Antioxidant, Radical Scavenging and Antimicrobial Activities of Red Onion (*Allium cepa* L) Skin and Edible Part Extracts

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Dedicated to the memory of Professor Dr. Valentin Koloini

The antioxidant, radical scavenging and antimicrobial activities of extracts from skin and edible part of red onion have been investigated. Crude extracts of red onion were obtained separately with acetone, ethanol and mixtures of solvents with water.

The amounts of isolated phenolic compounds and quercetin from onion skin were approximately 3 to 5 times higher as from the onion edible part. Antioxidant and radical scavenging activities of onion skin extracts were generally high, results were comparable to that of BHT. Extracts from onion edible part showed somewhat lower activity. Furthermore, high activity of skin extracts against bacteria *Escherichia coli, Pseudomonas fluorescens* and *Bacillus cereus* and fungi *Aspergillus niger, Trichoderma viride* and *Penicillium cyclopium* was observed. Antimicrobial activity of edible part extracts against tested microorganisms is generally lower, while for *Escherichia coli* no growth inhibition was observed.

Key words:
Red onion, extracts, total phenols, quercetin, antioxidant activity, antimicrobial activity

Introduction

Onion (*Allium cepa* L) is a common food plant rich in several phytonutrients associated with the treatment and prevention of a number of diseases. A review of the major volatile and non-volatile phytoconstituents of onion, identification methods and studies of biological effects was published recently by Lanzotti.[1] Extracts of red onion rich in phenolic compounds exhibit antiproliferative activity,[2] antimutagenic properties,[3] anticancer activities,[1,4] possess antiulcer,[1] antispasmodic[1,5] and antidiarrhoeal[1] activity. Onion is reported to be the richest source of flavonoids in the human diet.[6,7] The primary flavonoids found in onion are quercetin aglycone and glucosides (3,4'-di- and 4'-glucoside), and in some cases isorhamnetin monoglycoside or kaempferol monoglycoside.[8] As reported by Hertog *et al.*[9] it is ranked highest in quercetin content in a survey of 28 vegetables and 9 fruits. In addition, the presence of gallic, ferulic and protocatechuic acids in different layers of onion was also reported.[3,10]

There are many onion types, which differ in both onion colour and flavour, and contain different concentrations of phenolic compounds and flavonoids.[11–18] The antioxidative and antiradical activities were shown to be highly dependent on the content of phenolic compounds.[4,19–22] Generally, red cultivars contain the highest phenolics and flavonoids and show highest antioxidant activities among cultivars.[2,4,18] The antioxidant activity of the onion decreases significantly from the external leaves to the internal leaves.[23] Their health benefits and the methods used to determine the antioxidant activity is reviewed by Kaur *et al.*[24]

It has been reported previously, that bioactive compounds from onion exhibit antibacterial and antifungal activities. Essential oil extracts of onion, obtained by steam distillation, were shown to exhibit antibacterial activity against two bacteria, *Staphylococcus aureus*, *Salmonella enteritidis* and three fungi, *Aspergillus niger, Penicillium cyclopium* and *Fusarium oxysporum.*[25] Antifungal activity of onion bulb extract against *Aspergillus niger*, *A. flavus* and *A. fumigatus* was examined by Yin *et al.*[26] Further, phenolics and polyphenols from various plant materials are also known to be a group of compounds for which antimicrobial properties were reported.[11,12,27–30]

Onion skin contains significantly higher levels of flavonoids than the edible portion of the vegetables.[8,10,27,31,32] Therefore, the onion skin, which is a waste in onion processing, is especially interesting as a commercial source of phenolic compounds. In the present work, extracts from the skin and edible part of red onion were prepared separately. The aim

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was to investigate the content of phenolic compounds and quercetin in extracts and to ascertain and compare the antioxidant, radical scavenging and antimicrobial activities of extracts against three food-borne fungi: *A. niger, T. viride* and *P. cyclopium* and three bacteria as *E. coli, P. fluorescens* and *B. cereus.*

Materials and methods

Materials and chemicals

The red onion (var. Red baron) was supplied from Kmetijska zadruga Ptuj (Slovenia). All chemicals used for analysis were purchased from Merck (Germany). The HPLC-grade flavonoid standard quercetin was obtained from Acros (Belgium).

For the antifungal tests, potato dextrose agar (PDA) from Merck (Germany) was used. For antimicrobial tests nutrient agar based on meat was used and prepared from the following chemicals: sodium chloride, meat extract, peptone from meat (all purchased from Merck, Germany), and D(+)-glucose (supplied from Sigma, Germany).

Preparation of the extracts

The outer skin and edible part of red onion were separated and dried in a vacuum drier at 40 °C. Both parts of onion were ground, and sieve analysis of ground and dried material was carried out to determine the particle size distribution. Grinding was performed on a laboratory scale, in small quantities, so the heating of the raw material was minimal. Moisture content of plant material was determined using Karl Fisher Titrator (Mettler 99 Toledo DL31). In order to determine the optimal amount of solvent, the ground plant material was firstly extracted in an ultrasonic bath with specific amounts of solvent (ratio of solvent volume per g raw material (R); 10, 20, 50 and 100 mL g^{-1}) for 2 hours. Based on these experiments, the optimal ratio R determined was 20 mL g^{-1} and was used in all further experiments. Material was extracted in a round-bottom flask equipped with a condenser for 5 h at room temperature. The extraction mixture was constantly stirred with a magnetic stirrer. The extraction kinetics was followed by taking 1 mL of the solution from the extractor at specific time intervals, solvent was evaporated and the mass of extract and extraction yield were determined. After 5 h of extraction, the mixture was filtered using a 0.45-μm filter, the solvent was evaporated under vacuum and the yield of extraction was determined in wt. %. Residual plant material was subjected to second step of extraction where conditions were as follows: time 2 h, R = 20 mL g^{-1}. Extracts obtained from both steps were analyzed and the content of total phenols, quercetin and antioxidant and radical scavenging activities were determined. The antimicrobial activity of the extracts obtained in the first step of extraction was analysed.

Analysis of total phenols in extracts

The concentration of total phenols in extracts was measured by an UV spectrophotometric method based on a colorimetric oxidation/reduction reaction.[33,34] The oxidizing agent used was Folin-Ciocalteu reagent and the results were expressed in mg of gallic acid (GA) per g of extract (mg GA g^{-1} extract).

