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Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity

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Abstract

The antioxidant activities of the methanol extracts of selected varieties and parts of garlic and onion were determined by two methods: inhibition of lipid peroxidation induced by *tert*-butyl hydroperoxide in isolated rat hepatocytes and scavenging activity against diphenylpicrylhydrazyl radical. The total phenolics and the main flavonoids of the hydrolysed onion and garlic samples were also analysed. The antioxidant activities obtained by the two methods were compared. Both methods gave similar antioxidant activities for pure compounds and *Allium* extracts. However, the radical scavenging method had many benefits compared to the lipid peroxidation method, being easier, cheaper, more specific and reproducible. The radical scavenging activities also correlated positively with the total phenolics of the extracts. Onions had clearly higher radical scavenging activities than garlic, red onion being more active than yellow onion. The skin extracts of onion possessed the highest activities.
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Keywords: Antioxidant activity; Flavonoids; Garlic; Onion; Phenolics

1. Introduction

Diets rich in fruits and vegetables have been associated with decreased risks of several chronic diseases, such as coronary heart disease and some cancers (Block, Patterson, & Subar, 1992; Hertog et al., 1995; Lampe, 1999). These protective effects have been attributed partly to the various antioxidant compounds present in fruits and vegetables, for example, vitamins C and E, β-carotene and polyphenolics (Diplock et al., 1998). One group of polyphenolics, flavonoids, are found ubiquitously in the plant kingdom with a great variety of structures. In vegetables, quercetin glycosides are predominant, but glycosides of kaempferol, luteolin and apigenin are also present (Hertog & Hollman, 1996). Onion is one of the major sources of dietary flavonoids in many countries (Hertog et al., 1995; Knekt, Järvinen, Reunanen, & Maatela, 1996). In contrast to other vegetables, in onions the highest amounts of quercetin are found in the parts below the surface (Patil & Pike, 1995).

Plants belonging to the *Allium* family have been found to have antioxidant properties in several earlier studies (Cao, Sofic, & Prior, 1996; Gazzani, Papetti, Daglia, Berté, & Gregotti, 1998; Yin & Cheng, 1998). Especially, garlics and different garlic extracts have been shown to have antioxidant activity in different in vitro models. The antioxidant activity of *Allium* plants has mainly been attributed to a variety of sulphur-containing compounds and their precursors (Kim, Kubota, & Kobayashi, 1997; Lampe, 1999). According to Kim et al. (1997), allicin, diallyl disulphide and diallyl trisulphide appeared to be the main antioxidative compounds in the garlic volatiles. Animal tests have shown that allicin (diallyl thiosulphinate) can be responsible for garlic's antioxidative properties in low concentrations, although it can also act as a pro-oxidant in high concentrations (Lawson, 1998). However, Hirata and Matsushita (1996) demonstrated that alliin [(+)-S-allyl-L-cysteine sulphoxide], a precursor of allicin, does not have antioxidative activity in a linoleic acid oxidation system.

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The antioxidative potential of plant extracts and pure compounds can be measured using numerous in vitro assays. Each of these assays is based on one feature of antioxidant activity, such as the ability to scavenge free radicals or to inhibit lipid peroxidation. However, the total antioxidant activities of vegetables cannot be evaluated by any single method, due to the complex nature of phytochemicals (Chu, Chang, & Hsu, 2000). Two or more methods should always be employed in order to evaluate the total antioxidative effects of vegetables. Furthermore, although several methods have been used to test the antioxidant activities of various chemical compounds, often these methods cannot be used as such to measure the antioxidant activity of plant extracts due to their complex composition (Hodges, DeLong, Forney, & Prange, 1999; Janero, 1990; Yamaguchi, Takamura, Matoba, & Terao, 1998). Additionally, many of the methods used are either too expensive or too complex for testing of large series of samples.

In this study, we evaluated the suitability of two methods for screening of the antioxidant activities of a large number of samples consisting of fresh and processed vegetables commonly used in the food industry. *Allium* plants were chosen as test material because of their high antioxidative potential. Moreover, the role of plant phenolics in antioxidativity and/or in radical scavenging activity was investigated.

2. Materials and methods

2.1. Plant material

2.1.1. General

Onions (*Allium cepa* L.), chives (*Allium schoenoprasum* L.) and garlics (*Allium sativum* L.) were purchased from a wholesale market, except for the Finnish organic garlics which were a gift from a local organic farmer.

2.1.2. Sample extraction for HPLC

The plant material was freeze-dried and ground. Fifty milligrams of the ground plant material was hydrolysed in 5 ml of 1.2 M HCl in 50% aqueous methanol. After refluxing at 80 °C for 2 h, the extract was allowed to cool and was made up to 10 ml with 50% aqueous methanol and sonicated. The extract was then filtered through a 0.45-μm filter for organic solvents prior to injection. The hydrolyses were performed in triplicate.

