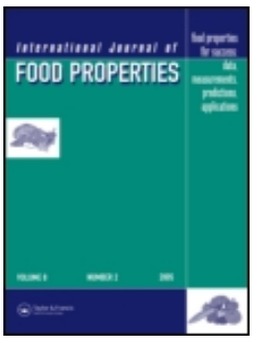

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EFFECTS OF COOKING ON THE BIOACTIVITY OF LOTUS ROOTS AND WHITE ONIONS

Myang Hee Im[1], Yong-Seo Park[1], Kyung-Sik Ham[2], Seong-Gook Kang[2], Buk-Gu Heo[3], Hanna Leontowicz[4], Maria Leontowicz[4], Jacek Namiesnik[5], Kasia Najman[4], and Shela Gorinstein[6]

[1]*Department of Horticultural Science, College of Natural Science, Mokpo National University, Muan, South Korea*
[2]*Department of Food Engineering, Mokpo National University, Muan, South Korea*
[3]*Naju Foundation of Natural Dyeing Culture, Naju, South Korea*
[4]*Department of Physiological Sciences, Warsaw University of Life Sciences, Warsaw, Poland*
[5]*Chemical Faculty, Gdansk University of Technology, Gdansk, Poland*
[6]*The Institute for Drug Research, School of Pharmacy, The Hebrew University–Hadassah Medical School, Jerusalem, Israel*

Bioactive compounds and antioxidant potentials were determined in fresh lotus roots and subjected to boiling for different periods of time (10, 20, 40, and 60 min). The obtained results were compared with the same indices in fresh and boiled white onions. It was found that fresh vegetables contained higher quantities of bioactive compounds than those boiled for 10 min, and these indices were significantly higher in fresh white onions than in fresh lotus roots ($P < 0.05$). Polyphenols (mg GAE/g) were 3.65 ± 0.2^a and 10.48 ± 0.5^b; flavanols (μg CE/g) -44.13 ± 2.8^a and 91.94 ± 4.3^b; flavonoids (mg CE/g) -0.54 ± 0.02^a and 1.04 ± 0.03^b; anthocyanins (mg CE/kg) -20.61 ± 1.2^a and 28.34 ± 1.3^b; and tannins (mgCE/g) -0.58 ± 0.02^a and 1.69 ± 0.03^b of lotus roots and white onions, respectively. The highest antioxidant potential was registered in fresh lotus roots and fresh white onions (10.1 ± 0.70^a and 15.0 ± 0.8^b; 8.1 ± 0.4^a and 23.05 ± 1.1^b; 18.7 ± 0.9^a and 26.9 ± 1.3^b μM TE/g for FRAP, DPPH, and CUPRAC, respectively ($P < 0.05$) and 26.9 ± 1.5^a and 24.14 ± 1.1^a μM TE/g for ABTS ($P > 0.05$). The influence on tumor cell lines Calu-6 (% of cell viability) of fresh lotus roots (75.61 ± 2.03) and boiling boiled for 10 min (76.54 ± 3.14) was significantly higher ($P < 0.05$) than for fresh white onions (94.18 ± 3.67) and 10 min (97.02 ± 4.16) white onion. By comparison, the tumor cell lines SNU-601 (% of cell viability) showed similar data for both fresh and boiled vegetables in the range of 86.45 ± 4.07 to 87.28 ± 2.42. In conclusion, the boiling led to a decrease of bioactivity; however, lotus demonstrated higher thermostability than white onions. Three-dimensional fluorescence can be used as an additional index for evaluation of properties. Fresh and boiled (for 10 min) lotus roots and white onions could be considered as a functional food with high antioxidative and antiproliferative activities.