Analysis of quercetin in extract

50 mg of the extract was dissolved in 20 mL of distilled water. 16 mL of this water solution was hydrolyzed using a solution of 5 mL 6 mol L^{-1} HCl and 24 mL pure ethanol. After refluxing at 90 °C for 2 h, the extract was cooled and diluted up to 50 mL, sonicated and analysed.[33,35]

The content of flavonol quercetin in hydrolyzed extract was determined by High Performance Liquid Chromatography (HPLC). The HPLC system consisted of a Varian 9012 pump and Varian diode array detector 9065 (Walnutcrek, California). As a stationary phase the Kromasil column C-18 250×4.6 mm with 5 μm particles size was used. The mobile phase consisted of 2 solvents A: methanol and B: 20 mmol L^{-1} phosphate buffer with pH = 3. Linear gradient elution was performed (35 min: 5–100 % A). Re-equilibration period of 5 min with 5 % A was used between individual runs. The flow rate was 0.8 mL min^{-1} and the detection was performed at 367 nm. The quantification was made with the help of an external standard.

Determination of antioxidant and radical scavenging activity

In order to determine the antioxidative and radical scavenging activity of quercetin, BHT (butylated hydroxytoluene) and that of plant extracts the following methods were applied: β-carotene bleaching test – (BCB method) and DPPH method.

BCB test. The antioxidant activity of test sample was elucidated on heat-induced oxidation of aqueous emulsion system of β-carotene and linoleic acid.[36] 1 mL of β-carotene chloroformic solution (0.2 mg mL^{-1}) was added to a boiling flask together with linoleic acid (0.02 mL) and Tween 20 (0.2 mL). After evaporation of solvent under vacuum by a rotary evaporator, 50 mL of distilled water saturated with oxygen was added to the flask and 0.2 mL of

methanolic solution of antioxidant (1 mg mL^{-1}) was added to 5 mL of so prepared emulsion. A control containing 0.2 mL of methanol and 5 mL of the emulsion was prepared. The mixtures were shaken and stored for 2 h at 50 °C. The absorbance of the samples was measured on the Varian UV/VIS spectrophotometer at 470 nm at the beginning (t = 0 min) and after the experiment (t = 120 min). Antioxidant activity of the sample(s) was calculated as percent inhibition of oxidation versus control sample (c), using the equation

$$\% \text{ antioxidant activity}_{\text{emulsion system}} = \\ = 100 \cdot \left[1 - \frac{(A_{s(0)} - A_{s(120)})}{(A_{c(0)} - A_{c(120)})}\right] \quad (1)$$

where A_s is the absorbance of sample at t = 0 min and t = 120 min, and A_c is the absorbance of control sample at t = 0 min and t = 120 min.

DPPH method. Radical scavenging activity of test sample against stable DPPH (2,2-diphenyl--2-picrylhydrazyl hydrate, Sigma-Aldrich Chemie, Steinheim, Germany) was determined spectrophotometrically (Varian UV/VIS spectrophotometer).[37]

Test sample solutions were prepared by dissolving 10 mg of sample (dry extract, BHT or quercetin) in 10 mL of methanol. The solution of DPPH in methanol (6 mol L^{-1}) was prepared daily, before UV measurements. 3 mL of this solution were mixed with 77 mL test sample solution. So prepared samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured at 515 nm. A blank sample containing the same amount of methanol and DPPH solution was prepared daily and absorption was measured. Radical scavenging activity was calculated by the following formula:

$$\% \text{ radical scavenging activity} = 100 \cdot \frac{(A_c - A_s)}{A_c} \quad (2)$$

where A_c is the absorption of control blank sample (t = 0 min) and A_s is the absorption of test sample solution (t = 15 min).

Statistical analysis

Extracts were prepared under the same conditions in three parallels. From each parallel, two samples were taken for further analyses (n = 5). The analytical methods were validated and on the 95 % confidence range, the results showed no statistical difference; the accuracy was acceptable. Results are presented as mean values ± standard deviation of 5 replicates.

Antimicrobial activity tests

Microbial strains. Organisms were obtained as lyophilized cultures from National Collection of Agricultural and Industrial Microorganism (Hungary). Organisms were as follows: three species of moulds, *Aspergillus niger, Trichoderma viride* and *Penicillium cyclopium,* and two species of Gram negative bacteria, *Escherichia coli* and *Pseudomonas fluorescens* and one species of Gram positive bacteria, *Bacillus cereus.*

Preparation of test microorganisms. Agar cultures of fungi and bacteria for antimicrobial tests were prepared as described by Vagi *et al.*[38]

The test fungi were maintained on PDA slopes and stored at 4 °C. Conidia were harvested in sterile distilled water containing approximately 10^5–10^7 conidia mL^{-1}. These conidia suspensions were used immediately after preparation for determining the antifungal activities of the onion extracts.

In the antibacterial experiments, test bacteria were grown on meat nutrient agar slopes for 24 hours at 30 °C, except for *E. coli* that was grown at 37 °C and then stored at 4 °C. Before the bacterial experiments were carried out, liquid medium was inoculated with freshly harvested bacteria and incubated for 24 hours at the adequate temperature. These bacteria suspensions (approximately 10^7–10^8 cell mL^{-1}) were used for inoculating the test medium containing the onion extracts.

Antifungal activity test. The antifungal activity test of onion extracts was carried out by the agar diffusion method as described in the literature.[25,38–40] Each PDA sterile plate contained 19 mL of molten medium and 1 mL alcoholic mixture of test sample (onion extracts in concentration of 20 g/100 mL; quercetin in concentration of 2 g/100 mL). As alcoholic control, PDA plates containing the same amount of medium and 1 mL 96 % ethanol were prepared. For all test fungi, PDA plates without materials were made and used as control plates. The solid plates were inoculated with 0.1 mL conidial suspension, measuring it into the holes (diameter 10 mm) in the centre of the medium. The plate was left undisturbed to allow diffusion of the sample into agar, and then the plate was incubated in the dark at 28 °C, and the diameter of the mycelial growth was measured. Incubation was stopped when the mycelial mass of control plates had filled the plates (12–33 days). Each test was run in triplicate and averages were calculated. The antifungal activity was determined in terms of percentage mycelial inhibition calculated by the following formula:

$$\text{Inhibition } (\%) = [(D_C - D_T)/D_C] \cdot 100 \quad (3)$$

where D_C is the colony diameter of the mycelium on the alcoholic control plate (mm), and D_T is the colony diameter of the mycelium on the test petri plate (mm).

