined as described by Santas *et al.* (2008). The absorbance was measured at 430 nm against a sample blank without reactants. Values were determined from a calibration curve prepared with rutin (ranging from 2 to 100 mg/l final concentration, $R^2 = 0.992$). Flavonoid content was expressed as rutin equivalent (RE) in mg/g DW.

Determination of proanthocyanidin (PAC) content. PAC contents in the extracts were determined by the DMAC colorimetric method (Prior *et al.* 2010). Absorbance of each sample was measured at 640 nm. Values were determined from a calibration curve prepared with procyanidin B2 (ranging from 2 to 100 mg/l final concentration, $R^2 = 0.995$). PAC content was expressed as procyanidin B2 equivalent (PBE)/10 g DW.

High performance liquid chromatography (HPLC) analysis. Identification of active ingredients in each layer of the yellow and red onion extracts, HPLC analysis method was used to identify and quantitative analysis for the phenolic acid and flavonoid compounds. HPLC analysis was performed using an Agilent 1200 chromatograph (Agilent, Santa Clara, USA) and chromatographic separations were performed on a LUNA C-18 column (5 µm × 250 mm × 4.6 mm) (Phenomenex, Torrance, USA). The composition of solvents and gradient elution conditions were described by Abdalbasit *et al.* (2010). The mobile phase was composed of solvent (A) water-acetic acid (95:5, v/v, pH 2.27) and solvent (B) acetonitrile. The solvent gradient was as follows: 0–15% B in 40 min, 15–45% B in 40 min, and 45–100% B in 10 minutes. A flow rate of 0.5 ml/min was used, and 20 µl of sample were injected. Samples and mobile phases were filtered through a 0.22 µm Millipore filter prior to HPLC injection. The wavelengths used for the quantification of the onion extracts with the diode detector were 280, 360, and 520 nm. Each treatment was analysed in duplicate.

Determination of antioxidant activity in emulsion system. The total antioxidant activity of onion extracts was determined according to a linoleic acid system (Ardestani & Yazdanparast 2007). The linoleic acid emulsion was prepared by mixing linoleic acid (0.280 g), Tween-20 emulsifier (0.280 g) and 50 ml of phosphate buffer (0.2M, pH 7.0). An aliquot of different extracts (0.5 ml) was mixed with linoleic acid emulsion (2.5 ml) and phosphate buffer (2 ml). The mixture reacted at 37°C for 60 min in the dark. The levels of peroxidation were determined according to the thiocyanate method by sequentially adding ethanol (5 ml, 75%), ammonium thiocyanate (0.1 ml, 30%), sample solution (0.1 ml), and ferrous chloride (0.1 ml, 20mM in 3.5% HCl). After mixing for 30 min, the antiradical activity was determined by reading the absorbance that was measured at 500 nm. The percentage of total antioxidant activity consumed was calculated from the following equation:

$$\text{Total antioxidant activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where:

A_0 – initial absorbance (no antioxidant)

A_1 – absorbance in the presence of the extracts

DPPH assay. The antioxidant capacity of the onion was measured using the DPPH method described by Sun *et al.* (2005) with minor modifications. Aliquots (1 ml) of extracts were added to 2 ml of DPPH solution (250 µmol/l) in methanol. The mixture was left in the dark at room temperature for 60 minutes. The absorbance was measured at 517 nm. The percentage of DPPH consumed was calculated from the following equation:

$$\text{Radical scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where:

A_0 – initial absorbance (no antioxidant)

A_1 – absorbance in the presence of the extracts

Statistical analysis. All experiments were carried out in quadruplicate, and expressed as mean ± standard deviation. Data were analysed using analysis of variance (SAS 9.0; SAS Inst., Cary, USA) and *t*-test to determine the statistical significance ($P < 0.05$).

