



The thermostability, bioactive compounds and antioxidant activity of some vegetables subjected to different durations of boiling: Investigation *in vitro*

Myang Hee Im^a, Yong-Seo Park^a, Hanna Leontowicz^b, Maria Leontowicz^b, Jacek Namiesnik^c, Kyung-Sik Ham^d, Seong-Gook Kang^d, Kasia Najman^b, Shela Gorinstein^{e,*}

^a Department of Horticultural Science, College of Natural Science, Mokpo National University, Muan 534-729, South Korea

^b Warsaw University of Life Sciences (SGGW), Faculty of Veterinary Medicine, Department of Physiological Sciences, Warsaw, Poland

^c Chemical Faculty, Gdansk University of Technology, Gdansk 80952, Poland

^d Department of Food Engineering, Mokpo National University, Muan, South Korea

^e The Hebrew University, School of Pharmacy, The Institute for Drug Research, Jerusalem, Israel

ARTICLE INFO

Article history:

Received 16 November 2009

Received in revised form

24 January 2010

Accepted 28 May 2010

Keywords:

Lotus

White onion

Bioactive compounds

Antioxidant activity

Thermostability

ABSTRACT

This research was performed in order to compare the water and acetone extracts of raw and boiled for 10, 20, 40 and 60 min Korean lotus roots (KLR) and Polish white onion (PWO) in the contents of their bioactive compounds, antioxidant activity and thermostability.

It was found that polyphenols (mg GAE/g), flavanols (μg GAE/g), flavonoids (mg CE/g), anthocyanins (mg CGE/kg) and tannins (mg CE/g) in water extract of raw lotus roots were 14.18 ± 0.7 , 8.41 ± 0.5 , 1.09 ± 0.06 , 21.3 ± 1.2 and 7.29 ± 0.4 , and of white onion – 11.11 ± 0.6 , 6.78 ± 0.3 , 0.71 ± 0.03 , 17.00 ± 0.9 and 1.64 ± 0.08 , respectively, and significantly higher in KLR ($P < 0.05$). The antioxidant activity of raw KLR water extract (139.4 ± 6.1 , 53.1 ± 3.6 and 89.3 ± 4.6 $\mu\text{mol TE/g}$ for DPPH, CUPRAC and ABTS, respectively) was significantly higher than in white onion (23.84 ± 1.8 , 31.9 ± 2.1 and 38.14 ± 2.6 for DPPH, CUPRAC and ABTS, respectively, $P < 0.05$).

The thermostability of the water KLR extract's of polyphenols, flavanols, flavonoids, anthocyanins and tannins was high and even after 60 min of boiling remains as 40.0, 42.3, 50.5, 41.4 and 41.0%, respectively. After 60 min of boiling the most thermostable compounds were flavonoids – remaining at 50.5% in water extract of KLR. Also after 60 min of boiling the thermostability of the antioxidant activity of water extracts of KLR remained significantly high: 40.6, 42.3, 46.3 and 43.6%, according to DPPH, FRAP, ABTS and CUPRAC assays, respectively.

Similar relationship was obtained with acetone extracts, but the value was lower than with the water ones. In conclusion, the contents of some bioactive compounds, the antioxidant activity and the thermostability in water and acetone extracts of KLR are significantly higher than the same indices in PWO. FTIR and fluorimetry can be used as additional markers for the characterization of bioactive compounds in vegetables.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Scientific investigations show that fruits and vegetables consumption prevents and treats some diseases (He, Nowson, & MacGregor, 2006; Ignarro, Balestrieri, & Napoli, 2007; Lichtenstein et al., 2006). Above mentioned health properties of these natural products depend on their antioxidants, mainly phenolics (Brand et al., 2004; Gorinstein et al., 2009; Hooper & Cassidy, 2006).

Among well known vegetables in the Europe and North America are onions (*Allium cepa*), which are frequently consumed after

cooking (Barzegar, Rajabi, Hassandokht, & Jabbari, 2008; Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008). The wide consumption of this vegetable is connected to the fact that people believed that onion cures some diseases. However, only recently the scientific investigations showed that onions contain high quantities of bioactive compounds which effective in prevention of cancer and heart diseases (Pellegrini et al., 2009; Prakash, Singh, & Upadhyay, 2007; Rune, Torgils, & Molund, 2007). Lotus roots, which are less known in Europe and North America, are very popular in countries of South-East Asia (Sridhar & Bhat, 2007; Wang, Yen, Liang, & Wu, 2003; Zhang, Yang, Xi, & Fu, 2008). Also lotus is mostly consumed after processing. We decided to compare these two vegetables (Polish white onion and Korean lotus roots) in order to find similarity features. It is known that processing leads to some

* Corresponding author. Tel.: +972 2 6758690.

E-mail address: gorin@cc.huji.ac.il (S. Gorinstein).

changes in the contents of the bioactive compounds and antioxidant activity of vegetables (Bushra, Farooq, & Shahid, 2008; Cavagnaro, Sance, & Galmarini, 2007; Erguder, Avci, Devrim, & Durak, 2007; Ferracane et al., 2008; Grzelak, Milala, Krol, Adamicki, & Badelek, 2009; Jimenez-Montral, Garcia-Diz, Martinez-Tome, Mariscal, & Murzia, 2009; Song & Thornalley, 2007). Therefore, we compared the contents of the bioactive compounds, antioxidant activity and thermostability in the water and acetone extracts of raw and boiled for 10', 20', 40' and 60' of Polish white onion (PWO) and Korean lotus roots (KLR).

In order to receive reliable antioxidant activity data four tests (ABTS^{•+}, FRAP, DPPH and CUPRAC) were used (Apak, Guclu, Ozyurek, & Karademir, 2004; Ozgen, Reese, Tulio, Scheerens, & Miller, 2006). We did not find published data of such investigations.

2. Materials and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent (FCR), FeCl₃·6H₂O, CuCl₂·2H₂O, 2, 9-dimethyl-1, 10-phenanthroline (neocuproine), and butylated hydroxyanisole (BHA) were purchased from Sigma Chemical Co., St. Louis, MO, USA. 2,4,6-tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade. Deionized and distilled water was used throughout.

2.2. Samples

Raw Polish white onions (*A. cepa*) were donated by the Polish firm "Elena".

The Korean white lotus roots were purchased by one of the investigators at a local market Gwangju, Republic of Korea. The samples were prepared according to the following steps of treatments: bulbs of white onions and lotus were washed, cleaned, peeled and cut with plastic knife for pieces before the heat treatment. The studied vegetables were boiled, starting from 10 min and increasing till 60 min. This protocol is applied in the present study, because it is similar for everyday food cooking (Gorinstein et al., 2009).