Keywords: ***Lotus roots, White onion, Bioactive compounds, Antioxidant potential, Antiproliferative activity.***

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Address correspondence to Yong Seo Park, Department of Horticultural Science, College of Natural Science, Mokpo National University, Muan 534-729, Korea. E-mail: ypark@mokpo.ac.kr

INTRODUCTION

Fruit and vegetable consumption treats and even prevents some diseases. The health properties of these natural products depend mainly on their phenolic compounds and to a lesser degree on dietary fibers.[1–6] Among the frequently used vegetables,[7–11] onions (*Allium cepa*) are the best known.[12,13] The consumption of these vegetables goes back to ancient times, however, only recently have scientific investigations showed that onion extracts are effective in the prevention of cardiovascular disease due to their hypocholesterolemic, hypolipidemic, antihypertensive, antidiabetic, antithrombotic, and antihyperhomocysteinemia effects.[14–16] Less is known about such properties of lotus roots.[9,17–19]

Vegetables lose some of their properties after processing.[15,20,21] Extracts of some vegetables in certain concentrations have cytotoxic properties.[3,20,22] Little has been found in scientific literature about the differences in properties between lotus roots and those of white onions. Therefore, in this investigation, lotus roots and white onions were subjected to different durations of boiling, and the changes in the contents of their bioactive compounds and antioxidant potential were compared. It was also important to know if lotus roots and white onions possess the same properties, for example if 100% methanol extracts of these vegetables affect human cancer cell lines (Calu-6 for human pulmonary carcinoma and SNU-601 for human gastric carcinoma). In order to receive reliable antioxidant potential data, four tests were used: (i) Ferric-reducing/antioxidant power (FRAP) assay; (ii) 2, 2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt ($ABTS^{\cdot}+$); (iii) 1, 1-Diphenyl-2-picrylhydrazyl method DPPH; (iv) Cupric reducing antioxidant capacity—CUPRAC.

MATERIALS AND METHODS

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), lanthanum (III) chloride heptahydrate, Folin-Ciocalteu reagent (FCR), $FeCl_3x6H_2O$, $CuCl_2x2H_2O$, 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. De-ionized and distilled water was used throughout.

Samples

White lotus (*N. nucifera Gaertn*), cv Muan, harvested in August, 2009, was purchased in the supermarket in Gwangju, Republic of Korea, and white onion (*Allium cepa*), cv Armstrong, harvested in September, 2008, was purchased in the market in Warsaw, Poland. The fresh samples were boiled for different periods of time (10, 20, 40, and 60 min) and then lyophilized and stored until used for experiments. Then the samples were extracted with 100% methanol.[15]

Preparation of Extracts

Lotus roots and white onions were freeze-dried (Alpha 2–4 Christ) and then ground to powder. The powder was stored at -20°C until extraction of antioxidant phytochemicals. Portions of 1 g of all freeze-dried samples were extracted three times with methanol (4 ml).

The extracts were used to determine the bioactive compounds, antioxidant potentials, and antiproliferative activities.

Fluorescence Measurements

Fluorescence spectra for all vegetable extracts in methanol at a concentration of 0.01 mg/mL were recorded on a model FP-6500, Jasco spectrofluorometer, serial N261332, (Jasco International Co. Ltd., Tokyo, Japan) 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 500 nm at 1.0-nm increments by varying the excitation wavelength from 250 to 450 nm at 10-nm increments. The scanning speed was set at 1000 nm/min for all measurements. All measurements were carried out in emission mode and with an intensity of up to 500.[23]

Determination of the Contents of the Bioactive Compounds

The studied bioactive compounds were determined as previously described.[15,16] For determination of polyphenols, 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% $NaNO_2$, 10% $AlCl_3X6H_2O$, and 1 M NaOH were measured at 510 nm.

The content of total flavanols was estimated using the *p*-dimethylamino cinnamaldehyde method, and then the absorbance at 640 nm was read. Absorbancies 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,[10] and the results were expressed as milligrams of cyanidin-3-glucoside equivalent (CGE). The extracts of condensed tannins (procyanidins) with 4% 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).

Determination of the Antioxidant Potential

A ferric-reducing/antioxidant power (FRAP) assay was used to measure the ability of the antioxidants in the investigated samples to reduce ferric-tripiridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}), which absorbs light at 593 nm. 2, 2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt ($ABTS^{\cdot+}$): $ABTS^{\cdot+}$ radical cation was generated by the interaction of ABTS (7 mM/L) and $K_2S_2O_8$ (2.45 mM/L). This solution was diluted with methanol until the absorbance in the samples reached 0.7 at 734 nm. 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). Reaction progress was monitored at 515 nm until the absorbance was stable. Cupric reducing antioxidant capacity (CUPRAC) 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.