2.1.3. Sample extraction for antioxidativity tests

Plant material was freeze-dried, ground and sieved (0.5 mm). Of the ground plant material 1.5 g was extracted with 10 ml methanol (HPLC-grade, Sigma) on a magnetic stirrer (1 h) and then in an ultrasonic bath (20 min). The extract was centrifuged at 3000 rpm for 20 min. The extraction was repeated once. The supernatants were evaporated to dryness at +40 °C using a rotavapor. The dry residue was dissolved in 1.5 ml methanol (radical scavenging method) or DMSO (lipid peroxidation method).

2.2. Analysis of flavonoids by HPLC

2.2.1. General

The flavonoids were analysed as aglycones from hydrolysed plant extracts using HPLC. The methods for sample extraction and HPLC separation were modifications of methods by Crozier, Lean, McDonald, and Black (1997), Häkkinen, Kärenlampi, Heinonen, Mykkänen, and Törrönen (1998), and Hertog, Hollman, and Venema (1992). The analysis were performed in triplicate.

2.2.2. HPLC separations

The hydrolysed samples were analysed using a Waters HPLC system comprising a Millenium[32] (Version 3.05.01) chromatography manager, a Waters 712 WISP automatic sample injector, a Waters 2487 Dual Wavelength Absorbance Detector and two Waters 6000A pumps. Reversed phase separations were carried out at room temperature using a 150×3.9 mm i.d., 5 μm C_{18} Symmetry column (Waters) fitted with a 20×3.9 mm i.d., 5 μm C_{18} Symmetry guard column (Waters). The mobile phase was a 25 min, 20–60% gradient of methanol in water with 300 μl l^{-1} trifluoroacetic acid, eluted at a low rate of 0.8 ml min^{-1}. After each analysis the column was washed with 100% methanol for 2 min, returned to 20% methanol and reequilibrated for 10 min before the next analysis. The eluted components were monitored at 280 and 340 nm.

2.2.3. Standards

The HPLC-grade flavonoid standards were purchased from Extrasynthese: apigenin (1102S), kaempferol (1124S), luteolin (1125S) and quercetin (1135S). The standard stock solutions were prepared by dissolving standards in methanol containing 0.2% phosphoric acid to a concentration of 200 μg ml^{-1}. For the calibration curves, ranging from 0.2 to 20 μg ml^{-1}, the standard stock solutions were diluted to final concentrations with 0.6 M HCl in 75% aqueous methanol.

2.3. Analysis of total phenolics

The amount of total phenolics was determined using Folin-Ciocalteu reagent (Singleton & Rossi, 1965). One-hundred microlitres of extract (three replicates) was diluted with the same amount of methanol. One hundred microlitres Folin–Ciocalteu reagent and 500 μl 20% Na_2CO_3 were added. The tubes were mixed and 200 μl 20% Na_2CO_3 were added. The tubes were centrifuged for 3 min at 14 000 rpm and allowed to stand for 20 min in the dark at room temperature. The absorbance was measured at 735 nm. The standard

curve was plotted using gallic acid. The amount of total phenolics was calculated as gallic acid equivalents (GAE) in milligrammes per kilogramme of freeze-dried plant material.

2.4. Analysis of antioxidativity

2.4.1. General analytical procedure

The antioxidant activity was assayed by measuring the inhibition of *tert*-butyl hydroperoxide-induced lipid peroxidation in isolated rat hepatocytes and the scavenging activity against diphenylpicrylhydrazyl radical. Apigenin (0051), kaempferol (0054), luteolin (0052), myricetin (0055), quercetin dihydrate (0066) and rutin trihydrate (0080), from Extrasynthese, were used as reference samples in the antioxidnt activity assays. The standards were dissolved in dimethyl sulphoxide (DMSO, Riedel-de Haën) (15 μM) for the lipid peroxidation assay and in methanol (200 μM) for the DPPH radical scavenging assay.

2.4.2. Analysis of antioxidativity by lipid peroxidation in rat hepatocytes

2.4.2.1. Animals. Male Wistar rats weighing 200–250 g were obtained from the National Public Health Institute (Helsinki, Finland). Rats had free access to food and water before anaesthesia.

2.4.2.2. Hepatocyte suspensions. The hepatocytes were isolated at the National Public Health Institute (Helsinki, Finland). Rats were anaesthetized with sodium pentobarbital (60 mg kg^{-1}). Hepatocytes were isolated by collagenase perfusion according to Lindros and Penttilä (1985). Cell suspensions showed a viability of 70–90% when assessed by the exclusion method with eosin. The incubation buffer of Malterud, Diep and Sund (1996) was used. Fatty acid-free bovine serum albumin (Sigma) was added at a concentration of 0.5% (m/V) immediately prior to use. The cell suspension was diluted to a concentration of 3×10^6 cells ml^{-1}.