2.3. Preparation of extracts

The shredded samples were freeze-dried (CHRIST ALPHA 2-4 LDplus Freeze Dryer, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and then ground to powder. The powder was stored at -20 °C in sealed bags until extraction of antioxidant phytochemicals. Defatted lyophilised vegetable samples were extracted from a 50 mg aliquot with 5 mL of water or 100% of acetone. The samples were extracted three times, and the extracts portions were combined (Gorinstein et al., 2009; Park et al., 2009; Pellegrini et al., 2009; Vinson, Hao, Su, & Zubik, 1998; Wu et al., 2004). The extracts were used for determination of the bioactive compounds, thermostability and antioxidant activity and kept until use at -20 °C for acetone extracts. The water extracts were done immediately. The extracted samples were divided into 20 groups named: LotusrawWater, OnionrawWater, LotusrawAcetone and OnionrawAcetone, which were extracted without processing, and LotusWater10', Onion Water 10', LotusAcetone10', OnionAcetone10', LotusWater20', Onion Water20', LotusAcetone20', OnionAcetone20', LotusWater40', OnionWater40', LotusAcetone40', OnionAcetone40', Lotus Water60', OnionWater60', LotusAcetone60', OnionAcetone60', which were boiled for 10', 20', 40' and 60', respectively.

2.4. Determination of the contents of the bioactive compounds

2.4.1. Fourier-transform infrared (FTIR) spectra of polyphenols

The presence of polyphenols (flavonoids and phenolic acids) in the investigated samples were studied by Fourier-transform infrared (FTIR) spectroscopy.

A Bruker Optic GMBH Vector FTIR spectrometer (Bruker Optic GMBH, Attingen, Germany) was used to record IR spectra. A potassium bromide microdisk was prepared from finely ground lyophilised powder of 2 mg of vegetable samples with 100 mg of KBr (Park et al., 2009).

2.4.2. Fluorescence measurements

Fluorescence spectra for water and acetone vegetables extracts were recorded on a model FP-6500, Jasco spectrofluorometer, serial N261332, Japan, 124 equipped with 1.0 cm quartz cells and a thermostat bath.

The widths of the excitation and the emission slits were set to 10.0 and 5.0 nm, respectively. The three dimensional spectra were collected with subsequent scanning emission spectra from 250 to 750 nm at 1.0 nm increments by varying the excitation wavelength from 250 to 500 nm at 10 nm increments. The scanning speed was set at 1000 nm/min for all measurements. All measurements were performed with emission mode and with intensity from -100 to 1000 (Wulf, Geyer, Nicolai, & Zude, 2005).

The studied bioactive compounds were determined as previously described (Gorinstein et al., 2009; Park et al., 2009).

To determine the total amount of polyphenols in the studied extracts, the Folin-Ciocalteu reagent (FCR) was used, and the measurement was performed at 765 nm with gallic acid as standard. Results were expressed as mg of gallic acid equivalent (GAE).

Flavonoids, extracted with 5 g/100 ml NaNO₂, 10 g/100 ml AlCl₃·6H₂O and 1M NaOH, were measured at 510 nm (Singleton, Orthofer, & Lamuela-Raventos, 1999).

The total flavanols amount was estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was read (Arnous, Makris, & Kefalas, 2001).

The absorbances for total anthocyanins were measured for two pH values (1.0 and 4.5) in a Beckman spectrophotometer at 510 nm, using the pH differential method. Results were expressed as milligrams of cyanidin-3-glucoside equivalent (CGE) (Lo Scalzo, Genna, Branca, Chedin, & Chassaigne, 2008).

The extracts of condensed tannins (procyanidins) with 4 ml/100 ml methanol vanillin solution were measured at 500 nm (+)-Catechin served as a standard for flavonoids, flavanols, and tannins, and the results were expressed as catechin equivalents (CE).

2.5. Determination of the antioxidant activity

The following 4 tests were used:

- (1) Ferric-reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants in the investigated samples to reduce ferric-tripyridyltriazine (Fe³⁺-TPTZ) to a ferrous form (Fe²⁺), which absorbs light at 593 nm.
- (2) 2,2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt (ABTS^{•+}): ABTS^{•+} radical cation was generated by the interaction of ABTS (7 mmol/L) and K₂S₂O₈ (2.45 mmol/L). This solution was diluted with methanol until the absorbance in the samples reached 0.7 at 734 nm.
- (3) 1,1-Diphenyl-2-picrylhydrazyl method (DPPH): DPPH solution (3.9 mL, 25 mg/L) in methanol was mixed with the samples extracts (0.1 mL). The reaction progress was monitored at 515 nm until the absorbance was stable.

(4) Cupric reducing antioxidant capacity (CUPRAC): This assay is based on utilizing the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidizing agent. The absorbance at 450 nm was recorded against a reagent blank (Apak et al., 2007; Ozgen et al., 2006; Pellegrini et al., 2009; Singh, Chidambara, & Jayaprakasha, 2002).

2.6. Thermostability

The thermostability was calculated as a ratio between the data after 60 min boiling and the data of raw vegetables (in %).

2.7. Statistical analysis

The results of this investigation are means \pm SD of five measurements. Differences between samples were tested by two-way ANOVA using GraphPad Prism, version 2.0. (GraphPad Software, San Diego, CA), following by Duncan's new multiple range test to assess differences groups means. The *P* values of <0.05 were considered significant.

3. Results

3.1. Bioactive compounds

3.1.1. FTIR spectra

The wavenumber of FTIR spectra for catechin at 827, 1039, 1115, 1143, 1286, 1478, 1511 and 1610 cm^{-1} were assigned to C–H alkenes, –C–O alcohols, C–OH alcohols, –OH aromatic, C–O alcohols, C–H alkanes, C=C aromatic ring, and C=C alkenes. Gallic acid showed the following wavenumbers (cm^{-1}): 866, 1026, 1238, 1450, 1542 and 1618.

Samples of raw white onion (upper curve, Fig. 1) and raw lotus roots (lower curve, Fig. 1) in the polyphenols region showed slightly different bands than the standards: 922, 1048, 1248 and 1634 cm^{-1} , but the wavelengths of the bands were similar in two vegetables samples.