Antiproliferative Activity

The antiproliferative activities were measured using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. Cells were harvested, counted

(3×10^4 cells/mL), and transferred into a 96-well plate and incubated for 24 h prior to the addition of lotus and onion methanol extracts. Serial dilutions of the extracts were prepared by dissolving compounds in dimethyl sulfoxide (DMSO) followed by dilution with RPMI-1640 medium to set the final concentration at 125, 250, 500, 1000, and 2000 $\mu g\ mL^{-1}$. Stock solutions of samples were prepared for cell lines at 90 μL and samples at 10 μL, and incubated for 72 h. MTT solution at 5 mg mL^{-1} was dissolved in 1 mL of PBS, and 10 μL of it was added to each of the 96 wells. The wells were wrapped with aluminum foil and incubated at 37°C for 4 h. The solution in each well containing media, unbound MTT, and dead cells were removed by suction, and 150 μL of DMSO was added to each well. The plates were then shaken, and optical density was recorded using a microplate reader at 540 nm. Distilled water was used as positive control and DMSO as solvent control. The effect of the lotus and onion extracts on the proliferation of cancer and normal cells was expressed as relative cell viability: percent viability = OD of lotus or onion extract treated sample/OD of none treated sample) $\times$ 100, where OD is optical density.[24]

Statistical Analysis

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

RESULTS

Fluorimetric Measurements

3-D fluorescence spectra (Fig. 1) illustrated as the elliptical shape of contours. The X-axis represents the emission spectra from 250 to 500–750 nm, while the Y-axis is the excitation spectra from 250 to 450–500 nm: 0.01 mg/ml of methanol extracts of boiled white onion (A), fresh onion(B), fresh lotus (C), and 0.005 mg/ml of methanol extracts of boiled lotus (D) and fresh lotus (E). In three-dimensional fluorescence spectra, the excitation and the emission wavelengths and the fluorescence intensity were used as the axes to investigate the information of the extracted bioactive compounds in the samples, and the contour spectra provided more information. The contour map [Aa (at 0.01 mg/ml peaks ex/em 275/300, 470; ex/em 350/390, 430); Ba (at 0.01 mg/ml peaks ex/em, 275/280, 460; ex/em 350/390, 430); Ca (at 0/01 mg/ml peaks ex/em, 275/310, 330, 410; ex/em 350/370, 390); Da (at 0.005 mg/ml peaks ex/em, 275/310, 550, 600; ex/em 350/390, 700); Ea (at 0.005 mg/ml peaks ex/em, 275/310, 550, 600; ex/em 350/370, 390, 700) displayed a view of the fluorescence spectra. The contour maps of methanol extracts showed for boiled and fresh white onion and lotus exactly the same profile of one main peak at location of ex/em 275/300 nm and the second peak at ex/em 275/600 appeared only for fresh and boiled lotus samples.

Bioactive Compounds

The significantly high contents (Table 1) of polyphenols, flavanols, flavonoids, anthocyanins, and tannins were registered in fresh samples of white onion ($P < 0.05$). However,

