The Canadian Society for Bioengineering
The Canadian society for engineering in agricultural, food, environmental, and biological systems.

CSBE | SCGAB

La Société Canadienne de Génie Agroalimentaire et de Bioingénierie
La société canadienne de génie agroalimentaire, de la bioingénierie et de l'environnement

Paper No. CSBE16-021

Screening Ontario grown onion varieties for antioxidant properties

Cynthya Maria Manohar

BioNano Laboratory, School of Engineering, University of Guelph Guelph, Ontario, N1G 2W1, Canada.

Abdul Murayyan

BioNano Laboratory, School of Engineering, University of Guelph Guelph, Ontario, N1G 2W1, Canada.

Suresh Neethirajan*

BioNano Laboratory, School of Engineering, University of Guelph Guelph, Ontario, N1G 2W1, Canada.

Jun Xue

Guelph Food Research Centre, Agriculture and Agri-Food Canada, N1G 5C9, Canada.

John Shi

Guelph Food Research Centre, Agriculture and Agri-Food Canada, N1G 5C9, Canada.

Written for presentation at the
CSBE/SCGAB 2016 Annual Conference
Halifax World Trade and Convention Centre
3-6 July 2016

ABSTRACT Agricultural by-products are in great demand in nutra-pharmaceutical and biomedical industries. Several studies have indicated that flavonoid based foods helps in reducing the incidence of cardiovascular diseases, breast cancer and diabetes. Onions are rich source of flavonoids and possess high levels of antioxidant activity. In our study, Ontario grown onion varieties namely Stanley, Safrane, Fortress, Lasalle and Ruby Ring were screened for their antioxidant properties. Here Low polarity water technology was used to extract the flavonoids from them and were identified by High Performance Liquid Chromatography. The free radical scavenging activities in the extracted samples were employed using 2,2-diphenyl-1-picrylhydrazyl(DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and Ferric reducing ability of plasma (FRAP) assays. The total polyphenolic content was higher in the red onion variety, namely the Ruby Ring (1.69±0.15 mg/g, $p<0.05$), when compared to the yellow onion variety. Stanley had the highest amount of total flavonoids (0.34±0.03 mg/g of onion, $p<0.05$). The DPPH, ABTS and FRAP scavenging activities were highest in the red onion variety (21.52±1.3 %; 95.8±0.3%; 0.20±0.002 mM respectively; $p<0.05$) when compared to the yellow onion variety. A very strong correlation between the total polyphenol content and antioxidant activities were also observed. The current study indicates that Ruby ring variety can be chosen to develop functional and health foods. The identification, enhancement and development of market quality traits such as antioxidants properties of Ontario-grown onions will enhance their marketability, export potential and sales. The results from this study will allow the farmers to grow preferred onion varieties with desired set of criteria for enabling the nutra-pharmaceutical industries.

Keywords: Flavonoids, Onions, Antioxidant activity, Total Polyphenols.

1. INTRODUCTION

Several epidemiological studies carried out indicated that consumption of vegetable and fruits helps in reducing many chronic diseases such as diabetic, cancer, coronary heart diseases and Alzheimer's disease (Hertog, 1996). One-third of cancer and cardio vascular diseases could be reduced by consuming proper diet. Free radicals in our body are created when cells use oxygen to generate energy.The formation and the activity of reactive oxygen species (ROS) leads to potentially harmful effects. Therefore in order to limit the levels of ROS, antioxidants are required. Antioxidants are present in many fruits and vegetables. Compounds such as phenolics, thiols and acids, that are present naturally in plants exhibit antioxidant activity. They scavenge free radical species and inhibit the production of reactive oxygen species. Antioxidants prevent proteins, lipids, DNA and cellular damage and death (Saija et al., 1995).

Cancer is one of the leading cause of death in people in both developed and developing nations. Cohort studies show that consumption of dietary rich food decreases the risk of cancer, immune dysfunctions and cardiovascular diseases. Naturally occurring flavonoids has received global attention since they are found in abundance in our daily diet as well as they impart several health benefits. Onions are richest sources of flavonoids and anthocyanins. Flavonoid has potential antioxidant, antibacterial, anti-inflammatory and anti-cancerous properties (Yamazaki et al., 2014).

Flavonoids are a set of polyphenolic plant secondary metabolites. Flavonoids, especially quercetin mono- and diglucosides are widely present in human diet. Epidemiological and in vitro and in vivo experimental data indicate that regular intake of onions is connected with a decreased risk of degenerative ailments. Onions (Allium cepa L.), are also a rich source of organosulfur compounds and most of the sulphur compounds present in them are in the form of cysteine derivatives, e.g. S-allyl cysteine sulfoxide. These compounds are also reported to have several potential health benefits, including preventing tumors and cancers. Polymerization of flavonoids in foods into large molecules are either by the plants themselves or during food processing. These are called tannins. The main flavonoid that is present in the Allium cepa is quercetin. The glycosides that constitute 80% of flavonoids in onion are quercetin 4 –O-β-glucoside and quercetin 3, 4-O-β-diglucoside. Other flavonoids that are present in onion are kaempferol, isorharmnetin derivatives, and myricetin. The glycosyl unit in these is identified as glucose. The bulb colour and the type depend on the amount of flavonoids present in them (Søltoft et al., 2009).

Onions are the second most common agricultural crop in the world. The pungency and flavour of onions are due to the sulphur compounds present in it. The great diversity of both the onion colour and flavour is due to their composition and concentration of phenolic compounds. The edible bulb portion of the red onion varieties will generally be higher in total flavonoids than the white onion varieties. Yellow onions will exhibit higher levels of quercetin flavonoid content than red onion varieties (Caridi et al., 2007).

Onion is one of the most important agricultural crops produced in Canada (mostly in the eastern provinces of Ontario and Quebec) with an annual production of 210,000 tons at a market value of $ 74 million (Gianessi, 2013). Although, onions are the major source of dietary flavonoids, due to genetic and environmental differences thorough screening of the onions for flavonoid and phenolic contents is needed. At present there is a mounting interest in the promotion of horticultural food crops with high amounts and desired composition of flavonoids. The differences in the phenolic and flavonoid content and their individual concentration amongst onion types will be a useful knowledge to the farmers or breeders in selecting variety with specific anticipated potential health benefits. Identification, enhancement and development of market quality traits such as antioxidants properties of onions will enhance their marketability as nutraceutical products, as natural antibiofilm coating agent for improved shelf life of packaged food and as a preservative in processed food (Yang et al., 2004).

In Ontario there are 17 varieties of onions are grown and out of these we chose five varieties since they are the most commonly used ones. Globally, the nutraceutical market is expected to grow at a rate of 6.4 % to reach US $ 204.8 billion by 2017. The demand for antioxidants and antimicrobial coatings and films for food industries is estimated to reach US $ 2.7 billion by the year 2018 (King et al., 2014). There is not enough scientific evidence to back up any nutraceutical claims of Ontario-grown onions. Till date, the health effects of Ontario grown onion extract have not been extensively explored. The unique properties of onion based flavonoids, including their biocompatibility, biodegradability, are that they are safe to eat and absence of toxicity make them more appealing to develop them as useful products than synthetic polymers or chemical based compounds for various nutraceutical and functional food applications.