2.4.2.3. Peroxidation in hepatocytes. Peroxidation in hepatocytes was performed according to Joyeux, Rolland, Fleurentin, Mortier, and Dorfman (1990) and Malterud et al. (1996), with some modifications. The inducing agent, *tert*-butylhydroperoxide (*t*-BuOOH, Merck), and the reference compound, promethazine hydrochloride (Sigma), were dissolved in water and the sample (plant extract or pure flavonoid) in DMSO (Riedel-de Haën). The hepatocyte cell suspension (1 ml) was pipetted into the incubation flasks (20-ml scintillation flasks). Into this suspension, 20 μl of *t*-BuOOH and 20 μl of the sample were added simultaneously. In the first experiment, a suitable concentration of *t*-BuOOH (0.25–24 mM) in buffer solution for hepatocytes was tested. On the basis of this experiment, a final concentration of 3.0 mM *t*-BuOOH was used in all later experiments. The final volume in each flask was 1.040 ml. Each sample was analysed in triplicate. Flasks were incubated under slow oscillation (170 rpm) at +37 °C for 30 min. The peroxidation reaction was stopped with 2 ml of 10% trichloroacetic acid (Merck) and the mixture was centrifuged (1300×*g*, 5 min). The supernatant fraction (2 ml) with 2 ml of 1% thiobarbituric acid (Fluka) in water, was heated in a boiling water bath for 10 min. The mixture was allowed to cool and the red colour obtained (malondialdehyde) was measured at 535 nm. The inhibition of the formation of thiobarbituric acid reactive substances (TBARS) was calculated as $(A_{uninh.}-A_{test})/(A_{uninh.}-A_{blank}) * 100$, where $A_{uninh.}$, A_{blank} and A_{test} are the absorbance values for uninhibited samples, blanks and the samples containing the test compound or the vegetable extract, respectively, as described in Malterud, Farbrot, Huse, and Sund (1993).

2.4.3. Analysis of antioxidativity by scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH)

The DPPH radical scavenging method used was a modification of the method of Malterud et al. (1993). Of a methanolic solution 2.8 ml of DPPH (45 μg ml^{-1}, Sigma) were rapidly mixed with 200 μl of a methanolic sample (plant extract or pure flavonoid) in a cuvette placed in the spectrophotometer. The absorbance at 515 nm was measured after 5 min. The initial absorbance of the DPPH solution was 1.2–1.3. The decline in radical concentration indicated the radical scavenging activity of the sample. Pyrogallol solution (125 μg ml^{-1}, Sigma) was used as a reference corresponding to 100% radical scavenging activity. Radical scavenging activity percentage was calculated as $(A_0 - A_{test})/(A_0 - A_{ref.}) * 100$, where A_0 is the initial absorbance (DPPH absorbance plus sample absorbance) and $A_{ref.}$ and A_{test} are the absorbance values after 5 min with pyrogallol solution and sample solution (corrected for the sample absorbance), respectively. The analyses were performed in triplicate except for $A_{ref.}$, which had six replicates.

For comparison, the radical scavenging activities of three different sulphur compounds, diallyldisulphide (1.5 mM, TCI Tokyo Kasei), diallyltrisulphide (1.4 mM, ICN Pharmaceuticals) and alliin (11.3 mM, Extrasynthese) were also measured.

3. Results

3.1. Flavonoids and total phenolics

Quercetin and kaempferol were found to be the most abundant flavonoids in the hydrolysed samples (Table 1). None of the analysed samples contained apigenin or

Table 1
Comparison of quercetin and kaempferol contents in various onions and garlics

		Quercetin		Kaempferol	
		mg/kg[a]	±S.D.	mg/kg[a]	±S.D.
Chives	Leaves	–	–	235	34.0
Giant onion	Leaves	24	2.4	90	7.8
	Stem	67	17.1	–[b]	–
	Edible part	85	12.42	–	–
Yellow spring onion	Leaves	7	1.3	295	27.9
	Stem	974	301	–[b]	
	Edible part	113	28.1	–	–
Red spring onion	Leaves	227	74.3	67	42.3
	Stem	2731	532	–	–
	Edible part	1274	375	–	–
Yellow onion	Skin	34 430	2211	–	–
	Edible part	1080	141	–	–
Red onion	Skin	83 477	2879	–	–
	Edible part	1926	266	–	–
Garlic, Finnish organically produced	Leaves	82	8.6	44	4.4
	Stem	–	–	–	–
	Skin	–	–	–	–
	Cloves	–	–	–	–
Garlic, Finnish giant	Stem	–	–	–	–
	Skin	–	–	–	–
	Cloves	–	–	–	–
Garlic, Hungarian	Cloves	–	–	–	–

–, Not detected.
[a] Lyophilized plant material.
[b] Trace amounts.

luteolin. In the onions the quercetin content varied from 7 mg to 83 g kg^{-1} (lyophilized plant material), depending on the variety and the part of the plant. The onion leaves contained mainly kaempferol, but quercetin was the major flavonoid in the other parts. The quercetin contents in the red onions were about two-fold higher than those of the yellow onions and, in the spring varieties, the differences were even bigger. Quercetin was not detected in chives. This result is in accordance with the observed trend that young leaves contain mainly kaempferol. Garlics did not contain quercetin or kaempferol in detectable amounts, except for the leaves of organic garlic.