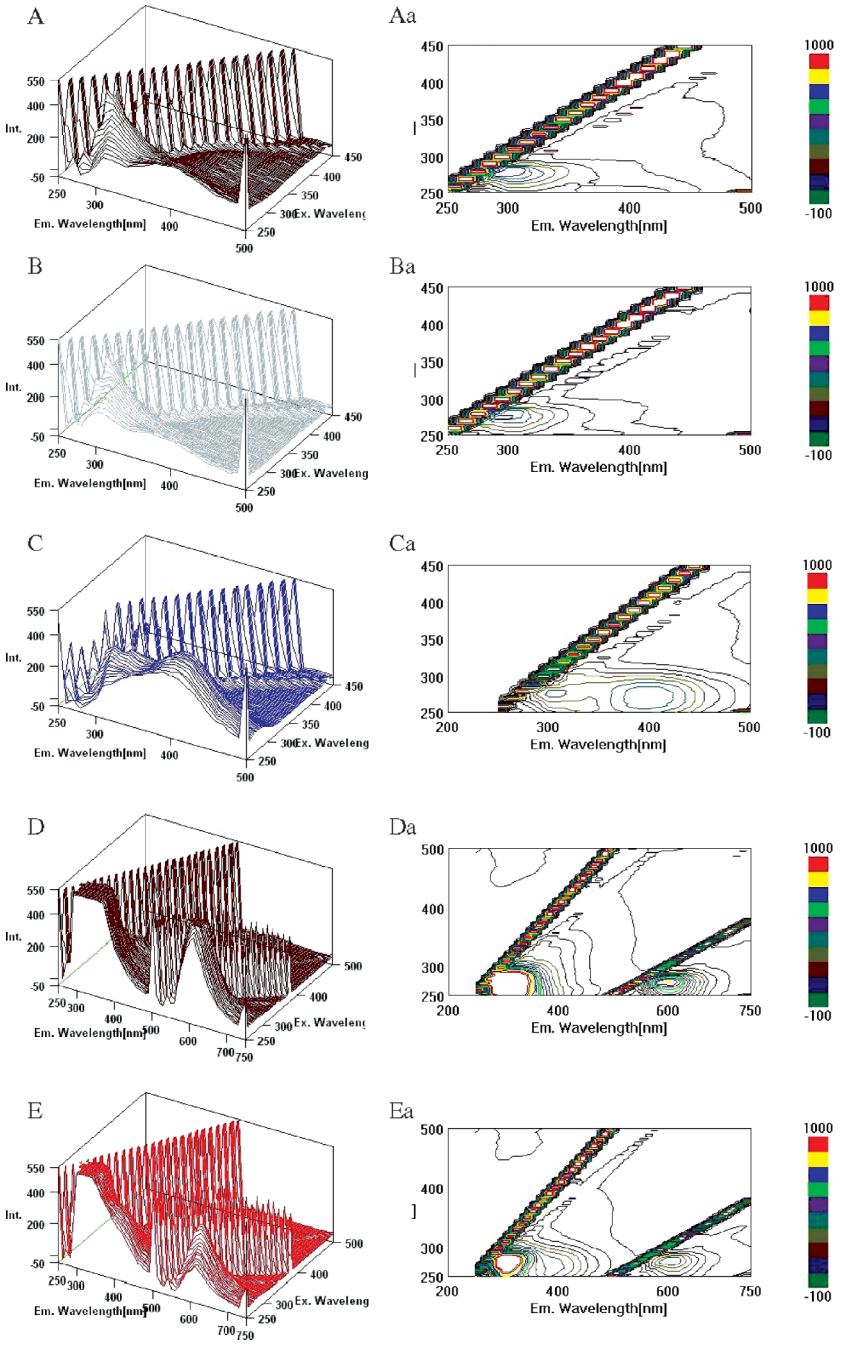

Figure 1 Three dimensional fluorescence map of 0.01 mg/ml of methanol extracts of processed white onion (A), fresh white onion (B), fresh lotus (C), lotus processed (D), lotus fresh (E), respectively. The contour map (Aa, Ba, Ca, Da, Ea) displayed a view of the corresponding fluorescence spectra. The three-dimensional spectra were with emissions from 250 to 500–750 nm and the excitation wavelengths from 250 to 450–500 nm, scanning speed was 1000 nm/min, emission mode and fluorescence intensity 500. Abbreviations: A-E on axis Z: Int, fluorescence intensity; X: Em. Wavelength, emission wavelength; Y: Ex. Wavelenth, excitation wavelength; Aa, Ba, Ca, Da, Ea on axis X: Em Wavelength, emission wavelength; Y: excitation wavelength; all the fluorescence intensity values from -100 to 1000 are presented (color figure available online).

Table 1 Bioactive compounds (per g DW) of methanol extracts of fresh lotus roots and white onions (WO) and subjected to boiling for different durations of time.