Antibacterial activity test. The antibacterial activity of onion extracts was tested by the broth dilution method.[38] The growth of bacteria was followed by measuring the optical density of bacterial suspension using densitometry. 50 mL nutrient liquid medium was measured into an Erlenmeyer flask. The flask with the medium was sterilized in an autoclave at 121 °C for 15 min.

Into each sterile flask 1 mL of alcoholic test sample solution of defined concentration (onion extracts in concentration of 20, 10 and 5 g/100 mL; quercetin in concentration of 2 g/100 mL) was added and inoculated with 1 mL bacterial suspension. An alcoholic control was prepared by addition of 1 mL of 96 % ethanol instead of test sample solution and then inoculated with 1 mL bacterial suspension. Bacteria control in the sterile medium was applied without any added material. *E. coli* and *B. cereus* were incubated at 37 °C and 30 °C, respectively. *P. fluorescens* was grown on a shaking platform at 30 °C. For obtaining the initial bacterial concentration (x_0) samples were taken straight after inoculation. To follow the bacterial growth, samples were taken after 3, 5, 8, 24 and 27 h of incubation. Due to the color of onion extracts, 1–2 mL samples were taken before inoculation, and were used as control solution during the density measurements. The optical density of the samples was measured by densitometry (Varian UV/VIS spectrophotometer) at 550 nm wavelengths. The inhibition is determined from the optical density of the sample (OD_x) after 27 hour of incubation compared to the optical density of alcoholic control (OD_{AC}) after the same time of incubation.

$$\text{Inhibition (\%)} = [(OD_{AC} - OD_x)/OD_{AC}] \cdot 100 \quad (4)$$

For verification of the results obtained by broth dilution method (VIS-method) after 27 hours viable counts of CFU (colony forming units) were done.

Statistical analysis. Experimental results were expressed as means ± standard deviation (SD) of three parallel measurements.

Results and discussion

Median particle size of ground onion skin and edible part plant material determined by sieve analysis was 0.25 mm. The moisture content of onion skin and the edible part was equal to 8.1 and 5.7 % (w/w), respectively.

Extraction kinetics study

Kinetic curves are presented in Figs. 1 and 2, where the yield of extraction is plotted as a function of time. Results show that the highest extraction yields and the highest initial extraction rates were obtained when mixtures of acetone or ethanol with water were used as solvents. Furthermore, the type and concentration of solvent (35 or 60 %) shows no significant influence on the course of extraction curve and final yields, which were for onion skin and edible parts from 41.4 ± 2.0 to 43.3 ± 2.1 % and 33.1± 1.6 to 37.2 ± 1.8 %, respectively. When pure acetone and ethanol were used as solvents, the yields obtained for onion skin and the edible parts were much lower and were from 6.4 ± 0.5 to 16.1 ± 0.8 % and 4.4 ± 1.0 to 8.0 ± 0.4 %, respectively.

Experimental extraction curves were analyzed with a mathematical model derived from Fick's second law.[41] The following equation, based on the as-

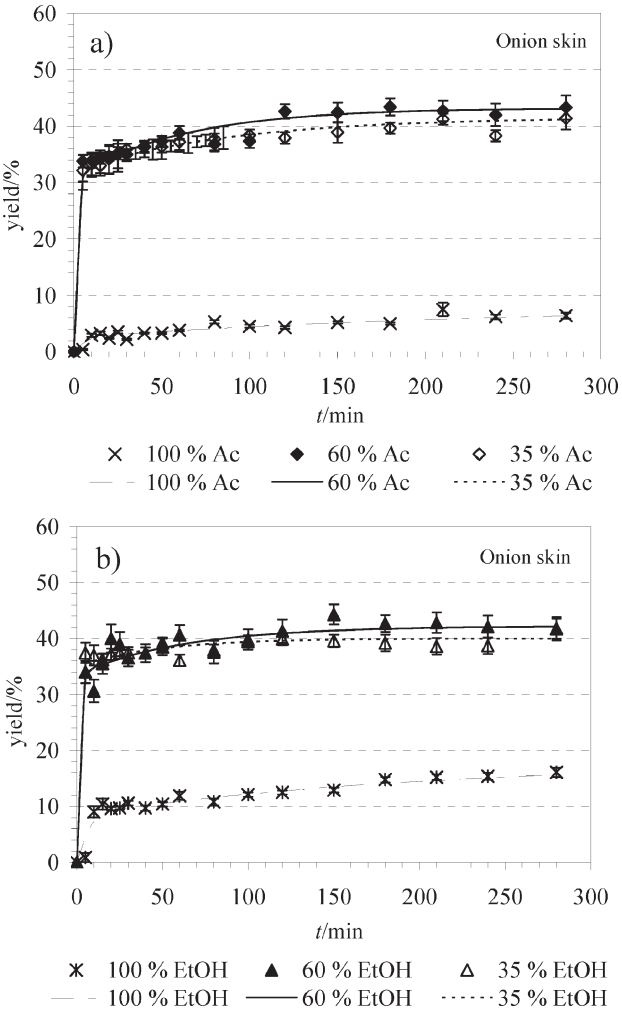


Fig. 1 – *Extraction kinetics of onion outer skin at room temperature and R = 20 mL g^{-1} with a) acetone (Ac) and water mixtures and b) ethanol (EtOH) and water mixtures (symbols represent experimental results, lines are obtained by model)*