3.1.2. Fluorimetric measurements

In order to introduce an additional analytical tool for differentiation and similarity between two vegetables in raw and processed

forms fluorescence was carried out. We are the first ones to apply 3D fluorescence for such purpose.

3D-fluorescence spectra [Fig. 2, acetone extracts of white onion boiled for 60 min (A), raw white onion (B), lotus boiled for 60 min (C) and raw lotus (D)] illustrated the elliptical shape of contours. The X-axis represents the emission spectra from 250 to 750 nm, while the Y-axis is the excitation spectra from 250 to 500 nm.

In three-dimensional fluorescence spectra the excitation and the emission wavelengths and the fluorescence intensity were used as the axes in order to investigate the information of the extracted bioactive compounds in the samples, and the contour spectra provided more information. The contour and cross view maps [(Aa, Ab (peaks ex/em 350/420); Ba, Bb (peaks ex/em 350/420); Ca, Cb (peak ex/em 350/390); Da, Db (peak ex/em 350/390))] displayed a view of the fluorescence spectra. For lotus samples the spectra was more condense than for onions. Fig. 3 presented the water extracts in a similar way as the acetone ones (Fig. 2) with a shift in their spectra: the contour and cross view maps [Aa, Ab (at 0.005 mg/mL peaks ex/em, 275/370; ex/em 350/430); Ba, Bb (peaks ex/em, 275/360; ex/em 350/430); Ca, Cb (peaks ex/em, 275/340; ex/em 350/430); Da, Db (peaks ex/em 275/280,310; ex/em 350/400,700)] displayed a view of the fluorescence spectra. All spectra of acetone and water extracts showed for raw and boiled white onion and lotus for 60 min not exactly the same profile of one main peak at location of ex 275 and 350 nm.

The results of the determination of the contents of the studied bioactive compounds are summarized in the Table 1. As can be seen (Table 1), the contents of polyphenols (mg GAE/g), flavanols (μg GAE/g), flavonoids (mg CE/g), anthocyanins (mg CGE/kg) and tannins (mg CE/g) in water extract of raw lotus roots were 14.18 ± 0.7 , 8.41 ± 0.5 , 1.09 ± 0.06 , 21.3 ± 1.2 and 7.29 ± 0.4 , and of white onion – 11.11 ± 0.6 , 6.78 ± 0.3 , 0.71 ± 0.03 , 17.0 ± 0.9 and 1.64 ± 0.08 , respectively. As can be seen, the contents of all studied bioactive compounds were significantly higher in water extract of lotus roots ($P < 0.05$).

Also after boiling for 60 min the significantly higher contents of polyphenols, flavanols, flavonoids, anthocyanins and tannins ($P < 0.05$) were registered in the water extracts of lotus roots (5.67 ± 0.3 mg GAE/g, 3.56 ± 0.12 μg CE/g, 0.55 ± 0.02 mg CE/g, 8.82 ± 0.6 mg CGE/kg and 2.99 ± 0.2 mg CE/g) vs. these indices in white onion (4.24 ± 0.2 mg GAE/g, 2.51 ± 0.11 μg CE/g, 0.25 ± 0.02 mg CE/g, 6.51 ± 0.4 mg CGE/kg and 0.56 ± 0.04 mg CE/g), respectively.

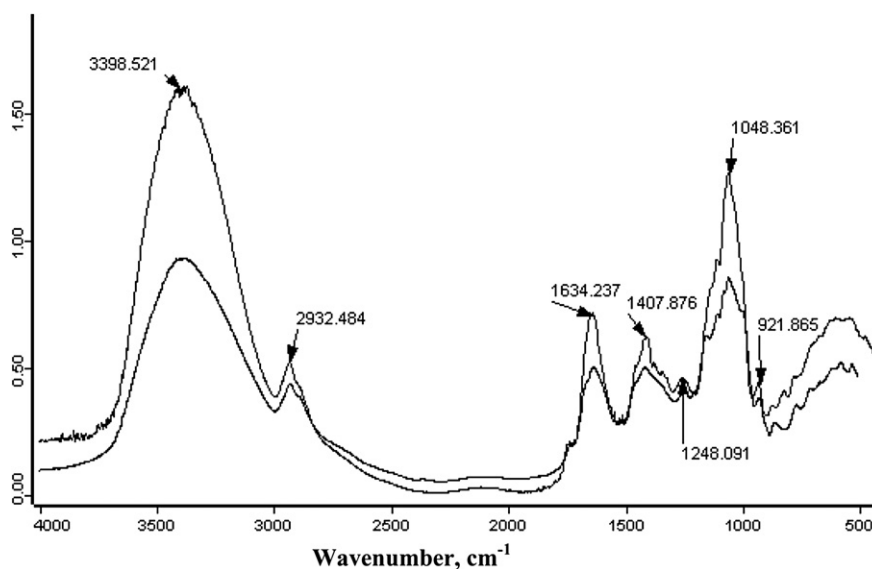


Fig. 1. FTIR spectra of raw white onion (upper curve), and raw lotus roots (low curve).