The amount of total phenolics varied widely in the *Allium* extracts and ranged from 75 to 80 000 GAE mg kg^{-1} freeze-dried plant material. The lowest levels were detected for garlic (75–700 GAE mg kg^{-1}), whereas the highest amounts of phenolics were detected for the dry skin of onions: 80 000 GAE mg kg^{-1} in red and 26 000 GAE mg kg^{-1} in yellow onion. Intermediate levels were found in onion bulbs and stems (800–3200 GAE mg kg^{-1}) (Table 2). These results are in accordance with those of Kähkönen et al. (1999), who detected total phenolics of 2500–3000 GAE mg kg^{-1} in onions.

Table 2
Total phenolics in onions and garlics

		Total phenolics	
		GAE mg/kg[a]	±S.D.
Giant onion	Edible part	845	104
Yellow spring onion	Stem	3160	248
	Edible part	1390	81.1
Red spring onion	Stem	2670	347
	Edible part	2000	167
Yellow onion	Skin	26 105	898
	Edible part	1550	90.5
Red onion	Skin	79 820	293
	Edible part	2075	147
Garlic, Finnish organically produced	Stem	295	52.9
	Cloves	75	8.8
	Skin	175	9.9
Garlic, Finnish giant	Cloves	115	12.9
	Skin	705	21.5
Garlic, Hungarian	Cloves	95	7.8

[a] Lyophilized plant material.

3.2. Lipid peroxidation

A suitable concentration of *t*-BuOOH (0.25–24 mM) in buffer solution for hepatocytes was tested in the first experiment. A concentration of 3.0 mM *t*-BuOOH was found to be the most suitable. This concentration is in the linear part of the curve and the absorbance of TBARS is rather high.

The lipid peroxidation method was first tested using reference flavonoids (15 μM): apigenin, kaempferol, luteolin, myricetin, quercetin, and rutin (Fig. 1). Myricetin was the most effective inhibitor of lipid peroxidation, followed by quercetin and kaempferol. The flavones, apigenin and luteolin, were weaker inhibitors than the flavonols. Rutin (quercetin glycoside) was the least effective. This agrees with the results of an earlier study of Joyeux, Lobstein, Anton, and Mortier (1995), who found that the anti-lipoperoxidation activity of flavonols seems to be directly proportional to the number of free phenolic hydroxyl groups. The *ortho*-hydroxylation on the B-ring appears to play an essential role in the activity. Additionally, Hopia and Heinonen (1999) observed that quercetin aglycone was a more active lipid peroxidation inhibitor in the methyl linoleate model than its glycoside derivatives (glucoside, rhamnoside and rutinoside). The linearity of the lipid peroxidation method was estimated, using pure flavonoids as test compounds. Myricetin and quercetin gave linear responses ($R^2=0.993$ and $R^2=0.990$) in concentrations of 2–14 and 2–20 μM, respectively. Inhibition percentages of lipid peroxidation were 10–60%. The reproducibility was excellent within one experiment (CV

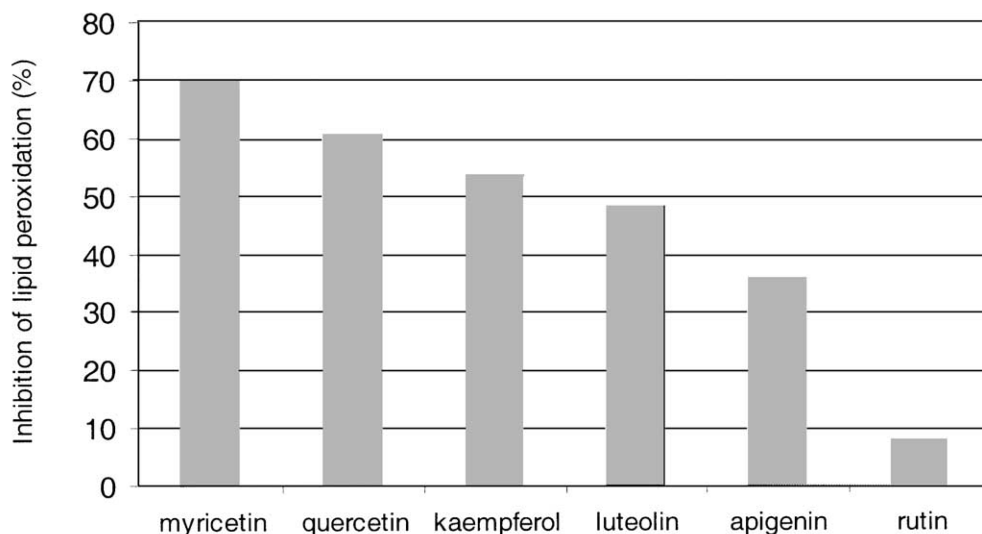

Fig. 1. Inhibition of *tert*-butylhydroperoxide-induced lipid peroxidation by flavonoids (15 μM) in rat hepatocytes.