RESULTS AND DISCUSSION

Total polyphenol (TP) content

The TP contents in the different layers of the red and yellow onion varieties are shown in Figure 1**a**. The TP contents were in the range from 6.06 ± 0.24 to 22.32 ± 1.62 GAE mg/g DW in yellow onions, and from 5.71 ± 0.20 to 18.58 ± 0.62 GAE mg/g DW in red onions. The TP contents in the yellow onions were higher than in the red onions. The inner layer of both varieties had the lowest TP contents followed by a continuous increase towards the outer part of the bulb. The TP contents in the different layers of yellow onions were similar to those of the red variety. Prakash *et al.* (2007) also investigated the TP contents in the different layers of onions, but the entire onion bulbs were divided into three parts only (outer, middle, inner). In this study, onion bulbs were divided into six layers according to their own characteristics. The results obtained from both varieties in the present study were consistent with the report by Prakash *et al.* (2007), although the onion varieties from other countries were different. The results suggested that the TP contents increase during the aging of the cells of the outer layer of onion bulbs, because it is known that the cells of the outer layer are more aged than those of the inner layer in a bulb.

Flavonoid content

The flavonoid contents in the different layers of both onion varieties were determined. The flavonoid contents from the inner to the outer parts in

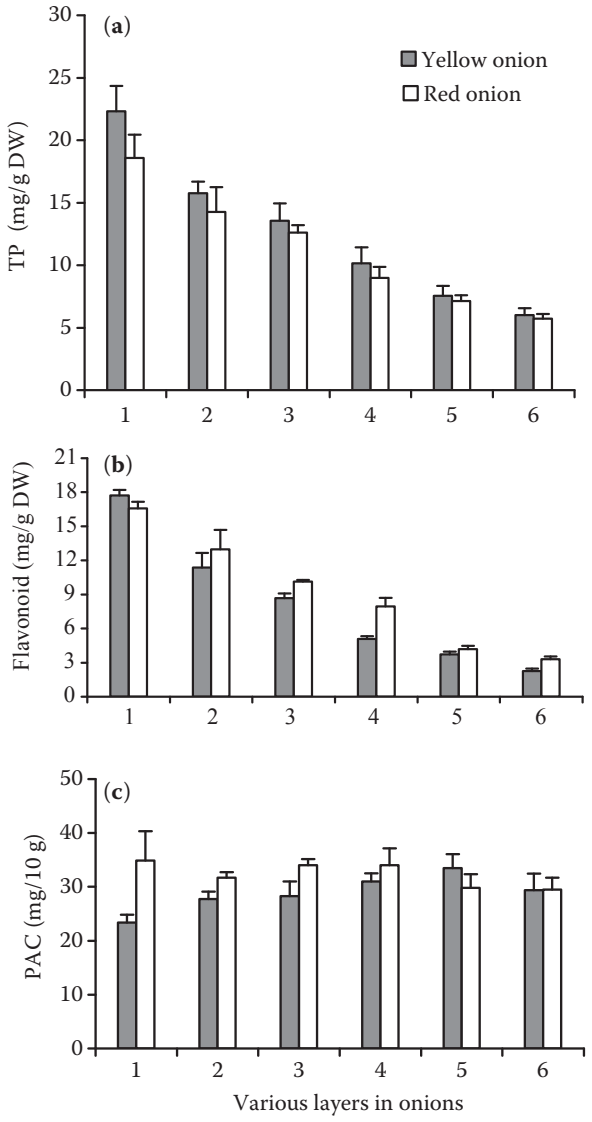

Figure 1. Contents of total phenols (TP), flavonoids, and proanthocyanidin (PAC) in each layer of yellow and red onions. (a) TP content expressed as GAE mg/g DW, (b) flavonoid content expressed as RE mg/g DW, (c) PAC content expressed as PBE mg/10 g DW

yellow onions varied from 2.25 ± 0.23 to 17.73 ± 0.70 RE mg/g DW, and from 3.31 ± 0.23 to 16.58 ± 0.41 RE mg/g DW in red onions (Figure 1b). The highest flavonoid content in the first layer was 7.9-fold compared to the sixth layer in yellow onion, and was only 5.0-fold in red onion. The flavonoid contents from the outer to the inner layers were decreasing more significantly compared with TP contents in onions. The flavonoid content in red onions was higher than that in yellow onions. Prakash *et al.* (2007) proved red onions to be richer in flavonols than yellow and pink onions. Some authors pointed out that flavonoids represent the main group of phenolic compounds in onion (Yang *et al.* 2004; Santas *et al.* 2008). The cause of changing regularity of flavonoids in different layers is the same as in polyphenols in onion. It is implied that flavonoid content increases during the aging of cells that constitute the layers of onion bulbs.