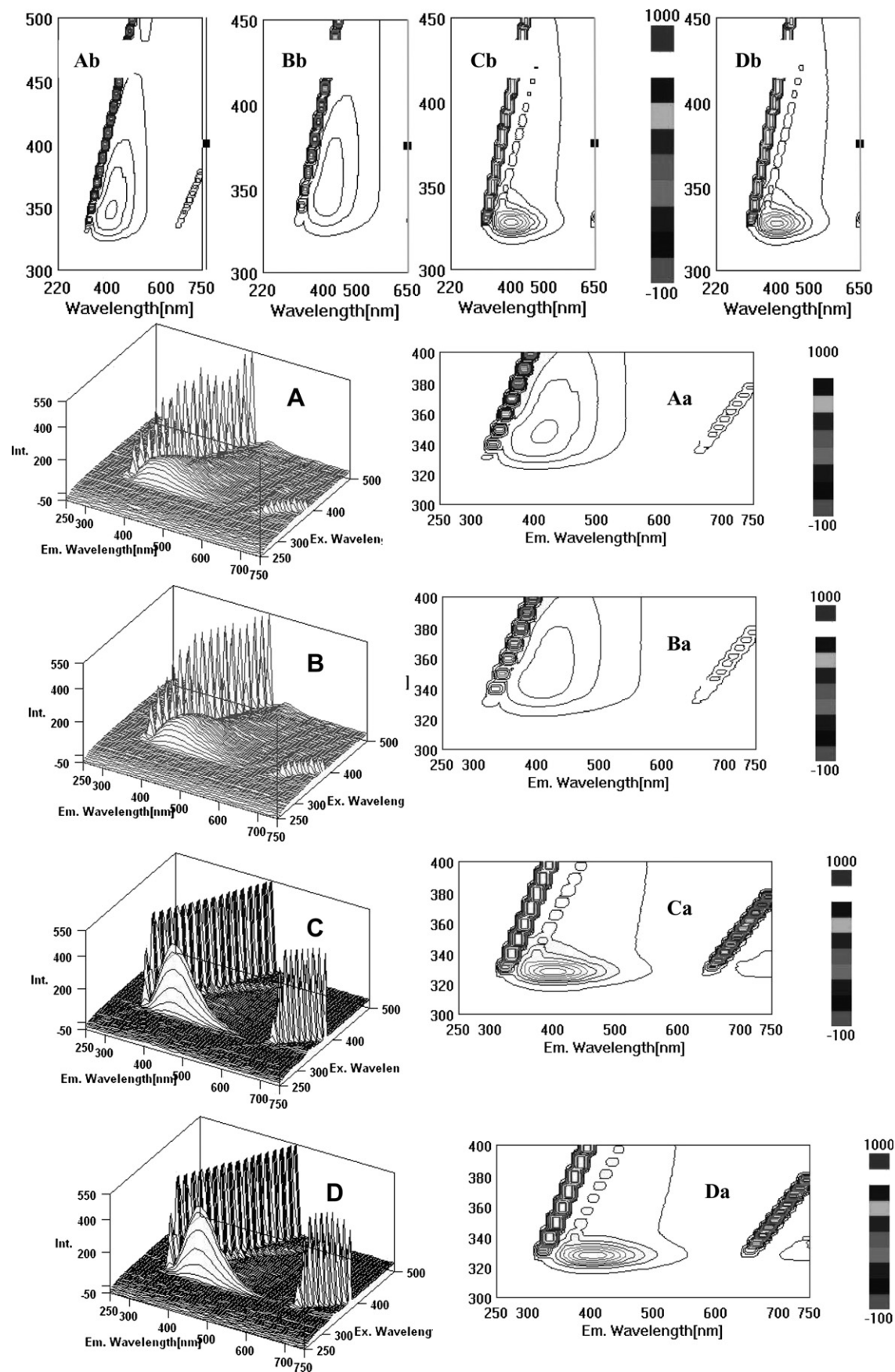


Fig. 2. Three dimensional fluorescence map of acetone extracts of white onion boiled for 60 min (A), fresh white onion (B), lotus boiled for 60 min (C), lotus fresh (D), respectively. The contour map (Aa, Ab, Ba, Bb, Ca, Cb, Da, Db) displayed a view of the corresponding fluorescence spectra. The three dimensional spectra were with emission from 250 to 750 nm and the excitation wavelengths from 250 to 500 nm, scanning speed was 1000 nm/min, emission mode and fluorescence intensity 1000. Abbreviations: A-E on axis Z: Int, fluorescence intensity; X: Em. Wavelength, emission wavelength; Y: Ex. Wavelength, excitation wavelength; (Aa, Ab, Ba, Bb, Ca, Cb, Da, Db) on axis X: Em Wavelength, emission wavelength; Y, excitation wavelength; all the fluorescence intensity values from -100 to 1000 are presented.

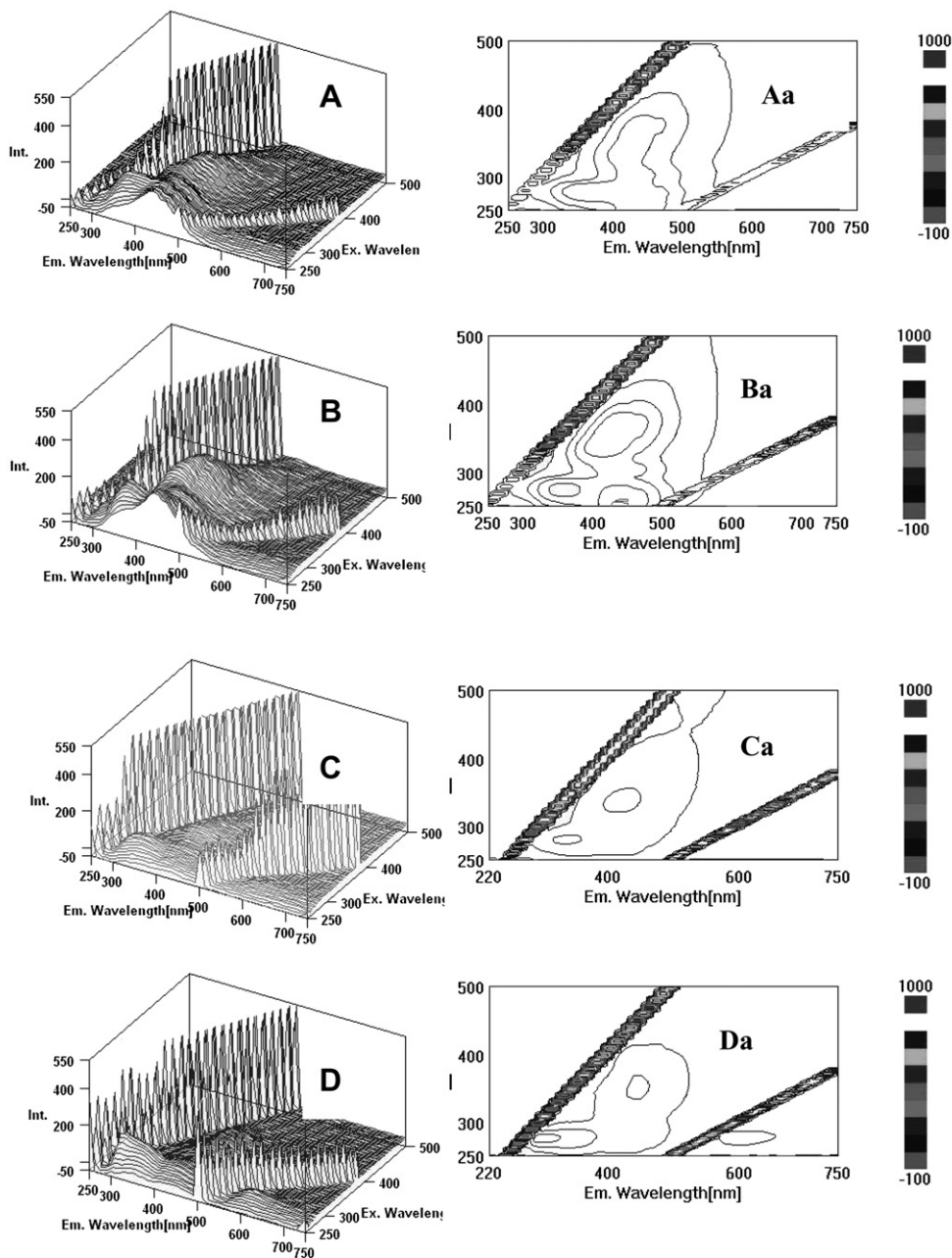
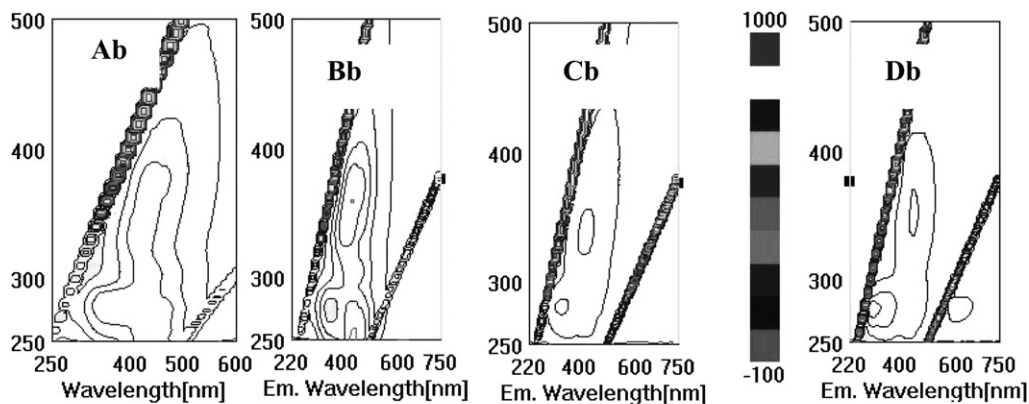