In the present study we screen Ontario grown onion varieties namely Stanley, Safrane, Fortress, Lasalle and Ruby Ring for their antioxidant properties, and isolated the flavonoids present in them. Extraction with low polarity water technology produced flavonoids and anthocyanins such as kampferol, quercetin, myricetin, isorhamnetin, cyanidin 3-(6''-malonylglucoside) delphinidin 3-glucoside, petunidin (glycosylglucoside), delphinidinaglycona from all these varieties. High Performance Liquid Chromatography analysed the extracted flavonoids and showed the presence of sulphur containing compounds. For all the five varieties both the total phenolic and flavonoid contents were estimated. The antioxidant efficacy of these extracts were evaluated by ferric reducing antioxidant power (FRAP) assay, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. These assays provided a better knowledge of the nutrient profile of the Ontario grown onion varieties as well as significant differences among these five varieties.

2. MATERIALS AND METHODS: Ontario grown onion varieties namely Stanley, Safrane, Fortress, Lasalle and Ruby Ring were used in this study.

2.1. Chemicals and reagents: Folin-Ciocalteu reagent (FCR), sodium carbonate, Aluminum Chloride, potassium acetate, Gallic acid, 2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), potassium persulfate, potassium hexacyanoferrate, ferric chloride and ascorbic acid were purchased from Sigma Aldrich Canada. 10% trichloroacetic acid were purchased from Labchem. HPLC grade water, methanol, formic acid, 96-well polystyrene microtiter plates were purchased from Fisher Scientific, USA.

2.2. Sample preparation and storage: Onion varieties sample were graciously donated by Holland Marsh Growers's Association of Ontario. Four medium sized onions of the same variety were cleaned, peeled and the skins of the onions were removed. The onions were then chopped, grounded and placed at -30ºC overnight. The paste from the five varieties were placed in a freeze drier for 72h. The freeze- dried material were grounded to a fine powder and stored in air tight container at -20ºC. The fine powder from the five varieties were selected for analysis.

2.3. Sample extraction: The flavonoids were extracted from onion samples by pressurized low polarity water technology at the facilities of the Guelph Food Research Centre of Agriculture and Agri-Food Canada. This type of extraction is environmentally friendly, ideal for extracting flavonoids-rich onion ingredients, and scalable for commercial use. The extractions were carried out using an automated Speed SFE NP model 7100 instrument (Applied Separation Inc., Allentown, PA, USA),equipped with a pump (Module 7100) and 10-mL thick-walled stainless cylindrical extractor vessel. The freeze dried onion powder (5 g) was mixed with 80 mL 0.1% formic acid in milli-Q water (v/v), and injected into extraction

vessel. The extraction parameters were: temperature at 60ºC, pressure at 150 bar, and extraction time of 60 min (Hawthorne et al., 1994).

2.4. High performance liquid chromatography (HPLC) Analysis: The samples were identified and analyzed using an Agilent 110 series HPLC system. The flavonoids were separated using a reverse phase C18 Luna column (Phenomenex, USA, 250 x 4.6 mm; 5 m). The mobile and stationary phase's solvent were A- 0.1 % formic acid: methanol (9:1; v/v) and solvent B- methanol. A continuous flow of mobile phase at 800µL/min were set up in the column at 254 nm. The following was the gradient program: 5% B for 1 min, a linear gradient to 50% B for 34 min, then to 100% B for 5 min, the isocratic elution for 4 min, followed by a min ramp back to 5 % B and re-equilibration for 6 min. The λ Absorbance Detector used for identification was set at 254 nm (sampling rate: 1.0/s). The flavonoids were quantified by integrating the peak areas (Søltoft et al., 2009).

2.5. Determination of Total Polyphenol Content: The total polyphenols contents (TPC) of the onion extracts were determined by Folin-Cicocalteau assay. Gallic acid was used as a standard. The onion extracts were oxidized with Folin-Ciocalteau reagent, and the reaction was neutralized with 7.5% sodium carbonate. The optical density (OD) was measured at 760 nm after 30 min in dark condition at room temperature. The OD values were measured by a plate reader (ELISA plate reader (Serial Number 80-2115-80 Amersham Biosciences Corp., USA). Gallic acid was used as a standard and the absorbance values were compared and calculated with those of standards (Singleton et al., 1999).

2.6. Determination of Total Flavonoid Amount: To determine the total flavonoid content in the five varieties a calorimetric method using aluminium chloride was used. A total of 0.5 mL of the onion extracts were mixed with 0.1ml of 10% aluminium chloride and 0.1mL of 1M potassium acetate and diluted with 2.8mL of distilled water. The samples were incubated at room temperature for 30 minutes. Using 96 well plate the OD values at 415nm were measured by a plate reader (ELISA plate reader (Serial Number 80-2115-80 Amersham Biosciences Corp., USA). The total flavonoid content is determined by a quercetin standard curve. Experiments were repeated thrice (Dewanto et al., 2002).

2.7. DPPH assay (2,2-diphenyl-1-picryl-hydrazyl-hydrate): DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), a stable free radical was used for determining free radical scavenging activity of the extracted onion samples. The DPPH radical scavenging method was determined as reported by Kedare and Singh (2011) with slight modifications. The reaction is initiated by mixing 500µL of the extracted sample in 3 mL of absolute ethanol. Then add 50µM of DPPH to the above mixture. The DPPH reacts with the antioxidant compound. The whole experiment is done in dark condition for 30 min. The change in color (from deep violet to light yellow) is read in a UV Visible spectrophotometer at 517nm.

Ascorbic acid and Quercetin were used as standard controls. Distilled water was used as blank. Using the following equation DPPH scavenging activity was calculated:

$$\text{Inhibition (\%)} = \{(A_{blank} - A_{sample})/A_{blank}\} \times 100 \quad (1)$$

Where A_{blank} is the absorbance of the control sample which contains all reagents except the test sample and A_{sample} is the absorbance of the test sample which contains all the reagents including the test compound.

2.8. ABTS assay (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid): Generation of ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation (blue/green ABTS•+ chromophore) through the reaction between ABTS and potassium persulphate is measured (Rael et al., 2004).The dye was diluted with distilled water to obtain a stock solution at 7mM. 2.45 mM potassium persulfate was added and the mixture will be allowed to stand in the dark condition for 12 to 16 h at room temperature. This resulted in the ABTS radical cation solution. The ABTS solution was then diluted with 80% ethanol to an absorbance of 0.70 at 734 nm and equilibrated at 30°C. 1mL of the ABTS reagent prepared was added to 100µL of the extracted samples. The mixture was incubated in dark condition for 10 mins at room temperature. Then the absorbance was measured at 734nm using a microplate reader. Deionized water was used as blank. Each sample were measured in triplicates and the percent inhibition (%) was calculated using the following equation:

Ascorbic acid and Quercetin were used as standard controls. Distilled water served as blank. Using the following equation DPPH scavenging activity was calculated:

$$\text{Inhibition (\%)} = \{(A_{blank} - A_{sample})/A_{blank}\} \times 100 \quad (1)$$

Where A_{blank} is the absorbance of the control sample and A_{sample} is the absorbance of the test sample.