Proanthocyanidin (PAC) content

In contrast to flavonoids and polyphenols, the distribution of PAC was not investigated in onions previously. The PAC contents in various layers were relatively steady, in contrast to flavonoids and polyphenols (Figure 1c). The PAC content in red onion was higher than that in the yellow variety except for the fifth layers. Aoyama and Yamamoto (2007) pointed out that red onion contains proanthocyanins, which not only impart red colour but also participate in their strong antioxidant capacity.

Identification of phenolic compounds by HPLC analysis

The quantities of gallic acid in yellow onions varied from 265.5 ± 14.8 to 445.0 ± 22.5 mg/100 g DW, ferulic acid from 87.7 ± 10.4 to 259.8 ± 17.0 mg/100 g DW, QDG (quercetin-3,4'-diglucoside) from 12.9 ± 2.6 to 146.5 ± 12.4 mg/100 g DW, Q4'G (quercetin-4'-glucoside) from 0.7 ± 0.1 to 4.3 ± 0.7 mg/100 g DW, quercetin from 63.6 ± 9.0 to 521.3 ± 25.1 mg/100 g DW (Table 1). In red onions, gallic acid varied from 202.0 ± 10.9 to 355.8 ± 20.3 mg/100 g DW, ferulic acid from 141.4 ± 13.0 to 282.0 ± 16.6 mg/100 g DW, QDG from 20.3 ± 3.0 to 177.1 ± 12.5 mg/100 g DW, Q4'G from 1.2 ± 0.2 to 8.9 ± 1.2 mg/100 g DW, quercetin from 149.6 ± 12.3 to 533.7 ± 28.2 mg/100 g DW. Q3'G (quercetin-3'-glucoside) was present only in the red variety and varied from 1.0 ± 0.1 to 5.3 ± 0.4 mg/100 g DW. In red and yellow onions, the results showed that the mean content of gallic acid was the highest among all compounds, and followed by quercetin, which was identified as the second major compound.

The amounts of ferulic acid, QDG, Q4'G and quercetin in red onion were higher than in the yellow variety, while gallic acid in the red variety was lower compared to yellow onion (Table 1). The contents of five compounds (gallic acid, feru-

Table 1. Phenolic contents (mg/100 g DW)in the different layers of red and yellow onions