2.6%) but only satisfactory between experiments (CV 15.3%).

The extracts of yellow onion were effective inhibitors of lipid peroxidation in rat hepatocytes, the dry skin again being the most effective. Concentrations of 10–80 mg ml^{-1} (based on the dry weight of the original extracted sample) resulted in 20–80% lipid peroxidation inhibition (Fig. 2). The edible part of onion was clearly less effective than the skin and a concentration of 1000 mg ml^{-1} (dry weight of the original extracted sample) resulted in only 40% lipid peroxidation inhibition. Garlic was a much less effective inhibitor of lipid peroxidation than yellow onion. Of the garlics tested, the best inhibition (25%) was obtained using Hungarian garlic (1000 mg ml^{-1}, dry weight of the original extracted sample). The giant garlic did not possess any inhibitory activity in two separate experiments at this same concentration. Yin and Cheng (1998) reported that garlic had a stronger inhibitory effect, on lipid oxidation in their liposome model, than onion. This difference between their results and the results of this study could be due to different methods of sample extraction. Yin and Cheng (1998) extracted the plant samples with distilled water, which results in a spectrum of compounds different from our method, in which methanol was used for extraction.

3.3. DPPH radical scavenging

Linearity of the radical scavenging method was estimated using quercetin, myricetin and yellow onion extract as test materials. For quercetin and myricetin, the method gave linear responses in the concentration range 25–250 μM (R^2=0.996 and R^2=0.993, respectively). The radical scavenging activities were 20–90%. The extract of the yellow onion gave a linear response (R^2=0.9947) in the extract concentration range 1–500 mg ml^{-1} (based on the dry weight of the original extracted sample). Among the flavonoids tested, quercetin (200 μM) was the most effective DPPH radical scavenger and it also reacted faster than the other flavonoids, especially rutin and kaempferol (Fig. 3).

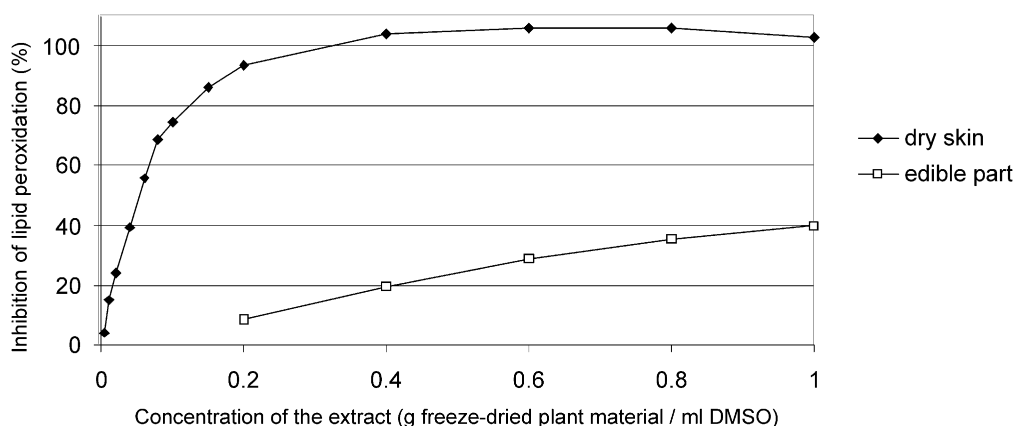

Fig. 2. Inhibition of lipid peroxidation in rat hepatocytes by methanol extracts of the dry skin and the edible part of yellow onion.