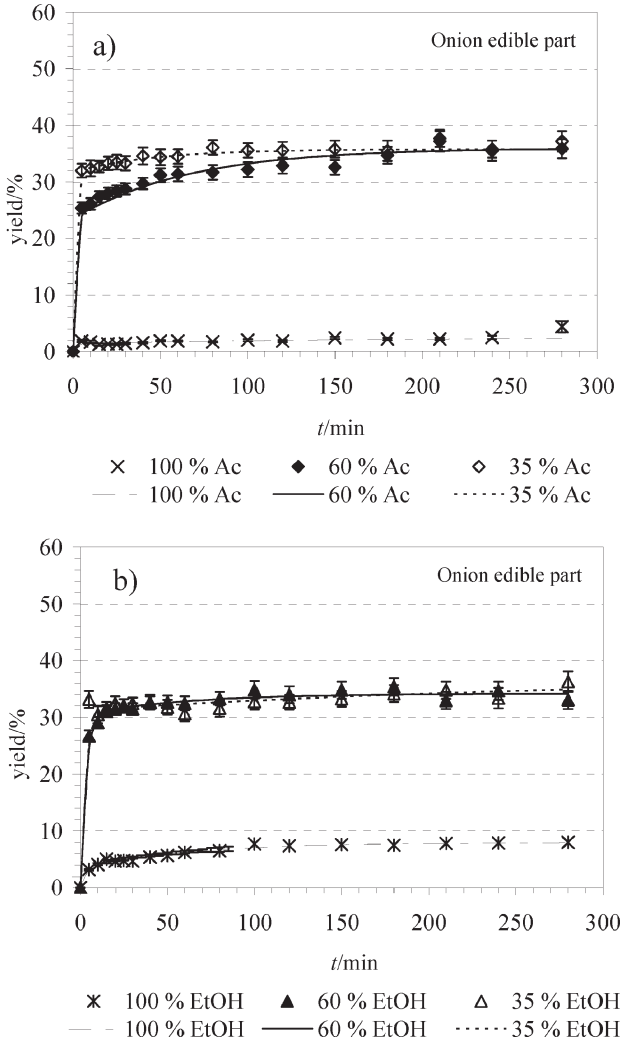


Fig. 2 – *Extraction kinetics of onion edible part at room temperature and R = 20 mL g^{-1} with a) acetone (Ac) and water mixtures and b) ethanol (EtOH) and water mixtures (symbols represent experimental results, lines are obtained by model)*

sumption that particles are spheres with radium r, was used:

$$\frac{\gamma_\infty - \gamma}{\gamma_\infty} = \frac{6}{\pi^2} \exp\left(-\frac{\pi^2 D\, t}{r^2}\right) \tag{5}$$

where γ and γ_∞ are the concentrations of the extracted constituent in the solution at time t and at infinite time, respectively. When two parallel diffusion processes are considered inside the solid (one faster and one slower),[42] following expression is obtained:

$$\frac{\gamma_\infty - \gamma}{\gamma_\infty} = \tag{6}$$

$$= \frac{6}{\pi^2}\left[f_1 \exp\left(-\frac{\pi^2 D_1\, t}{r^2}\right) + f_2 \exp\left(-\frac{\pi^2 D_2\, t}{r^2}\right)\right]$$

where f_1 and f_2 are the fractions of the solute, which are extracted with diffusion coefficients D_1 and D_2, respectively. In later stages of the extraction, only the second term on the right-hand side of eq. (6) remains significant. The parameter D_2 is obtained from the slope and the parameter f_2 from the intercept of the curve where $\ln[\gamma_\infty/(\gamma_\infty - \gamma)]$ is plotted as function of time t. In earlier stages of the extraction, the second exponential term is close to unity and with the addition of f_2 from the previous calculation, D_1 and f_1 can be determined.

The diffusion coefficients calculated with eq. (6) are summarized in Tables 1 and 2. The agreement of the model with the data is shown in Figs. 1 and 2 and the average absolute relative deviation (AARD) (Tables 1 and 2) is generally below 5 % except for pure solvents, where AARD is up to 29.83 ± 1.89 % for onion skin and up to 12.47 ± 1.42 % for the edible part. The diffusion coefficients calculated correspond to two extraction stages: fast, occurring in short time periods (D_1); and slow, occurring in long time periods (D_2). At operating conditions investigated, D_1 is in the order of magnitude from $0.530 \pm 0.029 \cdot 10^{-7}$ to $2.419 \pm 0.154 \cdot 10^{-7}$ cm^2 s^{-1}, while D_2 varies from $0.976 \pm 0.154 \cdot 10^{-9}$ to $5.752 \pm 0.412 \cdot 10^{-9}$ cm^2 s^{-1}.

Table 1 – *Diffusion coefficients obtained for extraction of outer onion skin*

	$D_1 \cdot 10^7$/cm^2 s^{-1}	$D_2 \cdot 10^9$/cm^2 s^{-1}	AARD/%
100 % EtOH	0.530 ± 0.029	1.319 ± 0.301	29.83 ± 1.89
100 % Ac	0.566 ± 0.051	1.135 ± 0.230	26.81 ± 1.38
60 % EtOH	1.819 ± 0.224	4.222 ± 0.385	4.06 ± 0.63
60 % Ac	1.789 ± 0.187	4.195 ± 0.243	3.50 ± 0.46
35 % EtOH	1.432 ± 0.131	5.541 ± 0.298	4.17 ± 0.85
35 % Ac	1.666 ± 0.219	2.955 ± 0.135	1.91 ± 0.44

Table 2 – *Diffusion coefficients obtained for extraction of onion edible part*

	$D_1 \cdot 10^7$/cm^2 s^{-1}	$D_2 \cdot 10^9$/cm^2 s^{-1}	AARD/%
100 % EtOH	0.875 ± 0.031	4.010 ± 0.301	3.79 ± 0.39
100 % Ac	2.009 ± 0.281	1.768 ± 0.347	12.47 ± 1.42
60 % EtOH	0.849 ± 0.028	4.485 ± 0.227	2.10 ± 0.43
60 % Ac	2.419 ± 0.154	4.010 ± 0.183	3.27 ± 0.87
35 % EtOH	0.866 ± 0.032	0.976 ± 0.154	3.38 ± 0.64
35 % Ac	2.015 ± 0.114	5.752 ± 0.412	1.21 ± 0.49

Content of phenolic compounds in extracts

The composition of extracts is presented in Table 3, where content of total phenols and quercetin in extracts obtained in two subsequent extraction steps can be observed. The highest concentrations of total phenols and quercetin in extracts were generally obtained with pure acetone. However, the best results regarding the amounts of isolated phenolic compounds and quercetin were obtained when 35 and 60 % acetone or ethanol aqueous mixtures were used as solvents for extraction. The amounts of isolated phenolic compounds and quercetin from onion skin were approximately 3 to 5 times higher as those from the edible part of onion.

In the case of the two-step extraction of red onion skin with 35 % and 60 % acetone or ethanol mixtures with water, the content of total phenolic compounds in extracts obtained after 1st and 2nd step was similar for both solvents, and was from 93.64 ± 1.74 to 150.63 ± 4.42 mg GA g^{-1} extract. The content of quercetin in 35 % and 60 % acetone or ethanol extracts was from 22.42 ± 0.35 to 64.80 ± 1.41 mg g^{-1} extract. Further, good linear correlation between total phenols and quercetin extracted per g of raw material was found (r^2 = 0.91) and indicates that quercetin in average presents 44 % of extracted phenolic compounds. This confirms literature reports that flavonoid quercetin is the main phenolic compound in onion.