Variety	Part	Gallic acid	Ferulic acid	QDG	Q4'G	Q3'G	Quercetin
Yellow	layer 1	$445.0^{a} \pm 22.5$	$259.8^{a} \pm 17.0$	$146.5^{a} \pm 12.4$	$4.3^{a} \pm 0.7$	–	$521.3^{a} \pm 25.1$
	layer 2	$332.5^{b} \pm 20.1$	$248.6^{a} \pm 19.5$	$71.9^{b} \pm 9.2$	$2.4^{ab} \pm 0.4$	–	$282.4^{b} \pm 18.2$
	layer 3	$308.6^{b} \pm 18.5$	$222.7^{a} \pm 16.9$	$64.7^{b} \pm 8.3$	$1.4^{b} \pm 0.2$	–	$247.1^{b} \pm 17.3$
	layer 4	$321.6^{b} \pm 17.5$	$121.3^{b} \pm 11.9$	$34.2^{c} \pm 6.9$	$1.4^{b} \pm 0.2$	–	$167.0^{c} \pm 12.2$
	layer 5	$265.5^{c} \pm 14.8$	$97.6^{c} \pm 10.7$	$23.3^{cd} \pm 5.3$	$0.8^{c} \pm 0.1$	–	$116.1^{d} \pm 10.7$
	layer 6	$312.1^{b} \pm 12.1$	$87.7^{d} \pm 10.4$	$12.9^{d} \pm 2.6$	$0.7^{c} \pm 0.1$	–	$63.6^{e} \pm 9.0$
Red	layer 1	$355.8^{a} \pm 20.3$	$282.0^{a} \pm 16.6$	$177.1^{a} \pm 12.5$	$8.9^{a} \pm 1.2$	$5.3^{a} \pm 0.4$	$533.7^{a} \pm 28.2$
	layer 2	$308.4^{b} \pm 20.0$	$227.0^{b} \pm 13.7$	$146.1^{a} \pm 11.1$	$7.1^{b} \pm 1.1$	$3.9^{b} \pm 0.3$	$360.2^{b} \pm 17.1$
	layer 3	$238.1^{c} \pm 13.9$	$203.0b \pm 15.2$	$72.3^{b} \pm 10.0$	$4.2^{c} \pm 0.6$	$2.9^{bc} \pm 0.2$	$313.8^{bc} \pm 16.5$
	layer 4	$202.0^{c} \pm 10.9$	$176.4^{c} \pm 14.0$	$69.9^{b} \pm 8.6$	$5.8^{c} \pm 0.7$	$3.3^{b} \pm 0.2$	$357.0^{b} \pm 14.1$
	layer 5	$219.6^{c} \pm 13.2$	$184.0^{c} \pm 12.6$	$44.5^{c} \pm 4.8$	$1.8^{d} \pm 0.1$	$2.1^{c} \pm 0.1$	$272.6^{c} \pm 12.0$
	layer 6	$218.3^{c} \pm 12.0$	$141.4^{d} \pm 13.0$	$20.3^{c} \pm 3.0$	$1.2^{d} \pm 0.2$	$1.0^{d} \pm 0.1$	$149.6^{d} \pm 12.3$

QDG – quercetin-3,4'-diglucoside; Q4'G – quercetin-4'-glucoside; Q3'G – quercetin-3'-glucoside

lic acid, QDG, Q4'G, and quercetin) in the outer layer (layer 1) were the highest in the bulb. Similar results were reported in the literature (Prakash *et al.* 2007; Zill-E-Huma *et al.* 2009). Amounts of five compounds decreased from the outer to the inner layers in both varieties. The outer layer of both varieties was found to be the richest source of gallic acid, ferulic acid, QDG, Q4'G, and quercetin. The amounts of QDG, Q4'G, and quercetin in red onions were higher than in the yellow variety. We pointed out that there is a continuous decrease in the content from the outer to inner layer, because the cells of the outer layer are more aged than those of the inner layer in a bulb. And there may be another point that the outer layers provide defence against predators and phenolic compounds have been implicated as defence chemicals in plants.

Antioxidant activity

The total antioxidant activity of both onions was measured using linoleic acid assay (Figure 2). The total antioxidant activity of extracts from yellow and red onions is shown in Figure 2a. In yellow onion, the percentage of total antioxidant activity in the different layers varied from 36.74 ± 1.15% to 80.58 ± 1.51%. In red onion, the percentage of antioxidant activity varied from 41.22 ± 3.0% to 81.96 ± 1.10%. For both varieties, the highest antioxidant percentage of extracts from the outer layer was almost 2.2-fold to the lowest percentage of extracts from the inner layer. And the total antioxidant activity decreased from the outer to the inner layers. The total antioxidant activity in red onion was higher than in the yellow variety.