Fig. 3. Three dimensional fluorescence map of water extracts of white onion boiled for 60 min (A), fresh white onion (B), lotus boiled for 60 min (C), lotus fresh (D), respectively. The contour map (Aa, Ab, Ba, Bb, Ca, Cb, Da, Db) displayed a view of the corresponding fluorescence spectra. The three dimensional spectra were with emission from 250 to 750 nm and the excitation wavelengths from 250 to 500 nm, scanning speed was 1000 nm/min, emission mode and fluorescence intensity 1000. Abbreviations: A-E on axis Z: Int, fluorescence intensity; X: Em. Wavelength, emission wavelength; Y: Ex. Wavelength, excitation wavelength; (Aa, Ab, Ba, Bb, Ca, Cb, Da, Db) on axis X: Em Wavelength, emission wavelength; Y, excitation wavelength; all the fluorescence intensity values from -100 to 1000 are presented.

Table 1
Bioactive compounds in water and acetone extracts from raw lotus and white onion and samples subjected to boiling for different duration of time.

Sample	Polyphenols mg GAE/g	Flavanols μ g CE/g	Flavonoids mg CE/g	Anthocyanins mg CGE/kg	Tannins mg CE/g
LotusrawWater	14.18 \pm 0.7 ^b	8.41 \pm 0.5 ^b	1.09 \pm 0.06 ^b	21.30 \pm 1.2 ^b	7.29 \pm 0.4 ^b
LotusrawAcetone	1.22 \pm 0.06 ^a	2.43 \pm 0.1 ^a	0.02 \pm 0.001 ^a	2.20 \pm 0.1 ^a	0.22 \pm 0.01 ^a
OnionrawWater	11.11 \pm 0.6 ^b	6.78 \pm 0.3 ^b	0.71 \pm 0.03 ^b	17.00 \pm 0.9 ^b	1.64 \pm 0.08 ^b
OnionrawAcetone	1.58 \pm 0.08 ^a	1.40 \pm 0.07 ^a	0.04 \pm 0.002 ^a	3.00 \pm 0.2 ^a	0.16 \pm 0.008 ^a
LotusWater10'	13.33 \pm 0.7 ^b	7.91 \pm 0.4 ^b	1.020 \pm 0.06 ^d	20.0 \pm 1.1 ^b	6.85 \pm 0.4 ^b
LotusAcetone10'	1.15 \pm 0.06 ^a	2.28 \pm 0.1 ^a	0.019 \pm 0.001 ^a	2.07 \pm 0.06 ^a	0.21 \pm 0.01 ^a
OnionWater 10'	10.44 \pm 0.6 ^b	6.37 \pm 0.3 ^b	0.670 \pm 0.05 ^b	16.00 \pm 0.9 ^b	1.54 \pm 0.07 ^b
OnionAcetone10'	1.48 \pm 0.06 ^a	1.32 \pm 0.06 ^a	0.038 \pm 0.002 ^a	2.82 \pm 0.02 ^a	0.15 \pm 0.008 ^a
LotusWater20'	10.78 \pm 0.6 ^b	6.39 \pm 0.4 ^b	0.830 \pm 0.06 ^b	16.19 \pm 0.8 ^b	5.54 \pm 0.3 ^b
LotusAcetone20'	0.93 \pm 0.06 ^a	1.85 \pm 0.09 ^a	0.015 \pm 0.001 ^a	1.67 \pm 0.06 ^a	0.17 \pm 0.01 ^a
OnionWater20'	8.44 \pm 0.5 ^b	5.15 \pm 0.3 ^b	0.540 \pm 0.03 ^b	12.9 \pm 0.6 ^b	1.25 \pm 0.06 ^b
OnionAcetone20'	1.20 \pm 0.06 ^a	1.06 \pm 0.01 ^a	0.03 \pm 0.0002 ^a	2.28 \pm 0.08 ^a	0.12 \pm 0.008 ^a
LotusWater40'	7.52 \pm 0.6 ^b	4.46 \pm 0.3 ^b	0.58 \pm 0.04 ^b	11.29 \pm 0.6 ^b	3.86 \pm 0.06 ^b
LotusAcetone40'	0.65 \pm 0.06 ^a	1.29 \pm 0.06 ^a	0.011 \pm 0.001 ^a	1.17 \pm 0.06 ^a	0.17 \pm 0.01 ^a
OnionWater40'	5.89 \pm 0.4 ^b	3.59 \pm 0.2 ^b	0.38 \pm 0.02 ^b	9.01 \pm 0.6 ^b	0.87 \pm 0.06 ^b
OnionAcetone40'	0.84 \pm 0.07 ^a	0.74 \pm 0.04 ^a	0.021 \pm 0.002 ^a	1.59 \pm 0.07 ^a	0.08 \pm 0.006 ^a
LotusWater60'	5.67 \pm 0.3 ^b	3.56 \pm 0.12 ^b	0.55 \pm 0.02 ^b	8.82 \pm 0.6 ^b	2.99 \pm 0.2 ^b
LotusAcetone60'	0.49 \pm 0.03 ^a	0.97 \pm 0.06 ^a	0.008 \pm 0.001 ^a	0.88 \pm 0.05 ^a	0.09 \pm 0.006 ^a
OnionWater60'	4.24 \pm 0.2 ^b	2.51 \pm 0.11 ^b	0.25 \pm 0.02 ^b	6.51 \pm 0.4 ^b	0.56 \pm 0.04 ^b
OnionAcetone60'	0.63 \pm 0.05 ^a	0.56 \pm 0.04 ^a	0.016 \pm 0.001 ^a	1.22 \pm 0.06 ^a	0.07 \pm 0.004 ^a