Sample	Polyphenols, mg GAE/g	Flavanols, μg CE/g	Flavonoids, mg CE/g	Anthocyanins, mg CGE/kg	Tannins mg CE/g
Lotusfresh	3.65 ± 0.2^{a}	44.13 ± 2.8^{a}	0.54 ± 0.02^{a}	20.6 ± 1.2^{a}	0.58 ± 0.02^{a}
WOfresh	10.48 ± 0.5^{b}	91.94 ± 4.3^{b}	1.04 ± 0.03^{b}	28.34 ± 1.3^{b}	1.69 ± 0.03^{b}
Lotus10′	3.42 ± 0.2^{a}	41.9 ± 2.6^{a}	0.49 ± 0.02^{a}	18.9 ± 0.9^{a}	0.51 ± 0.02^{a}
WO10′	8.06 ± 0.4^{b}	73.44 ± 3.2^{b}	0.86 ± 0.03^{b}	24.14 ± 1.2^{b}	1.41 ± 0.2^{b}
Lotus20′	3.11 ± 0.15^{a}	32.6 ± 2.12^{a}	0.41 ± 0.01^{a}	16.2 ± 0.8^{a}	0.39 ± 0.02^{a}
WO20′	6.9 ± 0.35^{b}	54.1 ± 2.8^{b}	0.49 ± 0.02^{a}	18.15 ± 0.9^{a}	0.95 ± 0.04^{b}
Lotus40′	2.69 ± 0.1^{a}	25.3 ± 1.3^{a}	0.34 ± 0.01^{a}	13.2 ± 0.7^{a}	0.21 ± 0.01^{a}
WO40′	4.7 ± 0.3^{b}	33.3 ± 1.8^{b}	0.43 ± 0.02^{a}	15.8 ± 0.8^{a}	0.72 ± 0.03^{b}
Lotus60′	2.5 ± 0.1^{a}	22.0 ± 1.3^{a}	0.29 ± 0.01^{a}	10.2 ± 0.5^{a}	0.19 ± 0.01^{a}
WO60′	3.3 ± 0.1^{a}	23.9 ± 1.4^{a}	0.31 ± 0.02^{a}	11.3 ± 0.6^{a}	0.27 ± 0.02^{a}

Valuesare means ± SD of five measurements. Means in columns without letters in common differ significantly ($P < 0.05$).

[a]All vegetables extractedat room temperature ina concentration of 25 mg lyophilized sample in 1 ml of methanol. 10, 20, 40, and 60 samples of fresh lotusand onion, boiled for 10, 20, 40, and 60 min, respectively.

GAE: gallicacid equivalents; DW: dry weight; CE: catechin equivalent.

the thermostability of the lotus roots was higher than in white onion and in samples subjected to boiling for 60 min (Table 1) the content of all studied bioactive compounds was comparable ($P > 0.05$).

Antioxidant Potential

The results of the determination of the antioxidant potential of the studied vegetables are summarized in Table 2. The significantly highest antioxidant potential ($P < 0.05$) was registered in fresh white onion (15.0 ± 0.8, 23.05 ± 1.1, and 26.9 ± 1.3 μM TE/g

Table 2 Antioxidant potentials (μMTE/g DW) of methanol extracts of fresh lotus roots and white onions (WO) and subjected to boiling for different duration of time.

Sample	FRAP	DPPH	CUPRAC	ABTS
Lotusfresh	10.1 ± 0.70^{a}	8.1 ± 0.4^{a}	18.7 ± 0.9^{a}	26.9 ± 1.5^{a}
WOfresh	15.2 ± 0.8^{b}	23.05 ± 1.1^{b}	26.9 ± 1.3^{b}	24.14 ± 1.1^{a}
Lotus10′	7.3 ± 0.5^{a}	7.2 ± 0.5^{a}	17.1 ± 0.8^{a}	23.8 ± 1.2^{a}
WO10′	14.05 ± 0.7^{b}	16.1 ± 0.8^{b}	21.44 ± 1.2^{b}	21.11 ± 1.1^{a}
Lotus20′	6.7 ± 0.3^{a}	6.8 ± 0.6^{a}	11.2 ± 0.6^{a}	20.5 ± 1.1^{a}
WO20′	11.2 ± 0.6^{b}	11.3 ± 0.6^{b}	16.3 ± 0.8^{b}	19.2 ± 0.9^{a}
Lotus40′	4.5 ± 0.3^{a}	5.2 ± 0.3^{a}	9.5 ± 0.5^{a}	17.8 ± 0.8^{a}
WO40′	7.2 ± 0.4^{b}	8.2 ± 0.4^{b}	13.3 ± 0.7^{b}	17.7 ± 1.7^{a}
Lotus60′	3.80 ± 0.2^{a}	4.1 ± 0.7^{a}	8.3 ± 0.4^{a}	13.8 ± 0.7^{a}
WO60′	4.1 ± 0.2^{a}	4.7 ± 1.0^{a}	9.1 ± 0.5^{a}	13.4 ± 0.7^{a}