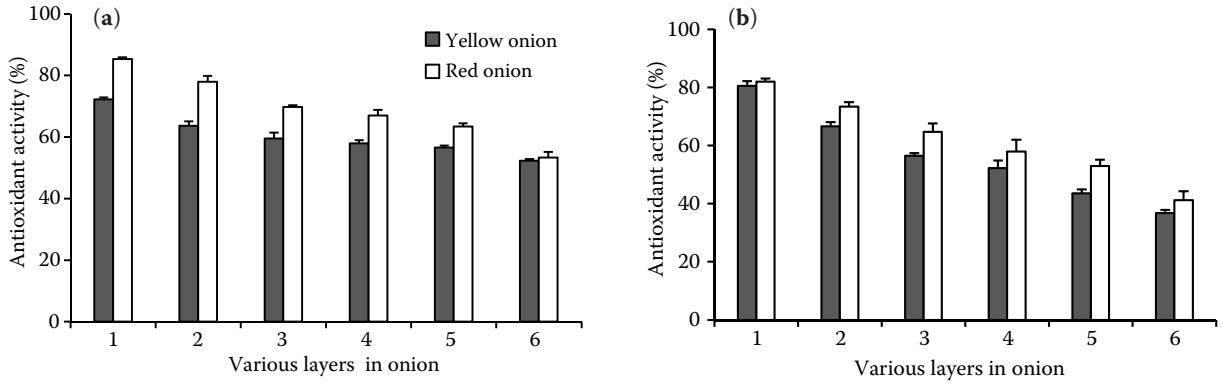

Figure 2. Antioxidant activity in layers of both onion varieties: (**a**) total antioxidant activity and (**b**) DPPH radical scavenging activity

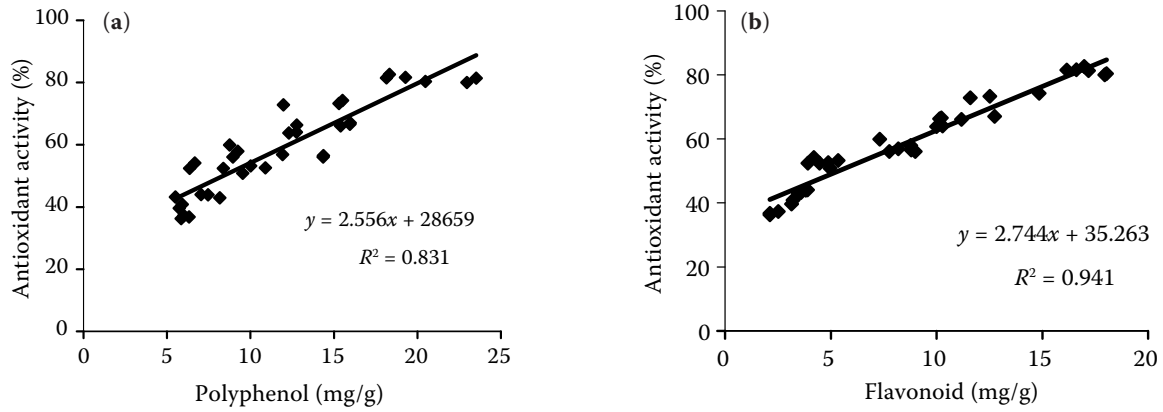

Figure 3. Correlation between (**a**) polyphenol and (**b**) flavonoid content and total antioxidant activity

The correlations between the total antioxidant activity and the polyphenol contents, and flavonoid contents were also determined in this study (Figure 3). The results showed that the correlation (R^2 = 0.941) between the flavonoid contents and the total antioxidant activity was better than between the polyphenol contents and the total antioxidant activity (R^2 = 0.831). The correlation value indicated that the flavonoids are mainly responsible for the total antioxidant activity. Flavonoids, known for their high antioxidant activity, are the main polyphenols present in onion bulbs. Nuutila *et al.* (2003) observed that onion extracts were effective in inhibiting peroxidation. Flavonoids are known as good inhibitors of lipid oxidation in onion (Lanzotti 2006).