2.9. Ferric Reducing Antioxidant Power (FRAP) Assay: The ferric reducing antioxidant power assay (FRAP) is an assay which is based on the reduction of Fe^{III+} to Fe^{II+} due to the action of antioxidants present in the sample. 0.25 µL of the different sample extracts was taken and mixed with25 µL of 0.2 M of phosphate buffer and 0.25mL of potassium hexacyanoferrate [K3Fe(CN)6] was added. The mixture was incubated in hot water bath for 20 min. Add 0.25mL of trichloroacetic acid solution (10% w/v) (Szydlowska-Czerniak et al, 2008). The tubes were then centrifuged for 10 min at 15,000g.The supernatant was mixed with ferric chloride solution and 0.5mL of water. Absorbance of the supernatant was measured at 593 nm against blank to determine the reducing power. Ascorbic acid (0µg/mL to 200µg/mL) acid was used as a standard and the absorbance values were compared and calculated with those of standards.

2.10. Statistics: All the experiments were repeated thrice on three different samples and the data was reported as mean ± standard errors. One-tailed t-tests was performed using an R statistical programming software.

3. RESULTS:

3.1 Identification of Flavonoids and anthocyanins by HPLC analysis: Eight common flavonoids were identified in yellow onion varieties and six anthocyanins were identified in the red onion variety. The HPLC analysis of these onion varieties is shown in the Figure 1. Quercetin, quercetin 7, 4'-diglucoside,kaempferol, isohamnetin, isohamnetin 4'-glucoside, quercetin 4-glucoside, and quercetin 3-glucoside with the retention times 20mins,22 min, 30min, 32min, 21 min and 12min were identified in yellow onion varieties. Cyanidin 3-(6''-malonylglucoside), delphinidin 3-glucoside, petunidin glucoside, delphinidin aglycon, petunidin (glucosylgucoside) and delphinidin glucosylglucoside with retention times 24min, 28min, 30min, 33min, 21min and 19 min were identified in red onion varieties. Quercetin is the major flavonoid found in the yellow onion varietyand cyanidin 3-(6''-malonylglucoside) is

the major anthocyanin compound. Delphinidin and petunidin does not have malonyl derivatives since malonylated derivatives is an exclusive feature of cyaniding (Gennaro et al., 2002).

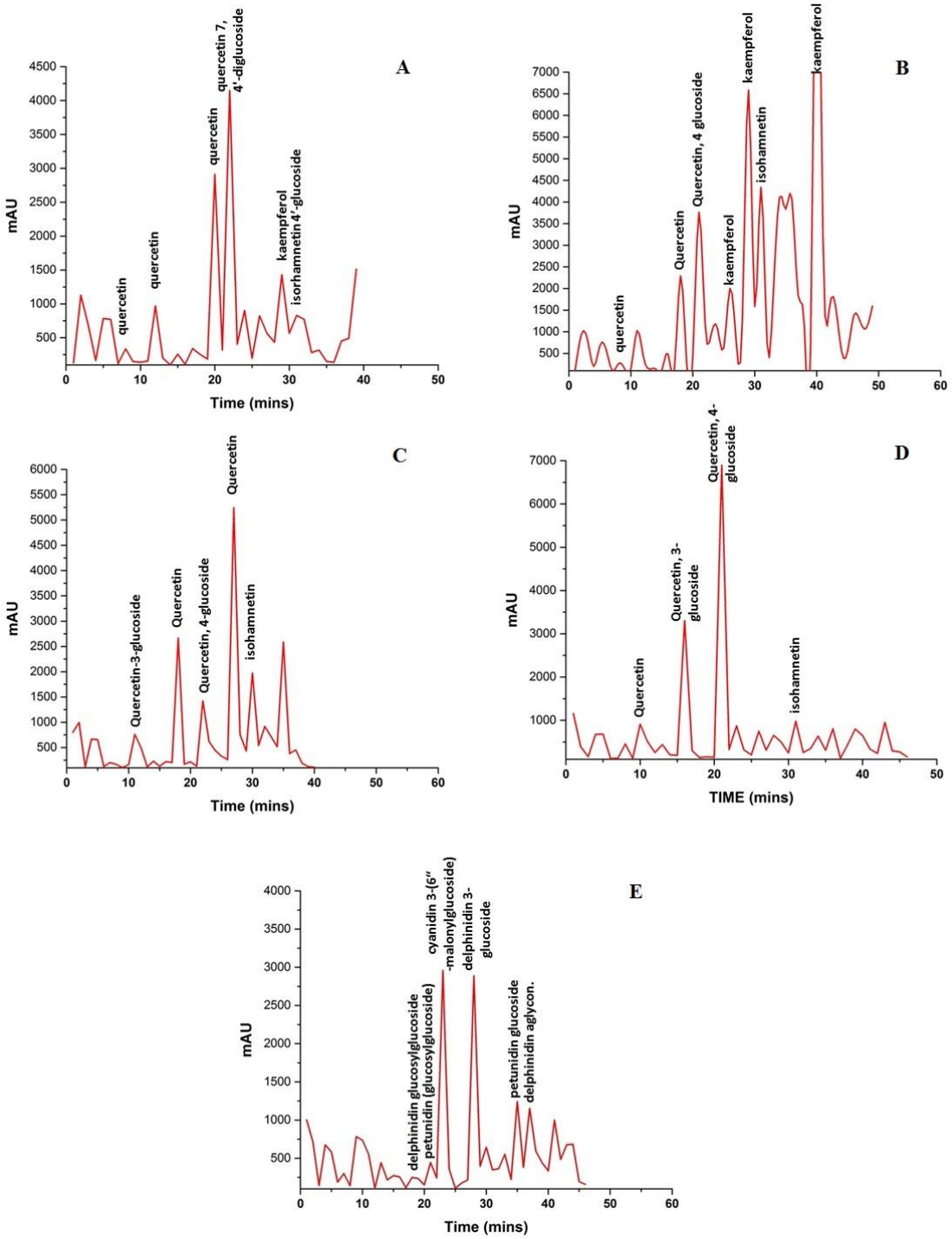

Figure 1. HPLC chromatogram at 254nm identified the flavonoids and anthocyanins extracted in various Ontario grown onion varieties A) Fortress B) Safrane C) Lasalle D) Stanley E) Ruby Ring.

3.2 Total Phenolic content of Ontario grown Onion varieties: The total phenolic content of the onion varieties are shown in Figure 2 Ruby Ring (red onionvariety) showed the highest phenolic content ($p<0.05$) at 1.95±0.05 mg of gallic acid equiv/g of sample, followed by the yellow onion varieties Stanley (1.69±0.13), Safrane (1.62±0.1), Lasalle (1.28±0.2) and Fortress (1.27±0.15). A statistical significant difference existed in the total phenolic content in various onion samples ($p<0.05$). There was a 0.3-fold difference in the total phenolic content between the Ruby ring (highest ranked) and Fortress (lowest ranked) varieties ($p<0.05$).