Values are means ± SD of five measurements. Means in columns without letters in common differ significantly ($P < 0.05$).

Fresh lotus and white onion extracted at room temperature; and 10′, 20′, 40′, and 60′ boiled for 10′, 20′, 40′, and 60′, respectively.

ABTS: 2, 2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt; CUPRAC: Cuprac reducing antioxidant capacity; DPPH: 1, 1-Diphenyl-2-picrylhydrazyl method; FRAP: ferric-reducing/antioxidant power.

according to FRAP, DPPH, and CUPRAC, respectively). The antioxidant potential of both vegetables, according to ABTS, was comparable ($P > 0.05$). As in the case of bioactive compounds, after boiling for 60 min, the antioxidant potential of both vegetables was comparably decreased. Therefore, the results of the determination of the antioxidant potential show that lotus root had higher thermostability. The results of this investigation differ from our previous study, but not significantly ($P > 0.05$).[15,16] It was expected since we used vegetables harvested in different climatic seasons.

Antiproliferative Activity

All the methanol extracts of the studied vegetables were tested for their antiproliferative activity on tumor cell lines Calu-6 for human pulmonary carcinoma and SNU-601 for human gastric carcinoma by the MTT assay (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide). No influence of fresh white onion on cell proliferation (% of cell viability) on tumor cell lines Calu-6 was observed (94.18 ± 3.67), compared to 75.61 ± 2.03% for fresh lotus. On the contrary—the influence of lotus and white onion on the tumor cell lines SNU-601 was nearly the same (86.45 ± 4.07 and 87.28 ± 2.42%). The boiled vegetables for 10 min lotus and white onion showed slightly lower antiproliferative activity than the fresh ones (Fig. 2).

DISCUSSION

The consumption of fruits and vegetables is important.[1] Among the most popular vegetables are onions, whose properties are well known.[12,13,15,16] The properties of lotus roots have been much less investigated.[18,19] It was shown that processing of natural products decreases the levels of bioactive compounds and their antioxidant potential.[15] What is the degree of such decreases and what are the benefits of consuming such vegetables? To answer these questions, it was decided to study the contents of important bioactive compounds, antioxidant potential in fresh lotus roots and white onion and to compare the data with those of these vegetables when subjected to different durations of boiling. Simultaneously, their antioxidant potential was studied.

Peaks in their 3D-fluorescence showed slight differences in the shift of their location before and after boiling. Three-dimensional fluorescence can be used as an additional index for evaluation of the product for consumers. Data from scientific literature demonstrate a wide variability of bioactive compounds and the antioxidant potential of vegetables.[2,11,24,25] We found that these indices were significantly higher in fresh white onion than in lotus root ($P < 0.05$): 10.48 ± 0.5^{b} and 3.65 ± 0.2^{a} mg GAE/g, 91.94 ± 4.3^{b} and 44.13 ± 2.8^{a} μg CE/g, 1.04 ± 0.03^{b} and 0.54 ± 0.02^{a} mg CE/g, 28.34 ± 1.3^{b} and 20.61 ± 1.2^{a} mg CGE/kg, and 1.69 ± 0.03^{b} and 0.58 ± 0.02^{a} mg CE/g of polyphenols, flavanols, flavonoids, anthocyanins and tannins, respectively (Table 1).