Antioxidant and radical scavenging activity of extracts

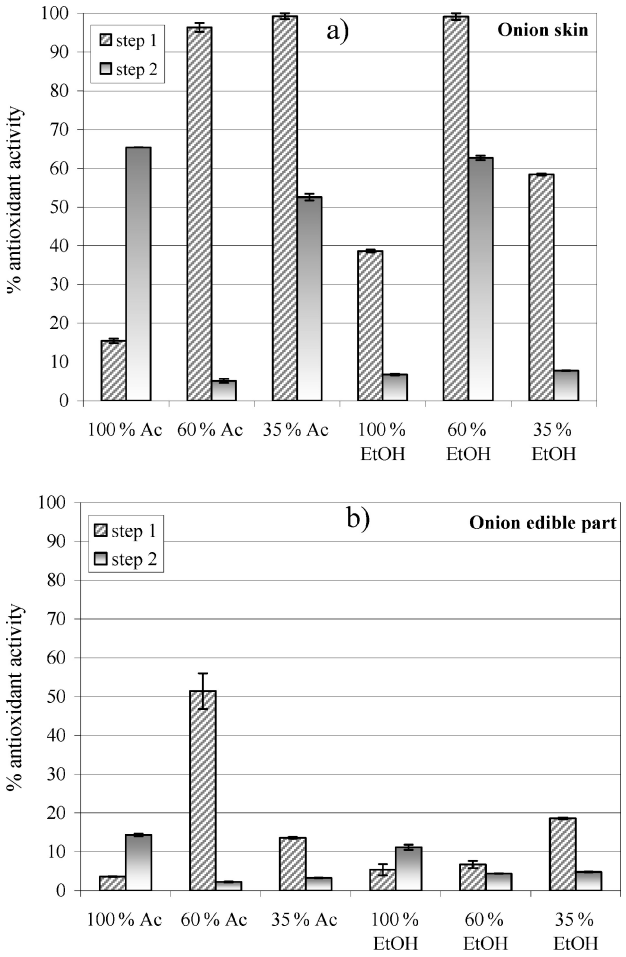
Fig. 3 presents antioxidative activity of onion skin and edible part extracts obtained by BCB method. Radical scavenging activity of extracts obtained with various solvents is presented in Fig. 4. Generally, much higher antioxidative and radical scavenging activities of skin extracts can be observed. The antioxidative activity by BCB test shows the highest activity of onion skin extracts obtained with 35 % and 60 % acetone and 60 % ethanol. The highest radical scavenging activity was observed for the pure acetone extract, which contained the highest amounts of phenolic compounds and quercetin. The good linear correlation between phenol content and radical scavenging activity determined by DPPH assay (r^2 = 0.884) confirms previous findings, that phenols are mainly responsible for the radical scavenging activity of extracts.[22] On the other hand, no correlation was found between phenol content and % Antioxidant activity values determined by BCB method.

Low antioxidant activity observed for onion edible part extracts is in agreement with the results reported by Kaur *et al.*[43] who have analyzed the antioxidant activities of aqueous and ethanolic extracts of 33 vegetables and ranked the onion edible part into the group with low antioxidant activity.

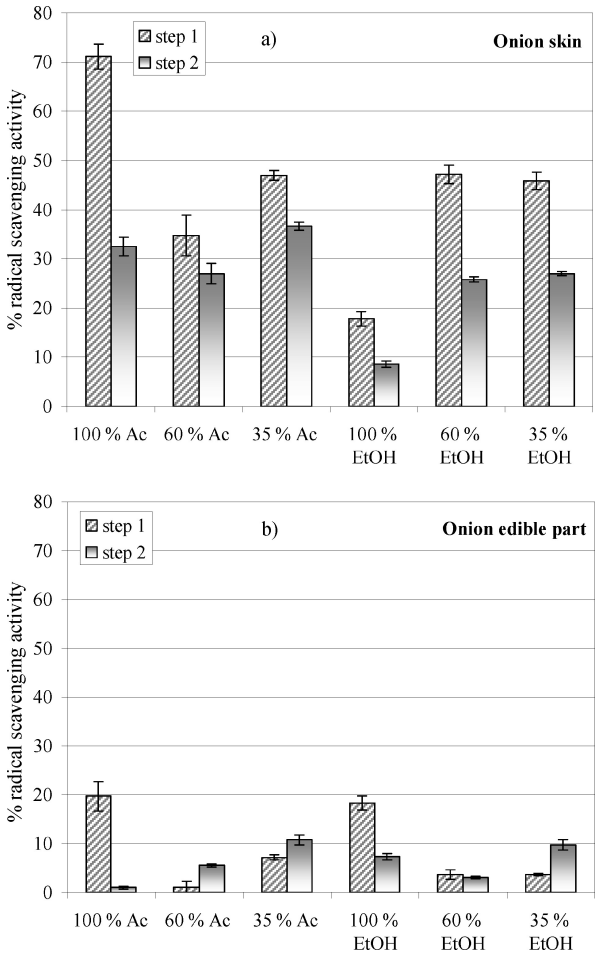
The antioxidant and radical scavenging activities of onion skin extracts obtained with 35 % acetone and 60 % ethanol are compared to activities of

Table 3 – *Extraction of onion outer skin and edible part: composition of extracts*

Material	Solvent	Total phenols/mg GA g^{-1} extract		Quercetin/mg g^{-1} extract	
		1. step	2. step	1. step	2. step
onion skin	100 % EtOH	63.62 ± 2.03	71.76 ± 1.59	61.88 ± 1.12	39.60 ± 0.81
	100 % Ac	208.42 ± 4.34	83.40 ± 2.13	163.14 ± 1.62	16.84 ± 0.45
	60 % EtOH	114.86 ± 1.15	104.52 ± 1.98	51.55 ± 1.05	32.24 ± 0.98
	60 % Ac	93.64 ± 1.74	97.48 ± 1.52	50.62 ± 0.86	22.42 ± 0.35
	35 % EtOH	113.38 ± 2.87	118.39 ± 3.09	33.56 ± 1.23	44.49 ± 0.44
	35 % Ac	129.83 ± 2.01	150.63 ± 4.42	64.80 ± 1.41	50.17 ± 0.52
edible part	100 % EtOH	49.88 ± 1.83	27.57 ± 0.95	8.22 ± 0.28	12.37 ± 0.31
	100 % Ac	57.82 ± 1.32	26.80 ± 1.03	13.76 ± 0.41	22.44 ± 0.24
	60 % EtOH	35.61 ± 0.85	20.93 ± 0.86	13.39 ± 0.35	1.13 ± 0.10
	60 % Ac	37.39 ± 1.37	30.11 ± 1.24	18.02 ± 0.24	0.47 ± 0.06
	35 % EtOH	31.23 ± 1.06	34.49 ± 1.32	8.20 ± 0.19	11.47 ± 0.12
	35 % Ac	33.95 ± 0.94	27.80 ± 1.08	10.26 ± 0.28	11.75 ± 0.09