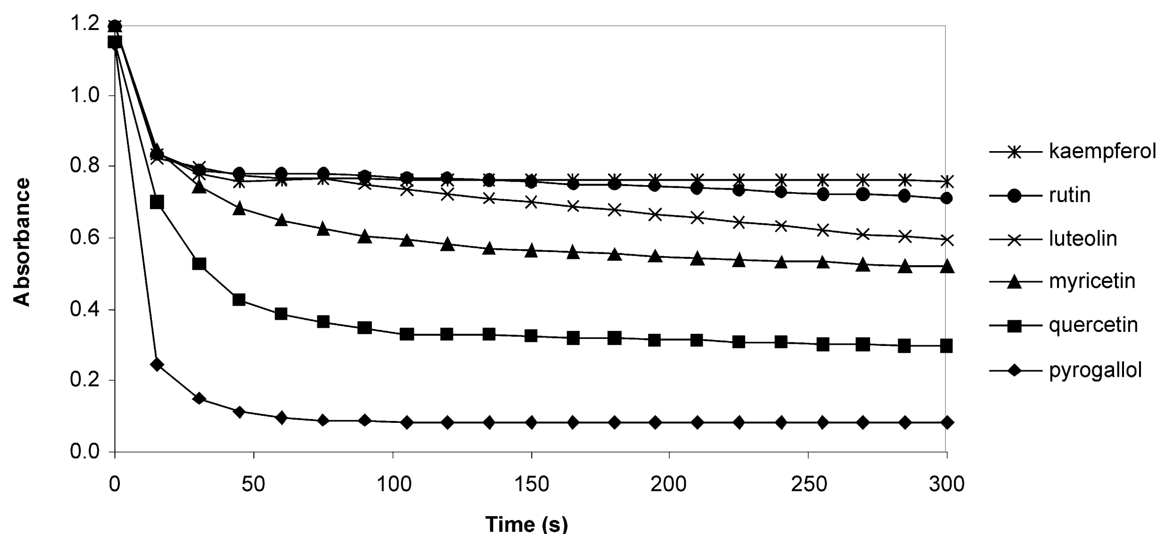

Fig. 3. Scavenging of the diphenylpicrylhydrazyl (DPPH) radical (114 μM) by myricetin, quercetin, rutin, kaempferol, luteolin (200 μM) and pyrogallol (1 mM).

DPPH radical scavenging activities of various onion extracts are presented in Table 3. The lowest antioxidant activities were detected for garlic. The edible parts of onions gave 15 times higher activities than did garlic, the red onion being more active than the yellow onion. Skin extracts of onion possessed the highest activities (more than 240 times higher than those of garlic), the red onion being again more active than the yellow onion. However, according to Miller, Rigelhof, Marquart, Prakash, and Kanter (2000), garlic is very high in antioxidants, its activity being about sixfold that of yellow onion (1300 Trolox equivalents/100 g vs. 200 Trolox equivalents/100 g). The difference is probably at least partially due to the different methods used. Miller and co-workers (2000) extracted the fresh vegetables using 50% methanol whereas, in our study, 100% methanol was used for extractions. Their procedure was developed specifically for cereal products, which contain mainly other antioxidants than flavonoids and sulphuric compounds. Cereal products are also very difficult to extract.

Table 3
Radical scavenging activities of the onion and garlic extracts

		Dilution used in the DPPH method[a] (mg/ml)	Radical scavenging activity of the dilution (%)
Giant onion	Edible part	67	44.4
Yellow spring onion	Stem	67	44.4
	Edible part	67	56.7
Red spring onion	Stem	67	–
	Edible part	67	79.8
Yellow onion	Skin	4.2	48.8
	Edible part	67	32.9
Red onion	Skin	4.2	74.7
	Edible part	67	44.5
Garlic, Finnish organically produced	Stem	1000	66.0
	Cloves	1000	62.1
	Skin	1000	63.7
Garlic, Finnish giant	Cloves	1000	60.9
	Skin	1000	95.8
Garlic, Hungarian	Cloves	1000	43.0

–, not measured.
[a] Based on the dry weight of the original extracted sample.

4. Discussion

Quercetin and kaempferol and their glycosides have been reported to be the most abundant flavonoids in the acid hydrolysed samples of onion (Chu et al., 2000; Price & Rhodes, 1997), as was also found in our study. The major flavonoids of onion bulb are quercetin-3,4′-O-diglucoside (Qdg) and quercetin-4′-monoglucoside (Qmg). Bilyk, Cooper, and Sapers (1984) studied eight onion varieties and found that the skin of some of the varieties contained quercetin, both as the aglycone and as glycosides. With some of the varieties, the skin also contained small amounts of kaempferol. Patil and Pike (1995) investigated the distribution of free and total quercetin contents in different rings of coloured onion cultivars. The dry skin of red varieties contained 27–30 g kg^{-1} f.w. of total quercetin and the dry skin of yellow varieties 10–17 g kg^{-1} f.w. In our study, the total quercetin content of dry skin of red onion (83 g kg^{-1} lyophilized plant material) was also approximately twice that of the dry skin of yellow onion (34 g kg^{-1} lyophilized plant material).

In the present study garlics did not contain quercetin or kaempferol in detectable amounts. Based on these results, the the hydrolysis method for analysis of flavonoids has been further developed in a later study, in

order to enhance detection of easily degradable aglycones (Nuutila, Kammiovirta, & Oksman-Caldentey, 2002). According to Bilyk and Sapers (1985) the kaempferol contents of green and white parts of garlic chives were 6 and 28 mg kg^{-1} f.w., respectively, and the quercetin content of the green part was 4 mg kg^{-1} f.w. Quercetin was not detectable in the white part of garlic chive using their method.