The same patterns were found for antioxidant potential (Table 2). The highest antioxidant potential was registered in fresh lotus roots and white onion (10.1 ± 0.70^{a} and 15.0 ± 0.8^{b}, 8.1 ± 0.4^{a} and 23.05 ± 1.1^{b}, 18.7 ± 0.9^{a} and 26.9 ± 1.3^{b} μM TE/g for FRAP, DPPH, CUPRAC, respectively ($P < 0.05$) and 26.9 ± 1.5^{a} and 24.14 ± 1.1^{a} μM TE/g for ABTS ($P > 0.05$). After being boiled for 60 min, the contents of the studied bioactive compounds and antioxidant potential were significantly decreased; however, these indices in both vegetables became comparable: a sign of the higher thermostability of lotus root. Our results were in accordance with the literature,[17] where the boiling of roots for 10 min

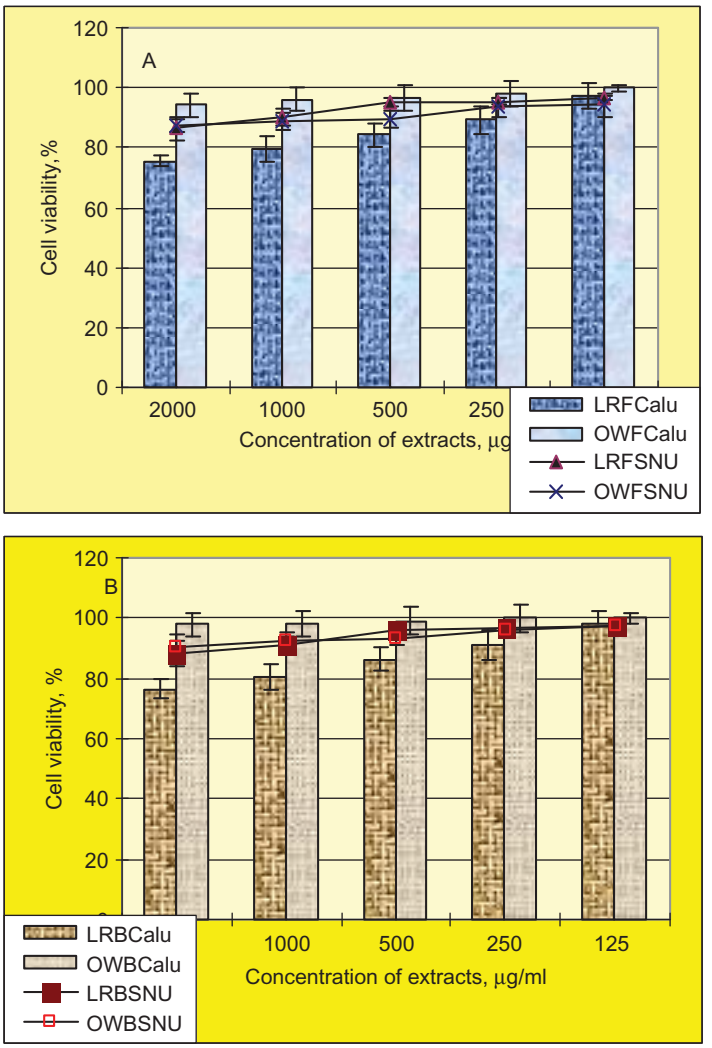

Figure 2 Proliferation rate of Calu-6 (for human pulmonary carcinoma) and SNU-601 (for human gastric carcinoma) cells treated with the lotus and white onions extracts. A, B, fresh lotus and white onion, boiled for 10 min lotus and white onion, respectively). Antiproliferative effects of the lotus and white onion were expressed as percent cell viability after exposure to treatment for 24 h. Abbreviations: LRF: lotus roots fresh; OWF: onion white fresh; LRB: lotus roots boiled; OWB: onion white boiled (color figure available online).

at 90°C was the optimal technological processing parameter of boiled lotus roots from fresh material.

As it was mentioned earlier, such different results compared to our previous data were expected: the used vegetables were harvested in different climatic seasons.[15] The bioactive compounds and their antioxidant activities in lotus roots, cv Muan, harvested in January, 2008[15,16] were higher than the same cultivar harvested in August, 2009 (Tables 1 and 2). The same conclusion can be reached from Chen et al.,[9] where a comparison of common vegetables was described. The ferric reducing/antioxidant power assay showed

that the lotus root was the strongest in antioxidant capacity among all 25 vegetables and followed by those in ginger, rape, garlic bulb, and white onion. According to FRAP, the antioxidant potential of lotus root was comparable with this index of the white onion (Table 2).