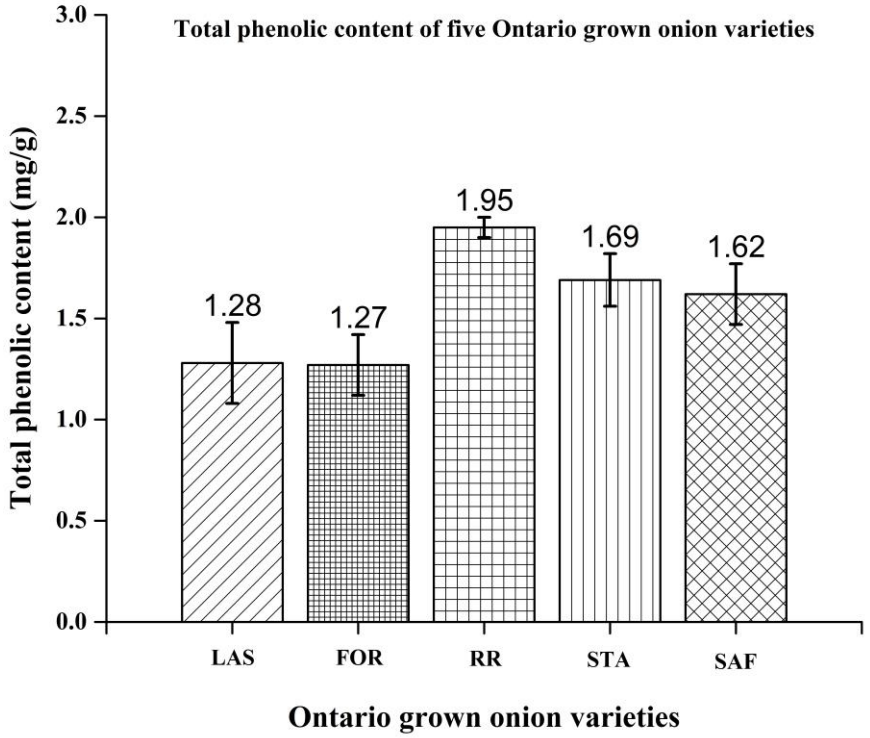

Figure 2. Total phenolic content of five onion varieties Lasalle (LAS), Fortress (FOR), Ruby Ring (RR), Stanley (STA) and Safrane (SAF) (mean ±SD, n= 5).

3.3 Total Flavonoid content of Ontario grown Onion varieties: The total flavonoid content of the five onion varieties are shown in Figure 3. Stanley showed the highest flavonoid content ($p<0.05$) at 0.33±0.03mg of quercetin equiv/g of sample, followed by the yellow onion varieties Ruby Ring (0.29±0.02), Fortress (0.15±0.02), Safrane (0.13±0.03) and Lasalle (0.12±0.02). A statistical significant difference existed in the total flavonoid content in various onion samples ($p<0.05$). There was a 2-fold difference in the total flavonoid content between the Stanley (highest ranked) and Lasalle (lowest ranked) varieties ($p<0.05$).

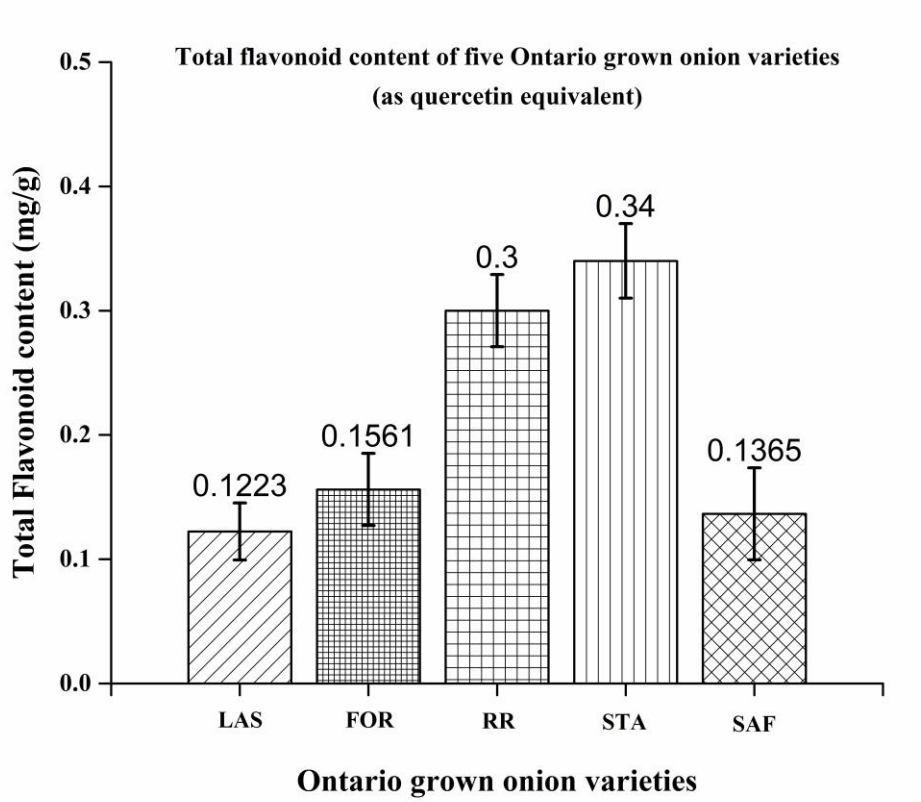

Figure 3. Total flavonoid content of five onion varieties Lasalle (LAS), Fortress (FOR), Ruby Ring (RR), Stanley (STA) and Safrane (SAF) (mean ±SD, n= 5).

3.4 Antioxidant activity: The radical scavenging activities of the different onion samples were determined by DPPH, ABTS and FRAP assays. The DPPH radical scavenging activities of different onion samples are shown in Figure 4. The DPPH scavenging activity in red onion variety (Ruby Ring) was highest when compared to the yellow onion varieties. Ruby Ring showed highest percent of inhibition 21.52±1.30 % ($p<0.05$) followed by Lasalle (15.46±3.88%), Fortress (13.44±4.19%), Stanley (11.38±1.96%) and Safrane (11.1±2.89%). DPPH radical scavenging activities of Ascorbic acid and Quercetin were 94.24±0.37% and 93.55±0.14%. A statistically significant difference existed in the DPPH radical scavenging activities of various onion samples ($p<0.05$).