F i g . 3 – *Antioxidant activity of red onion extracts determined by using β-carotene-linoleic acid emulsion system: a) skin extracts and b) edible part extracts*



F i g . 4 – *Radical scavenging activities of red onion extracts determined by using DPPH assay: a) skin extracts and b) edible part extracts*

quercetin and BHT in Table 4. The results summarized, show similar antioxidant activity of quercetin and BHT in water emulsion system (BCB test), while the radical scavenging activity (DPPH test) of quercetin is much higher than that of BHT. Red onion skin extracts show similar inhibition of β-carotene oxidation in emulsion system (99.15 ± 0.85 %) as BHT and quercetin, while the radical scavenging activity is comparable to that of BHT (47.14 ± 1.85 %).

T a b l e 4 – *Antioxidant and radical scavenging activity of quercetin, BHT and red onion skin extracts, obtained with 35 % acetone (Ac) and 60 % ethanol (EtOH) in 1st step of extraction at room temperature and R = 20 mL g^{-1}*

Sample	BCB Antioxidant activity/%	DPPH Radical scavenging activity/%
Quercetin	98.45 ± 1.06	95.70 ± 0.58
BHT	98.96 ± 1.04	58.22 ± 2.54
35 % Ac extract of onion skin	99.24 ± 0.74	46.95 ± 1.02
60 % EtOH extract of onion skin	99.15 ± 0.85	47.14 ± 1.85

Antifungal activity of extracts

Inhibition activities of onion extracts on the growth of *A. niger, T. viride* and *P. cyclopyum* are presented in Fig. 5. As can be seen from the results, the skin and edible part extracts show different inhibition degrees on the growth of the three test fungi.

All tested onion skin extracts showed antifungal activities on *A. niger*; the growth inhibition was from 9.3 ± 0.3 to 35.6 ± 4.4 %. Oppositely, the growth of *A. niger* was less or not inhibited by onion edible part extracts. This can be explained by higher concentration of active compounds in skin extracts. Furthermore, skin extracts obtained with 60 % solvent mixtures (acetone-water or ethanol-water) show higher inhibition of *A. niger* than extracts obtained with 35 % solvent mixtures.

Growth of *T. viride* was inhibited by all four onion skin extracts; the inhibition was from 41.5 ± 7.2 to 63.0 ± 6.2 %. Further, skin extracts obtained with the 60 % acetone and 60 % ethanol showed higher activity than extracts obtained with 35 % acetone and ethanol. Similar results were obtained also for

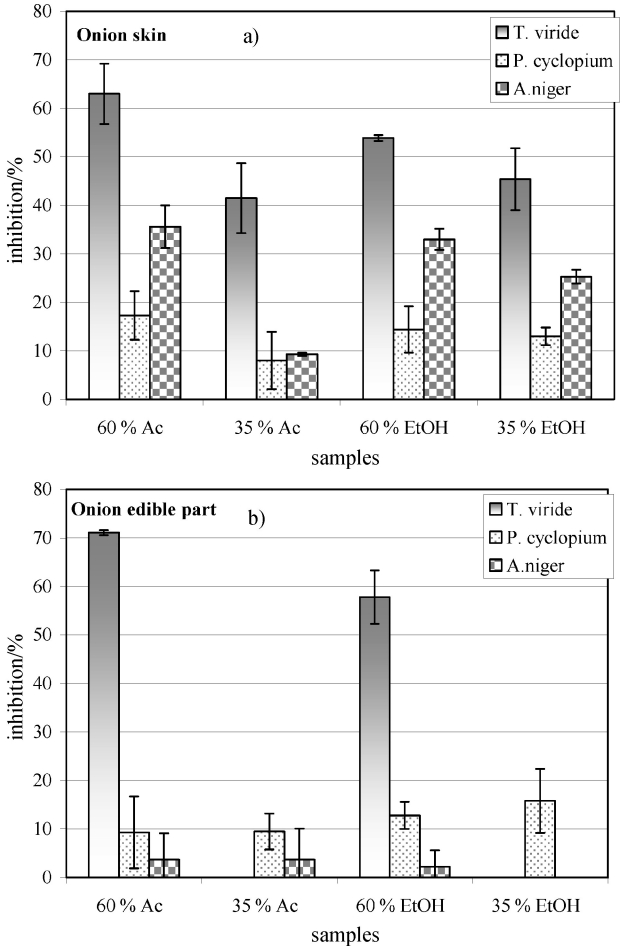


Fig. 5 – *The inhibition activity (%) of red onion extracts with concentration 20 g/100 mL against three tested fungi: a) skin extracts and b) edible part extracts*

edible part extracts, where high inhibition activities were observed for the 60 % acetone and 60 % ethanol extracts (71.1 ± 0.5 % and 57.8 ± 5.5 %, respectively), while no growth inhibition of *T. viride* was observed for 35 % acetone and 35 % ethanol extracts.

P. cyclopium was the only tested fungi, the growth of which was inhibited by all tested onion skin and edible part extracts, however inhibition was low. For skin extracts, the inhibition was between 17.3 ± 5.0 % and 8.0 ± 5.9 % and for edible part extracts inhibition was between 15.8 ± 6.6 % and 9.3 ± 7.4 %. Generally, it can be concluded that onion skin and edible part extracts have similar effects on *P. cyclopium* growth, and that the type of solvent has no significant effect on inhibitory activities of extracts.

For comparison, antifungal activity tests of pure quercetin were performed. To be approximately in the same concentration range as in extract solutions, the concentration of quercetin test solution was lower than that of extracts and was 2 g/100 mL. Pure quercetin showed no inhibition against *A. niger and T. viride* and showed only small inhibition against *P. cyclopium* (10.2 % ± 2.7 %).

Antibacterial activity of extracts

The results of antibacterial activity tests are summarized in Table 5. The onion skin and edible part extracts exhibited different antimicrobial activities against *B. cereus, E. coli* and *P. fluorescens*.