This resulted from different growth conditions and the different sources of the samples. There was a correlation between antioxidant potential and total polyphenol or total flavonoid. Different data were obtained by Santas et al.[12] The highest value of phenol content in Spanish onions was about 6.536.53 ± 0.16 mg GAE/g DW, which was lower than our data (Table 1), and the antioxidant potential was at 86.6 ± 2.97 and 29.9 ± 2.49 μM TE/g DW for ABTS and FRAP assays, which is higher than our data by about 3–4 times. As was mentioned previously and in this cited research, the obtained data depend on the solvent used and extraction conditions.

Combinations of polyphenols of fruits and vegetables can prevent cancer and other diseases.[24–26] Therefore, it was of great interest to investigate the antiproliferative effect of the studied vegetables. There are not many reports about the antiproliferative activity of the studied vegetables, but our results can be compared with a similar report of Jagadish[20] ethanol extracts of an edible mushroom *Agaricus bisporus*. They were examined before and after boiling for antioxidant and anticancer activities. The radical scavenging activities measured by ABTS and DPPH, total phenolic and flavonoid concentrations were similar in both extracts of fresh and boiled vegetables for 10 min. The decrease in total flavonoids was about 7.69% and for total polyphenols 21.7%[20] in comparison with our data for flavonoids of 9.3 and 17.3% and for polyphenols of 14.5 and 23.1% for lotus and white onion, respectively (Table 1). *A. bisporus* extracts inhibited cell proliferation of HL-60 leukemia by the induction of apoptosis. Our data showed that the methanol extracts of lotus and white onions inhibited as well cells Calu-6 and SNU-601 in relatively comparable values.

In order to investigate the antiproliferative activity of the studied by us vegetables, the tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) test was used for determination of cell viability in assays of cell proliferation and cytotoxicity. It is known that MTT is reduced in metabolically active cells to yield an insoluble purple formazon product.[22] Using this test we found a dose-dependent inhibition of cell proliferation, which was observed in both raw lotus roots and white onion methanol extracts only in concentration of 2000 $\mu g\ mL^{-1}$. The results of the experiment of Chen et al.[27] support our data. They investigated the effect of baicalein, silymarin, and their combination on two human liver-derived cell lines, HepG2 (hepatocellular carcinoma) and Chang liver (non-tumor liver cells). The viability at 48 h was 85.62% from 6.75 lg/ml baicalein treatment; but the viability was reduced to 49.67, 38.56, and 19.61% when 25, 50, and 100 lg/ml silymarin respectively, was added to the treatment.

Our data were similar to those of Melo et al.[21] Considering that food processing can affect the levels of nutrients in relation to fresh foods, the contents of antioxidants and their activity in the cited and present studies were examined; we examined the antioxidant capacity and polyphenol levels of ten vegetables including white onions after steam cooking and two vegetables after boiling for different periods of time, respectively. Methanol extracts of the vegetables were used in the cited literature and in our report. At the end of the reaction period (60 min), the scavenging capacity by DPPH maintained low for white onions. Nutrient composition and sensory profile of differently cooked green leafy vegetables (conventional, pressure, and microwave cooking) changed during the processing.[5,8] Thus, all cooked vegetables had antioxidant properties similar to some extent to what was found in our studies. The steam cooking did not dramatically affect the antioxidant

properties of the vegetables, and in our data only boiling for 10 min slightly changed these properties.

CONCLUSION

Methanol extract of fresh and boiled (for 10 min) lotus roots and white onion contain significantly higher quantities of bioactive compounds. Their antioxidant potentials are also significantly higher than in these vegetables processed for 20, 40, and 60 min. The thermostability of the lotus roots was higher than in white onion and in samples subjected to boiling for 60 min, and the content of all studied bioactive compounds was comparable ($P > 0.05$). The dose-dependent antiproliferative effect was registered in methanol extracts of both raw lotus roots and white onion, however, only in concentration of 2000 $\mu g\ mL^{-1}$. Lotus roots should be introduced into the European community to serve as a nutritional and beneficial vegetable.

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