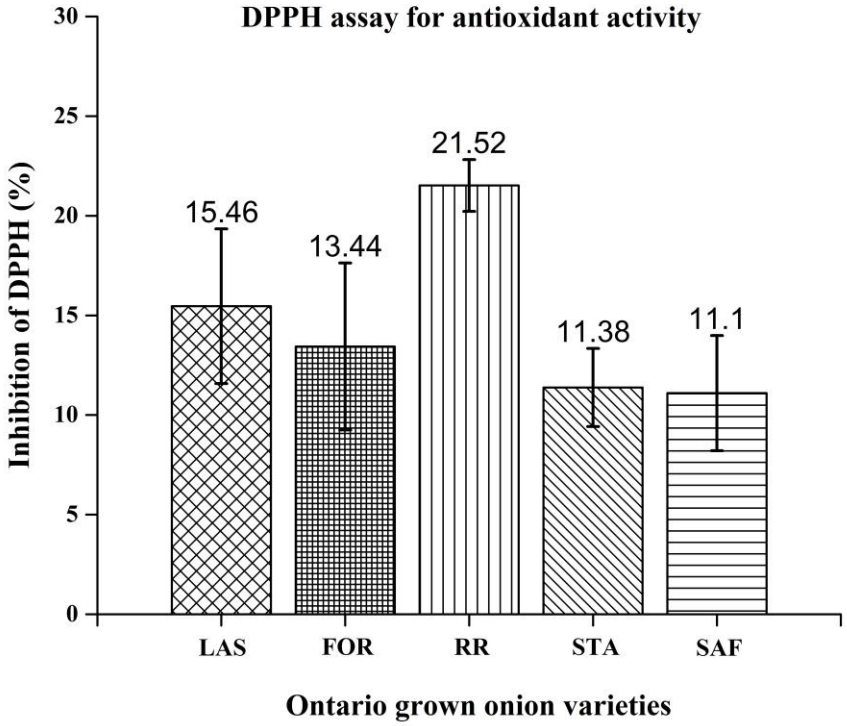

Figure 4. Standard controls ascorbic acid and quercetin was 94.24±0.37% and 93.55±0.14 %. Bar graph shows mean ± SD of % inhibition of DPPH (p-value= 0.009).

The ABTS radical scavenging activities of different onion samples are shown in Figure 5. The ABTS radical scavenging activity of red onion variety (Ruby Ring) was highest when compared to the yellow onion variety. Ruby Ring showed highest percent of inhibition 95.8±0.3% followed by Stanley (85.7±4.7%), Safrane (84.19±1.67%), Fortress (76.32±3.63%) and Lasalle (72.7±0.72%). ABTS radical scavenging activities of Ascorbic acid and Quercetin were 96.06±0.2% and 95.93±0.18%. A statistically significant difference existed in the ABTS radical scavenging activities of various onion samples ($p<0.05$).

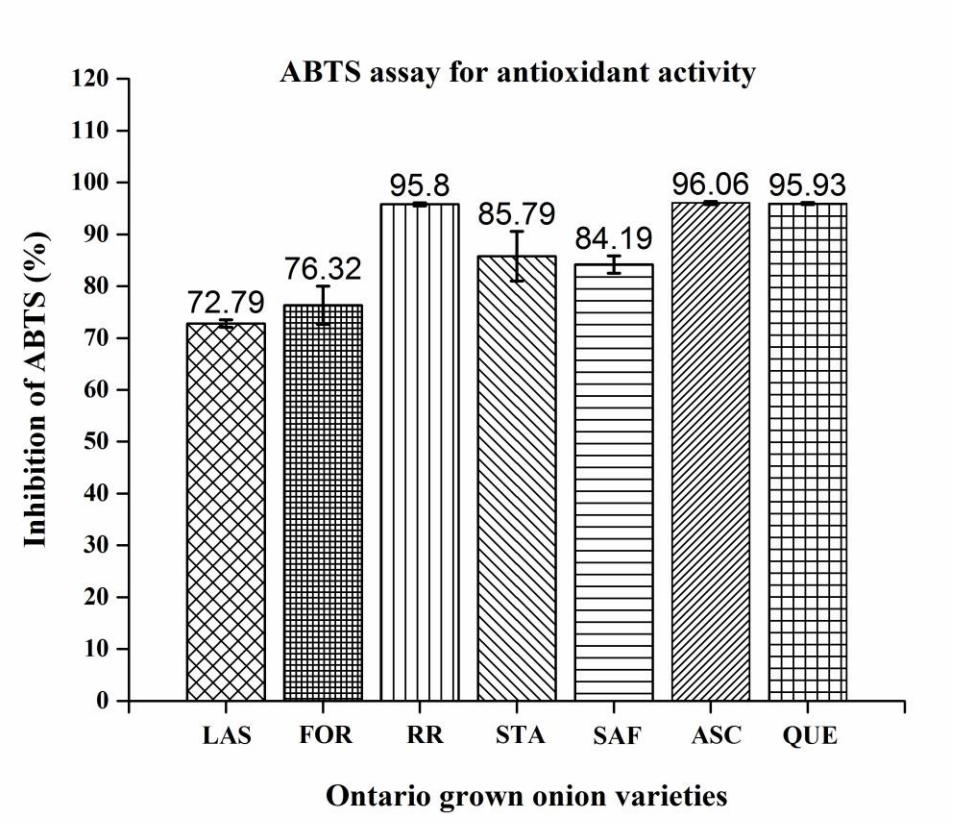

Figure 5. Standard controls Ascorbic acid (ASC) and Quercetin (QUE) was 96.06±0.28% and 95.93±0.18 %. Bar graph shows mean ± SD of % inhibition of ABTS; p=0.000.

The reducing power activity of the various onion samples are presented in Figure 6. In this assay Fe3+ was reduced to Fe2+in the presence of an antioxidant. Red onion variety showed the high absorbance indicating strong reducing power. Ruby Ring (red onion variety) showed the highest reducing power, 0.20±0.002 as mM (ascorbic acid equivalent)followed byStanley (0.18±0.003), Fortress (0.08±0.005), Safrane (0.06±0.003) and Lasalle (0.04±0.005). The data from these assays indicated that the red onion variety showed the highest radical scavenging activity compared to the yellow onion varieties. These results indicate that there is a possible relationship between the antioxidant activity and total flavonoid and total phenolic contents of the extracted samples.

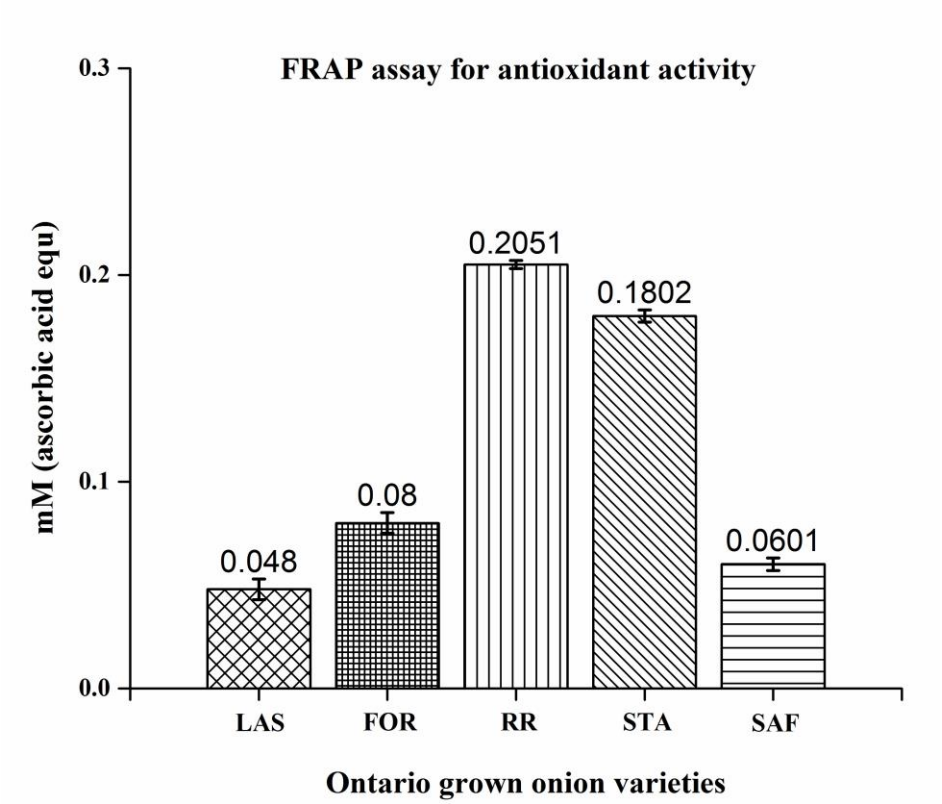

Figure 6. The reducing power activity of five onion varieties Lasalle (LAS), Fortress (FOR), Ruby Ring (RR), Stanley (STA) and Safrane (SAF) (mean ±SD, n= 5); p=0.000.