Values are means of five measurements \pm SD. Means in columns with different superscript letters separately for water and acetone extracts for raw and processed for 10, 20, 40 and 60 min vegetables. Values within a column with different superscript letters differ significantly ($P < 0.05$).

3.2. Antioxidant activity

The results of the determination of the level of antioxidant activity of water and acetone extracts of raw lotus and white onion and subjected to boiling for different duration of time are shown in the Table 2. As can be seen, the antioxidant activity (μ mol TE/g) of the raw lotus roots water extract according to DPPH, CUPRAC and ABTS assays (139.40 ± 6.1 , 53.10 ± 3.6 and 89.30 ± 4.6) was significantly higher than that of the raw white onion water extract (23.84 ± 1.8 , 31.90 ± 2.1 and 38.14 ± 2.6 , respectively).

Also after boiling for 60 min the high level of antioxidant activity was found in water extract of lotus roots (9.27 ± 0.6 , 58.97 ± 3.1 , 24.6 ± 1.5 and 38.9 ± 1.9 μ mol TE/g according to FRAP, DPPH, CUPRAC and ABTS tests, respectively) – higher than in water extract of white onion.

3.3. Thermostability

It was found that the thermostability (Table 1) of the polyphenols, flavanols, flavonoids, anthocyanins and tannins of the water extract of lotus roots were high and even after 60 min of boiling remains 40.0, 42.3, 50.5, 41.4 and 41.0%, respectively. The thermostability of polyphenols, flavanols, flavonoids and tannins of white onion after 60 min of boiling was only 38.0, 37.0, 35.2, 38.3 and 34.1%, respectively. As can be seen, the most thermostabile among biocompounds were flavonoids (50.5%) of water extract of lotus roots.

According to the calculation (a ratio between the data after 60 min boiling and the data of raw vegetables) the thermostability of the water lotus roots extract antioxidant activity even after 60 min of boiling remained significantly high: 40.6, 42.3, 46.3 and 43.6% according to DPPH, FRAP, ABTS and CUPRAC, respectively. The thermostability of the white onion water extract's antioxidant activity after 60 min boiling was 40.9, 43.8, 41.4 and 43.3% according to DPPH, FRAP, ABTS and CUPRAC assays, respectively.

As can be seen, the data of thermostability for lotus roots and white onion were comparable. The same relationship was found in acetone extracts of the studied vegetables.

4. Discussion

The vegetables are a part of the diet (He et al., 2006). It was shown that onions are among the most consumed vegetables (Gorinstein et al., 2009; Grzelak et al., 2009). On the other hand, lotus roots which are very popular in countries of South-East Asia are less known in Europe and North America. Processing of vegetables leads to changes in the contents of bioactive compounds and

Table 2
Antioxidant tests of water and acetone extracts of raw lotus and white onion and subjected to boiling for different duration of time.

Sample	FRAP, μ mol TE/g	DPPH, μ mol TE/g	CUPRAC, μ mol TE/g	ABTS, μ mol TE/g
LotusrawWater	22.8 \pm 1.8 ^b	139.4 \pm 6.1 ^b	53.1 \pm 3.6 ^b	89.3 \pm 4.6 ^b
LotusrawAcetone	1.81 \pm 0.1 ^a	1.56 \pm 0.1 ^a	2.18 \pm 0.2 ^a	4.03 \pm 0.2 ^a
OnionrawWater	24.5 \pm 1.9 ^b	23.84 \pm 1.8 ^b	31.9 \pm 2.1 ^b	38.14 \pm 2.6 ^b
OnionrawAcetone	2.98 \pm 0.2 ^b	2.34 \pm 0.2 ^a	2.28 \pm 0.2 ^a	5.79 \pm 0.3 ^a
LotusWater10'	21.50 \pm 1.3 ^b	129.98 \pm 5.7 ^b	51.84 \pm 3.1 ^b	82.3 \pm 4.6 ^b
LotusAcetone10'	1.61 \pm 0.1 ^a	1.41 \pm 0.1 ^a	1.89 \pm 0.1 ^a	3.63 \pm 0.2 ^a
OnionWater 10'	21.95 \pm 1.3 ^b	21.97 \pm 1.3 ^b	29.69 \pm 2.1 ^b	35.05 \pm 2.4 ^b
OnionAcetone10'	2.71 \pm 0.2 ^a	2.11 \pm 0.2 ^a	2.02 \pm 0.1 ^a	5.02 \pm 0.3 ^a
LotusWater20'	17.50 \pm 1.3 ^b	111.28 \pm 5.5 ^b	46.42 \pm 2.5 ^b	73.4 \pm 3.6 ^b
LotusAcetone20'	1.21 \pm 0.1 ^a	1.21 \pm 0.1 ^a	1.22 \pm 0.1 ^a	3.23 \pm 0.2 ^a
OnionWater20'	18.95 \pm 1.3 ^b	19.72 \pm 1.1 ^b	24.93 \pm 1.7 ^b	31.15 \pm 2.1 ^b
OnionAcetone20'	2.11 \pm 0.2 ^a	1.91 \pm 0.1 ^a	1.42 \pm 0.1 ^a	4.01 \pm 0.3 ^a
LotusWater40'	12.25 \pm 1.1 ^b	77.89 \pm 4.1 ^b	32.39 \pm 1.7 ^b	51.38 \pm 2.6 ^b
LotusAcetone40'	0.84 \pm 0.1 ^a	0.85 \pm 0.1 ^a	0.85 \pm 0.1 ^a	2.26 \pm 0.2 ^a
OnionWater40'	13.19 \pm 1.2 ^b	13.80 \pm 1.1 ^b	17.45 \pm 1.3 ^b	21.81 \pm 1.4 ^b
OnionAcetone40'	1.47 \pm 0.1 ^a	1.34 \pm 0.1 ^a	0.99 \pm 0.1 ^a	2.80 \pm 0.3 ^a
LotusWater60'	9.27 \pm 0.6 ^b	58.97 \pm 3.1 ^b	24.60 \pm 1.5 ^b	38.90 \pm 1.9 ^b
LotusAcetone60'	0.64 \pm 0.05 ^a	0.64 \pm 0.05 ^a	0.65 \pm 0.04 ^a	1.71 \pm 0.08 ^a
OnionWater60'	10.04 \pm 0.7 ^b	10.45 \pm 0.7 ^b	13.21 \pm 0.9 ^b	16.51 \pm 1.1 ^b
OnionAcetone60'	1.11 \pm 0.1 ^a	1.20 \pm 0.08 ^a	0.75 \pm 0.06 ^a	2.12 \pm 0.1 ^a