In the lipid peroxidation method, a concentration of 3.0 mM *t*-BuOOH was found to be the most suitable in our experiments. According to literature data, the concentration of *t*-BuOOH used by other investigators (Joyeux et al., 1990, 1995; Malterud et al., 1993, 1996; Miguez, Anundi, Sainz-Pardo, & Lindros, 1994) has been lower (1.5 mM). However, this can be explained by the lower extracellular pH (6.4 vs. 7.4) used in our study. Imberti, Nieminen, Herman, and Lemasters (1993) demonstrated that acidic pH can protect hepatocytes against lethal cell injuries from 100 μM *t*-BuOOH. Protection was greater at pH 6.5 than at 7.0. Our experiment showed that these protective effects probably also occur with higher concentrations of *t*-BuOOH.

A major problem in the thiobarbituric acid test in the lipid peroxidation method was the background caused by compounds in plant extracts, as previously already recognised. The thiobarbituric acid test is non-specific for malondialdehyde (MDA), and therefore non-lipid material, as well as fatty-peroxide-derived decomposition products other than MDA, can react positively with thiobarbituric acid (Janero, 1990). Using red cabbage as test material, Hodges and co-workers (1999) observed that without corrections for interfering compounds, MDA equivalents may be overestimated by up to 96.5% in the thiobarbituric acid test. In our study, the non-specific responses of three *Allium* extracts (Hungarian garlic, and the dry skin and bulb of yellow onion) were measured without hepatocytes after the *t*-BuOOH treatment and thiobarbituric acid test. The bulb of yellow onion gave the strongest responses. Interfering compounds were yellowish orange and their absorption maxima were at wavelengths of 450 and 380 nm. These interfering compounds distorted the results and therefore all the final results of the onion extracts have been corrected using the absorbances of the plant-derived compounds after the thiobarbituric acid test. No interfering compounds were detected in garlic.

In earlier studies, quercetin has been shown to be the most effective $ABTS^{\bullet +}$ radical scavenger, followed by myricetin, rutin, luteolin, apigenin, and kaempferol (Rice-Evans, Miller, & Paganga, 1996). The chemical properties of polyphenols, in terms of the availability of the phenolic hydrogens as hydrogen-donating radical scavengers, predict their antioxidant activity. In our study the radical scavenging activities of 100 μM myricetin and quercetin were equal but, at 250 μM, quercetin was more effective. All the sulphuric compounds tested had very low radical scavenging activities, below 3.5%.

The radical scavenging and antioxidant activity results for *Allium* plants obtained in this study are not in agreement with the earlier literature (Cao et al., 1996; Gazzani et al., 1998; Yin & Cheng, 1998). However, these contradictory results are most probably due to differences in the methodology and the experimental conditions used in the different studies. Due to the wide variety of potential antioxidant compounds, such as vitamins, flavonoids, phenolic acids and sulphur compounds (e.g. allicin) present in plants, differences in the method of sample extraction (e.g. solvent used) can result in a wide variation in the antioxidativity of the extract. In our study, there was an observable correlation between high radical scavenging/antioxidant activity and high amounts of total phenolics and flavonoids of the onion extracts (Tables 1–3). This result indicates that the phenolic compounds of *Allium* plants contribute to their antioxidative properties. In our experiment methanol extracts of garlic had only moderate antioxidant activities in both models when compared to onion. Neither did the tested pure sulphur compounds have radical scavenging activity. Yin and Cheng (1998) found aqueous extracts of garlic to have higher antioxidant activities than aqueous extracts of onion, but their results also suggested that compounds other than allicin, i.e. vitamins and phenolic compounds, were involved in determining the antioxidant activities of the extracts. According to Cao et al. (1996), among the 22 common vegetables studied, garlic had the highest antioxidant activity, with an antioxidant score (automated oxygen radical absorbance capacity assay) of 23.2 based on fresh weight of the vegetable. The antioxidant score of onion was 5.6. The difference was much smaller when the results were calculated per dry weight (55.1 vs. 49.5).

In conclusion, in this study two methods were compared: lipid peroxidation and DPPH radical scavenging. The two methods gave similar antioxidant activity trends for pure compounds and for *Allium* extracts. Scavenging of free radicals, which can initiate and propagate the lipid peroxidation cascade, is an important mechanism for the inhibitory activity towards lipid peroxidation. Thus, radical scavenging activity could be a good marker for antioxidant activity. In addition, the radical scavenging method had many benefits compared to the lipid peroxidation method. It was cheap and easy to perform, and the reproducibility of the method was better than that of the lipid peroxidation method, the reproducibility of which was clearly weaker, especially between the hepatocyte batches. The weakness of reproducibility of the lipid peroxidation method may also partly be due to the fact that the number of lipid peroxidation tests was restricted by the availability of hepatocytes. The sensitivity of the lipid peroxidation

test was not sufficient for all garlic samples. The DPPH radical scavenging method could also be enhanced even further using microtitre plates to improve the speed and efficiency of the method.