3.5 Correlations between Total Polyphenolic content (TPC), Total Flavonoid content (TFC) and Total Antioxidant Activity of the onion varieties: The correlations between total polyphenol content and total flavonoid content and total antioxidant activities are shown in Figure 7. Poor correlation was observed between TFC and DPPH activity and TPC and DPPH activity of the onions (0.23 and 0.43 respectively).

On the other hand excellent positive correlation was observed between TFC and FRAP activity (0.96), indicating increasing amounts of total flavonoid content in onions leading to increased FRAP activity. Also, excellent correlation was observed between TPC and ABTS activity (0.98), indicating increasing amounts of total phenolic content in onions leads to increase in the ABTS activity. Two linear regression relationships are developed for these activities as shown below and both the relations are statistically significant.

FRAP activity (as mM ascorbic acid equivalent) = 0.705 * TFC (as mg/g of onion as quercitin equivalent) – 0.032, R2 = 0.92, p = 0.01 (1)

ABTS activity (as % inhibition) = 34.94 +30.64 TPC (as mg/g), R2 = 0.97, p = 0.002 (2)

Although there was a good correlation between TFC and ABTS activity (0.72), the linear regression relation between these two parameters were not statistically significant.

ABTS activity (as % inhibition) = 69.19 +66.16 TPC (as mg/g), R2 = 0.53, p = 0.17 (3)

Similarly although there was a good correlation between TPC and FRAP activity (0.82), the linear regression relation between these two parameters were not statistically significant.
FRAP activity (as mM ascorbic acid equivalent) = 0.205 * TPC (as mg/g of onion as quercitin equivalent) – 0.207, R2 = 0.67, p = 0.09. (4)

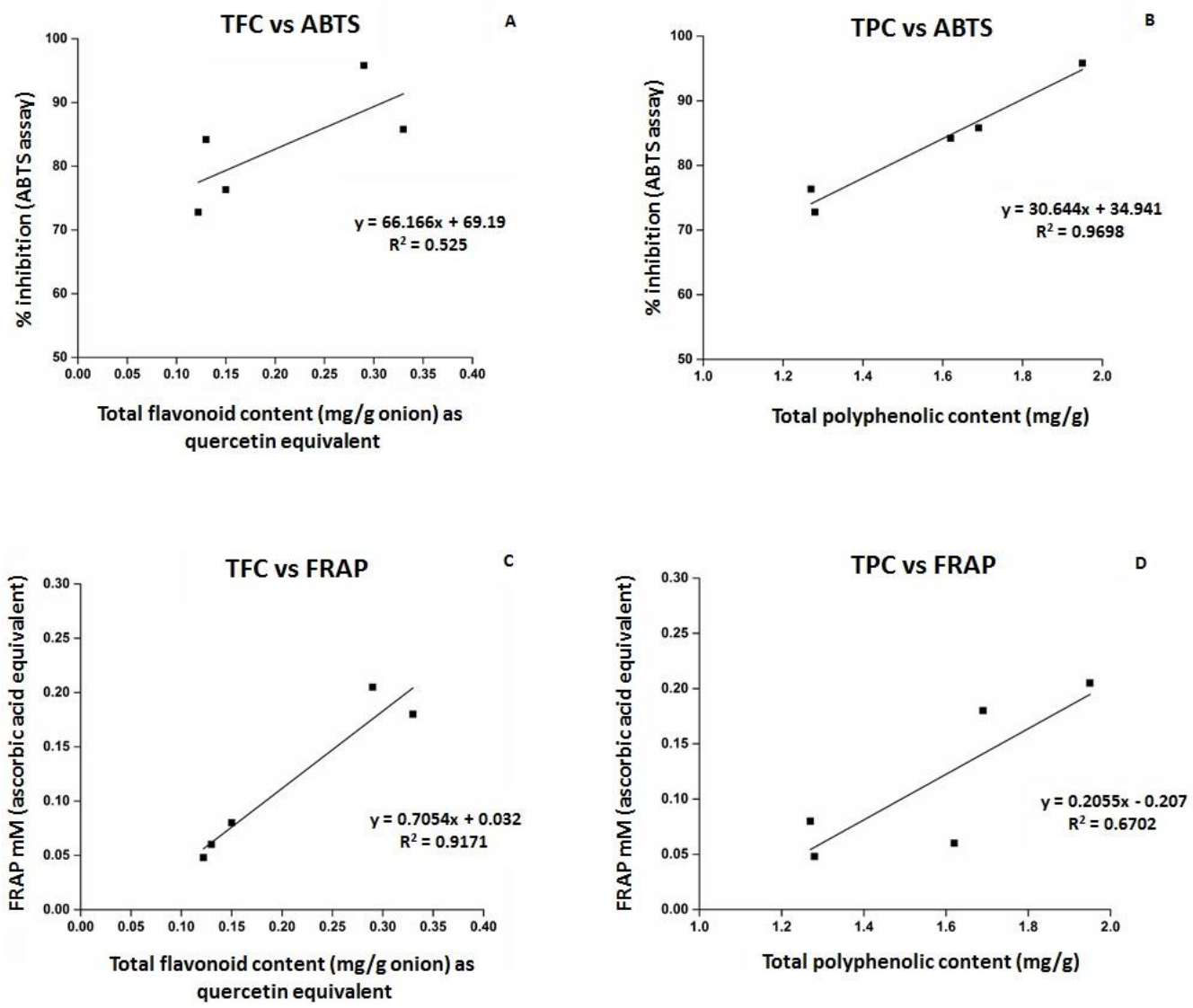

Figure 7. A) Correlation between total flavonoid content and percentage of antioxidant activity (ABTS scavenged effect) B) Correlation between total polyphenolic content and percentage of antioxidant activity (ABTS scavenged effect) C) Correlation between total flavonoid content and percentage of antioxidant activity (FRAP activity) D) Correlation between total polyphenolic content and percentage of antioxidant activity (FRAP activity).

4. DISCUSSION: Naturally occurring plant compounds such as phenolic exhibits high antioxidant activity that can protect against various chronic diseases such as cancer, cardiovascular, diabetes, and atherosclerosis. Onions contain strong antioxidant as well as free radical scavenging activities. Flavonoids extracted from onions possess high antioxidant activity. Quercetin in yellow onion variety and cyanidin 3-(6"-malonylglucoside) in red onion variety have been identified as major components and are in accordance with previously published papers (Gennaro et al., 2002; Sellapan et al., 2002; Caridi et al., 2007).Our antioxidant assay results showed that the red onion variety had higher antioxidant activity compared to other onion varieties.