Values are means \pm SD of 5 measurements.

Values in columns with different superscript letters are significantly different ($P < 0.05$).

Per g dry weight.

Abbreviations: FRAP, Ferric Reducing Antioxidant Power; DPPH, 1-Diphenyl-2-picrylhydrazyl method; CUPRAC, Cupric reducing antioxidant capacity; ABTS⁺, 2,2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt; TE, Trolox equivalent.

antioxidant activity (Ferracane et al., 2008; Miglio et al., 2008; Pellegrini et al., 2009). It was of interest to know the degree of such changes. Therefore, in this investigation were determined not only the contents of bioactive compounds and antioxidant activity of raw Korean lotus roots and Polish white onion, but also these indices in the samples subjected to boiling for 10, 20, 40 and 60 min. Simultaneously, the thermostability of the bioactive compounds and their antioxidant activity were studied.

A wide variability of bioactive compounds and the antioxidant activity of vegetables were shown (Grzelak et al., 2009; Lichtenstein et al., 2006; Yao et al., 2004). The composition of some onion cultivars (Barzegar et al., 2008) showed similar data (total phenolics in water extract – 58.3–180.7 mg TAE/100 g DW, and in methanol extract – 20.9–71.4 mg TAE/100 g DW) as our results. It was found that all cultivars are rich in bioactive compounds. However, their content differed significantly. The contents of all studied bioactive compounds were significantly higher in water extract of raw lotus roots ($P < 0.05$). It was found high level of antioxidant activity in lotus (Miao & Wu, 2008). Also other authors evaluated high contents of bioactive compounds in lotus roots (Rune et al., 2007). According to these authors, lotus roots are among the richest sources of dietary flavonoids and contribute to a large extent to the overall intake of flavonoids. Rune et al. (2007) found that red onions contain 415–1917 mg flavonols per kg FW. Also the antioxidant activity of raw lotus root water extract (Table 2) was significantly higher than in white onion ($P < 0.05$). After boiling for 60 min high contents of polyphenols, flavanols, flavonoids, anthocyanins and tannins were registered in lotus (Table 1) as an evidence of high thermostability of this vegetable. The data of Zhang et al. (2008) are in accordance with our results. Also after boiling for 60 min the high level of antioxidant activity was found in water extract of lotus roots (Table 1) – higher than in water extract of white onion. The results are in accordance with Jimenez-Montral et al. (2009).

As it was found that the thermostability of the polyphenols, flavanols, flavonoids, anthocyanins and tannins of the water extract of lotus roots was high and even after 60 min of boiling remains from 40 to 51% vs. 34 to 38% for white onion, respectively. The most thermostable among biocompounds were flavonoids in 72.65 and 57.64% after 60 min boiling of water lotus roots extract (50.5%). It has been reported in the literature that flavonoids, especially the flavan-3-ols catechin, epicatechin, galliccatechin, and epigallocatechin, are more thermostable and the extra-stability in aqueous solution is due to intermolecular H-bonding of flavonoids (Mohd Zainol, Abdul-Hamid, Abu Bakar, & Pak Dek, 2009).

The thermostability of the lotus roots water extract's antioxidant activity even after 60 min boiling remained relatively high from 40–46%, according to DPPH, FRAP, ABTS and CUPRAC, respectively, and significantly higher than of white onion.

In conclusion, the content of some bioactive compounds, the antioxidant activity and thermostability in water and acetone extracts of Korean lotus roots are significantly higher than the same indices of Polish white onion, and they were higher in water than in acetone extracts. FTIR and fluorimetry can be used as additional markers for the characterization of bioactive compounds in vegetables.

Acknowledgments

This study was supported by the Technology Development Program for Agriculture and Forestry, Ministry for Agriculture, Forestry and Fisheries, Republic of Korea.

The authors are thankful to Dr. Elena Katrich (School of Pharmacy, Hebrew University of Jerusalem) for her technical assistance in determination of antioxidant activity and 3 D fluorescence.