Table 5 – *Antibacterial activity (inhibition in %) of onion extracts against three bacteria (/ – experiments were not performed)*

c/g/100 mL	Onion skin extracts				Onion edible part extracts			
	60 % Ac	35 % Ac	60 % EtOH	35 % EtOH	60 % Ac	35 % Ac	60 % EtOH	35 % EtOH
B. cereus								
20	100.0	99.4 ± 1.1	97.3 ± 2.1	63.3 ± 2.5	98.4 ± 0.4	80.7 ± 0.2	79.2 ± 2.9	59.7 ± 4.9
10	95.0 ± 0.5	93.2 ± 1.9	89.9 ± 0.4	83.2 ± 1.1	70.1 ± 1.4	39.1 ± 3.2	37.7 ± 5.2	No inh.
5	84.3 ± 4.1	87.9 ± 1.2	83.3 ± 3.1	52.1 ± 1.3	63.3 ± 3.8	No inh.	26.0 ± 2.5	No inh.
E. coli								
20	97.9 ± 2.4	100.0	93.8 ± 4.1	80.6 ± 3.1	No inh.	No inh.	No inh.	No.inh.
10	88.2 ± 0.3	86.0 ± 5.3	81.4 ± 0.6	78.4 ± 2.9	No inh.	No inh.	No inh.	No inh.
5	85.5 ± 2.1	72.2 ± 3.9	84.3 ± 1.5	14.4 ± 4.5	/	/	/	/
P. fluorescens								
20	98.9 ± 1.0	98.3 ± 0.9	97.9 ± 0.3	97.6 ± 1.6	95.7 ± 0.7	7.2 ± 0.3	23.0 ± 4.2	9.2 ± 2.9
10	92.1 ± 1.1	95.9 ± 1.9	93.7 ± 1.7	94.5 ± 0.5	1.5 ± 2.7	3.2 ± 3.1	3.1 ± 2.2	6.1 ± 2.1
5	39.1 ± 0.6	71.7 ± 3.1	44.7 ± 1.9	42.4 ± 1.9	No inh.	No inh.	No inh.	No inh.

Generally, skin extracts showed significantly stronger inhibitory effects against three tested bacteria strains than the edible part extracts, and the inhibition activity increased with increasing concentration of extracts.

All tested skin extracts at different concentrations strongly inhibit the growth of *B. cereus*. Inhibition is 83.3 ± 3.1 % or higher even at low concentrations of extracts (5 g/100 mL), except for 35 % ethanol extract, where the lowest inhibition was observed (52.1 ± 1.3 %). On the other hand, edible part extracts exhibit relatively strong inhibition activities against *B. cereus* only at concentration 20 g/100 mL, while at lower concentrations weak or no inhibition activities were observed. An exception is observed with the 60 % acetone extract, where the inhibition activity in the concentration range of extract from 5 to 20 g/100 mL was from 63.3 ± 3.8 to 98.4 ± 0.4 %. The lowest activity was again observed for 35 % ethanol extract.

Inhibition activities of onion skin extracts on *P. fluorescens* growth (Table 5) showed high antibacterial activity against these food-borne bacteria at concentrations 20 and 10 g/100 mL, where inhibition was 92.1 ± 1.1 % or higher for all types of extraction solvents, while at concentration 5 g/100 mL inhibition activities were approximately 50 % lower. On the other hand, for edible part extracts high inhibition activity (95.7 ± 0.7 %) was observed when 60 % acetone was used as extraction solvent and conc. was 20 g/100 mL. For all other tested edible part extracts, low or no inhibition activities were observed.

All onion skin extracts possessed antibacterial activity against Gram-negative bacteria *E. coli.* High inhibition activities of all extracts (72.2 ± 3.9 % or higher) were generally observed in whole concentration range tested, except for 35 % ethanol extract, which showed low activity at concentration 5 g/100 mL. Oppositely, the growth of the *E. coli* was not inhibited by any of the tested edible part extracts. Apparently, onion edible part extracts do not contain bioactive compounds that inhibit the growth of *E. coli.*

For comparison, antibacterial activity tests of pure quercetin were performed and the concentration of quercetin test solution was 2 g/100 mL. Pure quercetin at this concentration showed no inhibition against *E. coli* while the inhibition of *B. cereus* and *P. fluorescens* growth was 16.3 % ± 1.1 % and 38.6 % ± 2.8 %, respectively.

Conclusion

Red onion skin is a rich source of phenolic compounds, especially of quercetin, which in average is found to represent 44 % of all extracted phenolic compounds. By using 35 % acetone or 60 % ethanol aqueous solutions, maximal amounts of quercetin extracted in two subsequent steps were 24.47 ± 0.48 and 19.98 ± 0.42 mg g^{-1} raw material, respectively. The amounts of isolated phenolic compounds and quercetin from onion skin were approximately 3 to 5 times higher as from the onion edible part.

Onion skin extracts also showed high antioxidant, radical scavenging and antimicrobial activities. Inhibition of β-carotene oxidation in emulsion system observed for skin extracts was similar to that for BHT and quercetin, while the radical scavenging activity of skin extracts determined by DPPH test was lower than that of pure quercetin, however it was comparable to that of BHT.

The results from this study indicate that onion skin extracts possess high activity against the growth of food poisoning bacteria such as *B. cereus, P. fluorescens* and *E. coli* for all tested concentrations and extraction solvents. On the other hand, onion edible part extracts showed lower or no inhibition activities against tested bacteria.

Antifungal activity of red onion extracts against *A. niger, T. viride* and *P. cyclopyum* was generally lower than their antibacterial activity. It was shown that both, skin and edible part extracts contain antifungal compounds that inhibit the growth of *P. cyclopium.* Further, all tested onion skin extracts showed antifungal activities on *A. niger* while the growth inhibition of *A. niger* was lower or not even observed for onion edible part extracts and tested concentrations. High inhibition activities were observed also against *T. viride* for all skin and edible part extracts obtained with 60 % solvent mixtures with water.

Antifungal and antibacterial activities of pure quercetin were generally much lower than that of extracts, or not even observed at concentrations investigated. Nevertheless, future studies are required to determine synergistic effects of bioactive compounds of onion extracts.

The results indicate that red onion skin and edible part extracts have the potential to be used as antioxidants and antimicrobial agents in the food, cosmetics and pharmaceutical industries.