DPPH is a stable highly coloured free radical that can absorb hydrogen atoms from polyphenol antioxidants with simultaneous formation of a colourless hydrazine (DPPH-H) (Dioufe *et al.* 2009). The DPPH method is frequently used to determine the antioxidant activity. The red onion extracts showed good antioxidant activity varying from 53.36 ± 1.77 to 85.53 ± 0.58%, and better than in the yellow variety ranging from 52.32 ± 0.50 to 72.25 ± 0.66% (Figure 2b). The results showed that the antioxidant activity remarkably decreased from the outer to the inner layer in onions, which is consistent with flavonoid contents. The difference in the TP contents of vegetable varieties is reflected by the antioxidant activity of extracts, as reported previously (Sellappan & Akoh 2002). In the present study, the antioxidant values in the outer layer of red onions were in a similar range to the previous results (Dhan *et al.* 2007).

There is a better correlation between the flavonoid content and DPPH value (R^2 = 0.826) compared with TP contents and DPPH value (R^2 = 0.629), which confirm that the antioxidant

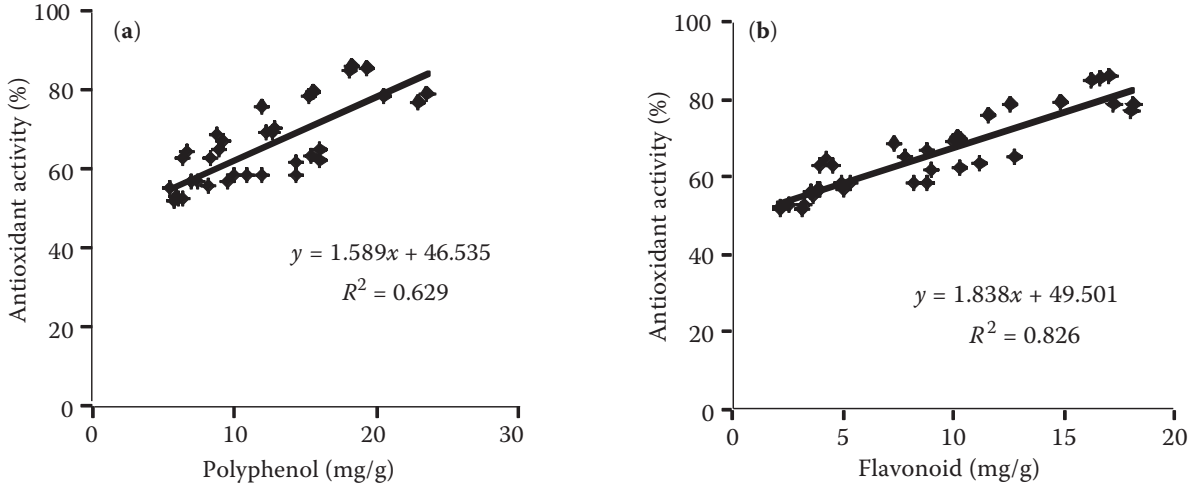

Figure 4. Correlation between (**a**) polyphenol and (**b**) flavonoid content and DPPH

activity is mainly mediated by flavonoids in the onions (Figure 4). Several studies have reported a good correlation between the TP content of plant extracts and antioxidant activity (Bahorun *et al.* 2004). But other studies have reported a poor correlation (Sellappan & Akoh 2002). In this study, the correlation between polyphenol/flavonoid contents and the total antioxidant activity was better than that between polyphenol/flavonoid contents and DPPH value.

CONCLUSIONS

The phenolic content, antioxidant activity, and the correlation between phenolic content and antioxidant capacity were studied in both red and yellow onion. The major polyphenols were identified as gallic acid and ferulic acid, the major quercetin as QDG and Q4'G. A decreasing tendency was observed in TP and flavonoid content from the outer to the inner layers in both varieties. The outer layers had the highest antioxidant activity of extracts followed by a continuous decrease towards the inner layers. The antioxidant activity of the onion extracts was better correlated with the flavonoid contents in a statistically significant manner compared to the TP contents, which indicated that mainly flavonoids were responsible for the antioxidant capacity. Onions were rich sources of polyphenols and flavonoids, and showed the promising antioxidant and free radical scavenging activities. From this study it could be concluded that there is a real possibility of using those onion for developing natural ingredients with bioactivated properties.

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