References

- Apak, R., Güçlü, K., Demirata, B., Özyürek, M., Çelik, S. E., Bektasoglu, B., et al. (2007). Comparative evaluation of total antioxidant capacity assays applied to phenolic compounds, and the CUPRAC assay. *Molecules*, *12*, 1496–1547.
- Apak, R., Guclu, K., Ozyurek, M., & Karademir, S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agriculture and Food Chemistry*, *52*, 7970–7981.
- Arnous, A., Makris, D. P., & Kefalas, P. (2001). Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. *Journal of Agriculture and Food Chemistry*, *49*, 5736–5742.
- Barzegar, M., Rajabi, A., Hassandokht, M. R., & Jabbari, A. (2008). Chemical composition of different cultivars of onion (*Allium cepa* L.) produced in Iran. *Horticulture, Environment and Biotechnology*, *49*, 121–127.
- Brand, K., Christensen, L. P., Hansen-Møller, J., Hansen, S. L., Haraldsdottir, J., Jespersen, et al. (2004). Health promoting compounds in vegetables and fruits. A systematic approach for identifying plant components with impact on human health. *Trends in Food Science and Technology*, *15*, 384–393.
- Bushra, S., Farooq, A., & Shahid, I. (2008). Effect of different cooking methods on the antioxidant activity of some vegetables from Pakistan. *International Journal of Food Science & Technology*, *43*, 560–567.
- Cavagnaro, P. F., Sance, M. M., & Galmarini, C. R. (2007). Effect of heating on onion (*Allium cepa* L.) antiplatelet activity and pungency sensory perception. *Food Science and Technology International*, *13*, 447–453.
- Erguder, B. I., Avci, A., Devrim, E., & Durak, I. (2007). Effects of cooking techniques on antioxidant enzyme activities of some fruits and vegetables. *Turkish Journal of Medical Science*, *37*, 151–156.
- Ferracane, R., Pellegrini, N., Visconti, A., Graziani, G., Chiavaro, E., Miglio, C., et al. (2008). Effects of different cooking methods on antioxidant profile, antioxidant capacity, and physical characteristics of artichoke. *Journal of Agricultural and Food Chemistry*, *56*, 8601–8608.
- Gorinstein, S., Jastrzebski, Z., Leontowicz, H., Leontowicz, M., Namiesnik, J., Najman, K., et al. (2009). Comparative control of the bioactivity of some frequently consumed vegetables subjected to different processing conditions. *Food Control*, *20*, 407–413.
- Grzelak, K., Milala, J., Krol, B., Adamicki, F., & Badelek, E. (2009). Content of quercetin glycosides and fructooligosaccharides in onion stored in a cold room. *European Food Research and Technology*, *228*, 1001–1007.
- He, F., Nowson, C., & MacGregor, G. (2006). Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. *The Lancet*, *367*, 320–326.
- Hooper, L., & Cassidy, A. (2006). A review of the health care potential of bioactive compounds. *Journal of the Science of Food and Agriculture*, *86*, 1805–1813.
- Ignarro, L. J., Balestrieri, M. L., & Napoli, C. (2007). Nutrition, physical activity, and cardiovascular disease: an update. *Cardiovascular Research*, *72*, 326–340.
- Jimenez-Montral, A. M., Garcia-Diz, L., Martinez-Tome, M., Mariscal, M., & Murzia, M. A. (2009). Influence of cooking methods on antioxidant activity of vegetables. *Journal of Food Science*, *74*, H97–H103.
- Lichtenstein, A. H., Appel, L. J., Brands, M., Carnethon, M., Daniels, S., Franch, H., et al. (2006). Diet and lifestyle recommendations revision 2006. A scientific statement from the American Heart Association Nutrition Committee. *Circulation*, *114*, 82–96.
- Lo Scalzo, R., Genna, A., Branca, F., Chedin, M., & Chassaigne, H. (2008). Anthocyanin composition of cauliflower (*Brassica oleracea* L. var. botrytis) and cabbage (*B. oleracea* L. var. capitata) and its stability in relation to thermal treatments. *Food Chemistry*, *107*, 136–144.
- Miao, Y., & Wu, Y. (2008). Research on processing technology of boiled fresh lotus roots flexible can. *Shipin Gongye Keji*, *29*, 205–207.
- Miglio, C., Chiavaro, E., Visconti, A., Fogliano, V., & Pellegrini, N. (2008). Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. *Journal of Agriculture and Food Chemistry*, *56*, 139–147.
- Mohd Zainol, M. K., Abdul-Hamid, A., Abu Bakar, F., & Pak Dek, S. (2009). Effect of different drying methods on the degradation of selected flavonoids in Centella asiatica. *International Food Research Journal*, *16*, 531–537.
- Ozgen, M., Reese, R. N., Tulio, A. Z., Jr., Scheerens, J. C., & Miller, A. R. (2006). Modified 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) methods. *Journal of Agriculture and Food Chemistry*, *54*, 1151–1157.
- Park, Y.-S., Towantakavanit, K., Namiesnik, J., Sliwka-Kaszynska, M., Kusznierewicz, B., Kozłowska-Tylingo, K., et al. (2009). Partial characterization of three Korean white lotus cultivars. *Journal of Agriculture and Food Chemistry*, *57*, 4391–4397.
- Pellegrini, N., Miglio, C., Del Rio, D., Salvatore, S., Serafini, M., & Brighenti, F. (2009). Effect of domestic cooking methods on the total antioxidant capacity of vegetables. *International Journal of Food Sciences and Nutrition*, *60*, 12–22.
- Prakash, D., Singh, B. S., & Upadhyay, G. (2007). Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). *Food Chemistry*, *102*, 1389–1393.
- Rune, S., Torgils, F., & Molund, V. I. (2007). Onions: a source of unique dietary flavonoids. *Journal of Agriculture and Food Chemistry*, *55*, 10067–10080.
- Singh, R. P., Chidambara, M., & Jayaprakasha, G. K. (2002). Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. *Journal of Agriculture and Food Chemistry*, *50*, 81–86.

- Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods of Enzymology*, 299, 152–178.
- Song, L., & Thornalley, P. J. (2007). Effect of storage, processing and cooking on glucosinolate content of Brassica vegetables. *Food and Chemical Toxicology*, 45, 216–224.
- Sridhar, K. R., & Bhat, R. (2007). Lotus – a potential nutraceutical source. *Journal of Agricultural Technology*, 3, 143–155.
- Vinson, J. A., Hao, Y., Su, X., & Zubik, L. (1998). Phenol antioxidant quantity and quality in foods: vegetables. *Journal of Agriculture and Food Chemistry*, 46, 3630–3634.
- Wang, L., Yen, J. H., Liang, H. L., & Wu, M. J. (2003). Antioxidant effect of methanol extracts from lotus plumule and blossom (*Nelumbo nucifera* Gertn). *Journal of Food Drug Analysis*, 11, 60–66.
- Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. J. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agriculture and Food Chemistry*, 52, 4026–4037.
- Wulf, J. S., Geyer, M., Nicolai, B., & Zude, M. (2005). Non-destructive assessment of pigments in apple fruit and carrot by laser-induced fluorescence spectroscopy (LIFS) measured at different time-gate positions. *Acta Horticulturae*, 682, 1387–1393, (Proceedings of the 5th International Postharvest Symposium, 2004).
- Yao, L. H., Jlang, Y. M., Shi, J., Tomas-Barberan, F. A., Datta, N., Singanusong, R., et al. (2004). Flavonoids in food and their health benefits. *Plant Foods for Human Nutrition*, 59, 113–122.
- Zhang, M., Yang, R., Xi, Y., & Fu, J. (2008). Antioxidant activity of fruits and vegetables. *Hebei Nongye Daxue Xuebao*, 31, 97–101.