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List of symbols

A_c – absorption of control sample, dimensionless

A_s – absorption of test sample solution, dimensionless

AARD – average absolute relative deviation, %

D_1 – diffusion coefficient in short time period, $cm^2\ s^{-1}$

D_2 – diffusion coefficient in long time period, $cm^2\ s^{-1}$

D_C – colony diameter of the mycelium on the alcoholic control plate, mm

D_T – colony diameter of the mycelium on the test petri plate, mm

f_1 – fractions of the solute extracted in short time period, dimensionless

f_2 – fractions of the solute extracted in long time period, dimensionless

OD_x – optical density of the sample, dimensionless

OD_{AC} – optical density of alcoholic control, dimensionless

R – ratio of solvent volume and mass of raw material, mL g^{-1}

r – sphere radium, cm

t – time, s

γ – concentration of the extracted constituent in the solution at time t, g dm^{-3}

γ_∞ – concentration of the extracted constituent in the solution after infinite time, g dm^{-3}

References

1. *Lanzotti, V.,* J. Chromatogr. A **1112** (2006) 3.
2. *Gorinstein, S., Park, Y. S., Heo, B. G., Namiesnik, J., Leontowicz, H., Leontowicz, M., Ham, K. S., Cho, J. Y., Kang, S. G.,* Eur. Food Res. Technol. **228** (2009) 903.
3. *Singh, B. N., Singh, B. R., Singh, R. L., Prakash, D., Singh, D. P., Sarma, B. K., Upadhyay, G., Singh, H. B.,* Food Chem. Toxicol. **47** (2009) 1161.
4. *Jeong, C. H., Heo, H. J., Choi, S. G., Shim, K. H.,* Food Sci. Biotechnol. **18** (2009) 108.
5. *Naseri, M. K. G., Yahyavi, H., Arabian, M.,* Iran J. Pharm. Res. **7** (2008) 155.
6. *Miean, K. H., Mohamed, S.,* J. Agr. Food Chem. **49** (2001) 3106.
7. *Gennaro, L., Leonardi, C., Esposito, F., Salucci, M., Maiani, G., Quaglia, G., Fogliano, V.,* J. Agr. Food Chem. **50** (2002) 1904.
8. *Suh, H. J., Lee, J. M., Cho, J. S., Kim, Y. S., Chung, S. H.,* Food Res. Int. **32** (1999) 659.
9. *Hertog, M. G. L., Hollman, P. C. H., Katan, M. B.,* J. Agr. Food Chem. **40** (1992) 2379.
10. *Prakash, D., Singh, B. N., Upadhyay, G.,* Food Chem. **102** (2007) 1389.
11. *Yang, J., Meyers, K. J., Van der Heide, J., Liu, R. H.,* J. Agr. Food Chem. **52** (2004) 6787.
12. *Sellappan, S., Akoh, C.,* J. Agr. Food Chem. **50** (2002) 5338.
13. *Shon, M.-Y., Choi, S.-D., Kahng, G.-G., Nam, S.-H., Sung, N.-J.,* Food Chem. Toxicol. **42** (2004) 659.
14. *Crozier, A., Lean, M. E. J., McDonald, M. S., Black, C.,* J. Agr. Food Chem. **45** (1997) 590.
15. *Nuutila, A. M., Puupponen-Pimia, R., Aarni, M., Oksman-Caldentey, K. M.,* Food Chem. **81** (2003) 485.
16. *Marotti, M., Piccaglia, R.,* J. Food Sci. **67** (2002) 1229.
17. *Aoyama, S., Yamamoto, Y.,* Food Sci. Technol. Res. **13** (2007) 67.
18. *Kaur, C., Joshi, S., Kapoor, H. C.,* J. Food Biochem. **33** (2009) 184.
19. *Proteggente, A. R., Pannala, A. S., Paganga, G., van Buren, L., Wagner, E., Wiseman, S., van de Put, F., Dacombe, C.,* Free Radical Res. **36** (2002) 217.
20. *Benkeblia, N.,* Braz. Arch. Biol. Techn. **48** (2005) 753.
21. *Makris, D. P., Boskou, G., Andrikopoulos, N. K.,* J. Food Compos. Anal. **20** (2007) 125.
22. *Santas, J., Carbó, R., Gordon, M. H., Almajano, M. P.,* Food Chem. **107** (2008) 1210.
23. *Leon, H. C. L., Galdon, B. R., Rodriguez, E. M. R., Romero, C. D.,* Cyta J. Food **7** (2009) 53.
24. *Kaur, C., Kapoor, H. C.,* Int. J. Food Sci. Tech. **36** (2001) 703.
25. *Benkeblia, N.,* Lebensm. Wiss. Technol. **37** (2004) 263.
26. *Yin, M., Tsao, S.,* Int. J. Food Microbiol. **49** (1999) 49.
27. *Tsuchiya, H.,* J. Chromatogr. B **720** (1998) 225.
28. *Cowan, M. M.,* J. Clin. Microbiol. Rev. **12** (1999) 564.
29. *Cushnie, T. T. P., Lamb, A. J.,* Int. J. Antimcrob. Ag. **26** (2005) 343.
30. *Cook, N. C., Samman, S.,* J. Nutr. Biochem. **7** (1996) 66.
31. *Chu, Y. H., Chang, C. L., Hsu, H. F.,* J. Sci. Food Agr. **80** (2000) 561.
32. *Wach, A., Pyrzyńska, K., Biesaga, M.,* Food Chem. **100** (2007) 699.
33. *Škerget, M., Kotnik, P., Hadolin, M., Rižner-Hraš, A., Simonič, M., Knez, Ž.,* Food Chem. **89** (2005) 191.
34. AOCS, Official methods and recommended practices of American Oil Chemists' Society, 4th ed., American Oil Society, Champaign, 1990.
35. *Wang, H., Helliwell, K.,* Food Res. Int. **34** (2001) 223.
36. *Kulisic, T., Radonic, A., Katalinic, V., Milos, M.,* Food Chem. **85** (2004) 633.
37. *Miliauskas, G., Venskutonis, P. R., van Beek, T. A.,* Food Chem. **85** (2004) 231.
38. *Vagi, E., Simandi, B., Suhajda, A., Hethelyi, E.,* Food Res. Int. **38** (2005) 51.
39. *Ozcan, M., Boyraz, N.,* Eur. Food Res. Technol. **212** (2000) 86.
40. *Singh, G., Marimuthu, P., Catalan, C., deLampasona, M. P.,* J. Sci. Food Agr. **84** (2004) 1878.
41. *Crank, J.,* The Mathematics of Diffusion, Oxford University Press, Oxford, 1975.
42. *Osburn, J. O., Katz, D. L.,* T. Am. Inst. Chem. Eng. **40** (1944) 511.
43. *Kaur, C., Kapoor, H. C.,* Int. J. Food Sci. Tech. **37** (2002) 153.