Table 1 Comparison of Total flavonoid content (TFC) of Ontario grown onions (current study) with other varieties

VARIETY	TFC IN MG/100 G	REFERENCES
KING-MIDAS	13.3	Rhodes and Price (1996)
SBO 133	12.2	Rhodes and Price (1996)
RED	91.8	Rhodes and Price (1996)
PINK	71.1	Rhodes and Price (1996)
BROWN	80.3	Rhodes and Price (1996)
FROM TENERIFE (TEXAS, MASCA, GUAYONJE, SAN JUAN, CARRIZALBAJO AND CARRIZAL ALTO)	7 to 9	Rodriguez Galdon et al (2008)
GOLDEN BRONZE "DORATA DENSITY" FROM ITALY	97.9	Marotti and R.Piccaglia (2002)
FESTIVAL, TAMARA, DAYTONA, DORATA DENSITY, CASTILLO AND SANATANA FROM ITALY	52.5 to 93.3	Marotti and R.Piccaglia (2002)
INDIAN ONION VARIETIES (PUNJAB WHITE, PUNJAB NAROYAAND PRO-6)	10.0 to 13.2	Majid et al (2016)
ONTARIO GROWN ONION VARIETY - FORTRESS	15.6	Current study
ONTARIO GROWN ONION VARIETY - LASALLE	12.2	Current study
ONTARIO GROWN ONION VARIETY - RUBY RING	29.0	Current study
ONTARIO GROWN ONION VARIETY - SAFRANE	13.6	Current study
ONTARIO GROWN ONION VARIETY - STANLEY	33.7	Current study

Table 2 Comparison of Total phenolic content (TPC) of Ontario grown onions (current study) with other varieties

VARIETY	TPC IN MG/100 G	REFERENCES
WHITE, YELLOW, RED, SWEET ONIONS AND SHALLOT (USA)	142 to 428	Lu et al (2011)
YELLOW ONIONS	44.9	Siddiq et al (2013)
RED AND YELLOW ONIONS FROM SOUTH KOREA	20 and 54	Shim et al (2016)
INDIAN ONION VARIETIES (PUNJAB WHITE, PUNJAB NAROYA ,AND PRO-6)	22.0 to 32.2	Majid et al (2016)
FROM TENERIFE (TEXAS, MASCA, GUAYONJE, SAN JUAN, CARRIZALBAJO AND CARRIZAL ALTO)	25.3 to 75.9	Rodriguez Galdon et al (2008)
YELLOW	30 to 65	Lombard (2000)
EUROPEAN	250 to 300	Kahkonen et al (1999)
GIANT, YELLOW SPRING AND RED SPRING ONIONS	84 to 316	Nuutila et al. (2003)
ONTARIO GROWN ONION VARIETY - FORTRESS	127	Current study
ONTARIO GROWN ONION VARIETY - LASALLE	128	Current study
ONTARIO GROWN ONION VARIETY - RUBY RING	195	Current study
ONTARIO GROWN ONION VARIETY - SAFRANE	162	Current study
ONTARIO GROWN ONION VARIETY - STANLEY	169	Current study
WHITE, YELLOW, RED, SWEET ONIONS AND SHALLOT (USA)	142 to 428	Lu et al (2011)

The current study on Ontario onions show that the percentage to vary between 8.0 to 19.8%.The flavonoid content of Ontario grown onions in the current study was 15.6, 12.2, 29.0, 13.6 and 33.7 mg/ 100 g for Fortress, Lasalle, Ruby Ring, Safrane and Stanley respectively. The current study indicates total polyphenolic content to range between 127.9 to 195.1 mg/100 g which appears to be high. Recent studies have shown that polyphenols such as flavonoids from fruits and vegetables are major groups that prevents free radical damage (Bernardi et al., 2008). In the present study radical scavenging activity significantly increased hence it was necessary to calculate the correlation between the total phenolic content and total antioxidant activity. A very good correlation was found between total phenol contents and ABTS scavenging activity. Our results are in accordance with

previously studied research articles which indicated that a high correlation between total phenols and antioxidant activity (Lu et al., 2011; Shim et al., Yang et al., 2004).

Santas et al (2008) studied two Spanish onion varieties, namely white onion and Calcot de Valls, and observed that white onions contained higher phenol content than Calcot onions. The values were between 2.57 ± 0.51 to 6.53 ± 0.16 mg gallic acid equivalents (GAE)/g dry weight (gallic acid equivalent/g DW). Also higher phenol content was associated with higher antioxidant capacity. White onion extracts had the highest antioxidant activity (86.6 ± 2.97 and 29.9 ± 2.49 µmol Trolox/g DW for Trolox equivalent antioxidant capacity (TEAC) and Ferric Reducing Ability of Plasma (FRAP) assays, respectively). Whereas the values for the Calcot variety were 17.5 ± 0.46 and 16.1 ± 0.10µmol Trolox/g DW. Thus these results indicated that the antioxidant activity of many plants extracts is due to their phenolic compounds.

Reactive oxygen species (ROS) plays a vital role in several chronic diseases such as cancer, diabetics and cardiovascular which are the major health issues in North America. Epidemiological, in vitro and in vivo studies suggest that regular consumption of dietary rich food especially flavonoids from onions reduces risk of degenerative disorders. The quality of flavonoid extracts from onions will depend on the technological processes involved in its extraction. The five Ontario onion varieties showed significant differences. HPLC results showed the different flavonoids present in the sample. Thus in this study the chemical characterization of polyphenols present in the five varieties of Ontario grown onions suggested that phenolic enriched extract will exhibit strong antioxidant activity. It also specified that synergistic combination of uncharacterized molecules will explain the differences in quantification in biological activities measured from the extracts of different varieties of onions. Hence, successful screening and characterization will allow the producers to grow best and preferred candidates of onion varieties. Through this research it will also be possible to select quality varieties and develop processing techniques to extract active components. In conclusion these results will be helpful in developing biocompatible, biodegradable and eco-friendly antioxidant products from onions for the food and pharmaceutical industries. Flavonoids from Ontario grown onions show strong potential to be used as natural antioxidant food or for manufacturing coatings and thin films and novel nutraceuticals products for food industries.

4. CONCLUSIONS: Among all vegetables, onions are the most widely and largely consumed vegetable. Onions are rich in phenolics such as flavonoids. Flavonoids have potential antioxidant, antibacterial, anti-inflammatory and anti-cancerous properties. Epidemiological studies indicate that flavonoid based foods could help to reduce diseases that are associated with reactive oxygen species (ROS) and also the incidence of cardiovascular diseases, breast cancer, diabetes, inflammation and osteoporosis. There is currently a growing interest in the development of agronomically important food crops with optimized levels and composition of flavonoids. Knowledge of differences in the phenolic and flavonoid content and their individual concentration amongst onion types will be a useful knowledge to the farmers/breeders in selecting onions with specific anticipated health benefits. Identification, enhancement and development of market quality traits such as antioxidants properties of onions will enhance their marketability as nutraceutical products, as natural antibiofilm coating agent for improved shelf life of packaged food and as a preservative in processed food. In this study we have demonstrated that the Ontario grown onions have good total polyphenolic and total flavonoid content which reflect in their measured antioxidant activities.

The Ruby ring variety showed highest antioxidant activity (as measured in all the three antioxidant assays) and also the total polyphenolic content. Yellow onion variety Stanley showed highest total flavonoid content. Quercetin was identified as the major compound in yellow onion varieties. A strong correlation was observed in total polyphenolic and total flavonoid content against the observed antioxidant activities. Thus our current research work can provide consumers with increased awareness in the health benefits of different varieties of onions. In addition knowledge of specific differences in the flavonoid and phenolic contents among these various types of onions may be of potential value to breeders in selecting the best onion variety with specific anticipated health benefits.

Acknowledgements. The authors sincerely thank the Ontario Ministry of Research and Innovation (051455) and the Ontario Ministry of Agriculture, Food and Rural Affair for funding this study. The authors also thank the Holland Marsh Growers Association for providing onion samples.

REFERENCES

Hertog, M. G. 1996. Epidemiological evidence on potential health properties of flavonoids. Proceedings of the Nutrition society 55(1B): 385-397.

Saija, A., Scalese, M., Lanza, M., Marzullo, D., Bonina, F., and Castelli, F. 1995. Flavonoids as antioxidant agents: importance of their interaction with biomembranes. Free Radical Biology and Medicine 19(4): 481-486.

Yamazaki, S., Miyoshi, N., Kawabata, K., Yasuda, M., and Shimoi, K. 2014. Quercetin-3-O-glucuronide inhibits noradrenaline-promoted invasion of MDA-MB-231 human breast cancer cells by blocking β 2-adrenergic signaling. Archives of biochemistry and biophysics 557:18-27.

Søltoft, M., Christensen, J. H., Nielsen, J., and Knuthsen, P. 2009. Pressurised liquid extraction of flavonoids in onions. Method development and validation. Talanta 80(1): 269-278.

Caridi, D., Trenerry, V. C., Rochfort, S., Duong, S., Laugher, D., and Jones, R. 2007. Profiling and quantifying quercetin glucosides in onion (Allium cepa L.) varieties using capillary zone electrophoresis and high performance liquid chromatography. Food chemistry 105(2): 691-699.

Leonard Gianessi, Canadian Onion Production Depends on Fungicides, Crop Life Foundation, Washington DC, Report 77 Available at: www.croplife.org, April 2013

Yang, J., Meyers, K. J., van der Heide, J., and Liu, R. H. 2004. Varietal differences in phenolic content and antioxidant and antiproliferative activities of onions. Journal of agricultural and food chemistry 52(22): 6787-6793.

King, M. (2014). Food preservative market is shifting towards natural preservatives to align with consumer demands.

Hawthorne, S. B., Yang, Y., and Miller, D. J. 1994. Extraction of organic pollutants from environmental solids with sub-and supercritical water. Analytical Chemistry 66(18): 2912-2920.

Singleton, V. L., Orthofer, R., and Lamuela-Raventos, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in enzymology 299: 152-178.

Dewanto, V., Wu, X., Adom, K. K., and Liu, R. H. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of agricultural and food chemistry 50(10): 3010-3014.

Kedare, S. B., and Singh, R. P. 2011. Genesis and development of DPPH method of antioxidant assay. Journal of food science and technology 48(4): 412-422.

Rael, L. T., Thomas, G. W., Craun, M. L., Curtis, C. G., Bar-Or, R., and Bar-Or, D. 2004. Lipid peroxidation and the thiobarbituric acid assay: standardization of the assay when using saturated and unsaturated fatty acids. BMB Reports 37(6):749-752.

Szydłowska-Czerniak, A., Dianoczki, C., Recseg, K., Karlovits, G., and Szłyk, E. 2008. Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods. Talanta 76(4): 899-905.

Gennaro, L., Leonardi, C., Esposito, F., Salucci, M., Maiani, G., Quaglia, G., and Fogliano, V. 2002. Flavonoid and carbohydrate contents in Tropea red onions: effects of homelike peeling and storage. Journal of Agricultural and Food Chemistry 50(7): 1904-1910.

Sellappan, S., and Akoh, C. C. 2002. Flavonoids and antioxidant capacity of Georgia-grown Vidalia onions. Journal of Agricultural and Food Chemistry 50(19): 5338-5342.

Rodríguez Galdón, B., Rodríguez Rodríguez, E. M., and Díaz Romero, C. 2008. Flavonoids in onion cultivars (Allium cepa L.). Journal of food science 73(8): C599-C605.

Rhodes, M. J., and Price, K. R. 1996. Analytical problems in the study of flavonoid compounds in onions. Food Chemistry 57(1):113-117.

Marotti, M., and Piccaglia, R. 2002. Characterization of flavonoids in different cultivars of onion (Allium cepa L.). Journal of food science 67(3): 1229-1232.

Majid, I., Dhatt, A. S., Sharma, S., Nayik, G. A., and Nanda, V. 2016. Effect of sprouting on physicochemical, antioxidant and flavonoid profile of onion varieties. International Journal of Food Science and Technology 51(2): 317-324.

Lombard, K. A., Geoffriau, E., and Peffley, E. 2002. Flavonoid quantification in onion by spectrophotometric and high performance liquid chromatography analysis. HortScience 37(4): 682-685.

Lu, X., Wang, J., Al-Qadiri, H. M., Ross, C. F., Powers, J. R., Tang, J., and Rasco, B. A. 2011. Determination of total phenolic content and antioxidant capacity of onion (Allium cepa) and shallot (Allium oschaninii) using infrared spectroscopy. Food Chemistry 129(2): 637-644.

Siddiq, M., Roidoung, S., Sogi, D. S., and Dolan, K. D. 2013. Total phenolics, antioxidant properties and quality of fresh-cut onions (Allium cepa L.) treated with mild-heat. Food chemistry 136(2): 803-806.

Shim, S. M., Yi, H. L., and Kim, Y. S. 2011. Bioaccessibility of flavonoids and total phenolic content in onions and its relationship with antioxidant activity. International journal of food sciences and nutrition 62(8): 835-838.

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., and Heinonen, M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. Journal of agricultural and food chemistry 47(10): 3954-3962.

Nuutila, A. M., Puupponen-Pimiä, R., Aarni, M., and Oksman-Caldentey, K. M. 2003. Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. Food chemistry 81(4): 485-493.

Bernardi, A. P. M., López-Alarcón, C., Aspée, A., Rech, S. B., Von Poser, G. L., Bridi, R., ... and Lissi, E. 2008. ANTIOXIDANT ACTIVITYIN SOUTHERN BRAZIL HYPERICUM SPECIES. Journal of the Chilean Chemical Society 53(4): 1658-1662.

Yang, J., Meyers, K. J., van der Heide, J., and Liu, R. H. 2004. Varietal differences in phenolic content and antioxidant and antiproliferative activities of onions. Journal of agricultural and food chemistry 52(22): 6787-6793.

Santas, J., Carbo, R., Gordon, M. H., and Almajano, M. P. 2008. Comparison of the antioxidant activity of two Spanish onion varieties. Food Chemistry 107(3): 1210-1216.