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References

Bilyk, A., Cooper, P. L., & Sapers, G. M. (1984). Varietal differences in distribution of quercetin and kaempferol in onion (*Allium cepa*) tissue. *Journal of Agricultural and Food Chemistry*, *32*, 274–276.

Bilyk, A., & Sapers, G. M. (1985). Distribution of quercetin and kaempferol in lettuce, kale, chive, garlic chive, leek, horseradish, red radish and red cabbage tissues. *Journal of Agricultural and Food Chemistry*, *33*, 226–228.

Block, G., Patterson, B., & Subar, A. (1992). Fruits, vegetables and cancer prevention: a review of the epidemiological evidence. *Nutrition and Cancer*, *18*, 1–29.

Cao, G., Sofic, E., & Prior, R. L. (1996). Antioxidant capacity of tea and common vegetables. *Journal of Agricultural and Food Chemistry*, *44*, 3426–3431.

Chu, Y-H., Chang, C-L., & Hsu, H-F. (2000). Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture*, *80*, 561–566.

Crozier, A., Lean, M. E. J., McDonald, M. S., & Black, C. (1997). Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and cellery. *Journal of Agricultural and Food Chemistry*, *45*, 590–595.

Diplock, A. T., Charleux, J.-L., Crozier-Willi, G., Kok, F. J., Rice-Evans, C., Roberfroid, M., Stahl, W., & Vinâ-Ribes, J. (1998). Functional food science and defence against reactive oxidative species. *British Journal of Nutrition*, *80S*, S77–S112.

Gazzani, G., Papetti, A., Daglia, M., Berté, F., & Gregotti, C. (1998). Protective activity of water soluble components of some common diet vegetables on rat liver microsome and the effect of thermal treatment. *Journal of Agricultural and Food Chemistry*, *46*, 4123–4127.

Häkkinen, S. H., Kärenlampi, S. O., Heinonen, I. M., Mykkänen, H. M., & Törrönen, A. R. (1998). HPLC method for screening of flavonoids and phenolic acids in berries. *Journal of the Science of Food and Agriculture*, *77*, 543–551.

Hertog, M. G. L., Hollman, P. C. H., & Venema, D. P. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agricultural and Food Chemistry*, *40*, 1591–1598.

Hertog, M. G. L., & Hollman, P. C. H. (1996). Potential helath effects of dietary flavonol quercetin. *European Journal of Clinical Nutrition*, *50*, 63–71.

Hertog, M. G. L., Kromhout, D., Aravanis, C., Blackburn, H., Buzina, R., Fidanza, F., Giampaoli, S., Jansen, A., Menotti, A., Nedeljkovc, S., Pekkarinen, M., Simics, B. S., Toshima, H., Feskens, E., Hollman, P. C. H., & Katan, M. B. (1995). Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Archives of Internal Medicine*, *155*, 381–386.

Hirata, R., & Matsushita, S. (1996). Reducing activity level of alliin. *Bioscience, Biotechnology, and Biochemistry*, *60*, 484–485.

Hodges, D. M., DeLong, J. M., Forney, C. F., & Prange, R. K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*, *207*, 604–611.

Hopia, A., & Heinonen, M. (1999). Antioxidant activity of flavonol aglycones and their glycosides in methyl linoleate. *Journal of the American Oil Chemists' Society*, *76*, 139–144.

Imberti, R., Nieminen, A.-L., Herman, B., & Lemasters, J. J. (1993). Mitochondrial and glycolytic dysfunction in lethal injury to hepatocytes by t-butylhydroperoxide: protection by fructose, cyclosporin A and trifluoperazine. *The Journal of Phamacology and Experimental Therapeutics*, *265*, 392–400.

Janero, D. R. (1990). Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radical Biology & Medicine*, *9*, 515–540.

Joyeux, M., Lobstein, A., Anton, R., & Mortier, F. (1995). Comparative antilipoperoxidant, antinecrotic and scavenging properties of terpenes and biflavones from Ginkgo and some flavonoids. *Planta Medica*, *61*, 126–129.

Joyeux, M., Rolland, A., Fleurentin, J., Mortier, F., & Dorfman, P. (1990). Tert-butylhydroperoxide-induced injury in isolated rat hepatocytes: a model for studying antihepatotoxic crude drugs. *Planta Medica*, *56*, 171–174.

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J.-P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry*, *47*, 3954–3962.

Kim, S. M., Kubota, K., & Kobayashi, A. (1997). Antioxidative activity of sulfur-containing flavor compounds in garlic. *Bioscience, Biotechnology, and Biochemistry*, *61*, 1482–1485.

Knekt, P., Järvinen, R., Reunanen, A., & Maatela, J. (1996). Flavonoid intake and coronary mortality in Finland: a cohort study. *British Medical Journal*, *312*, 478–481.

Lampe, J. W. (1999). Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. *American Journal of Clinical Nutrition*, *70*, 475S–490S.

Lawson, L. D. (1998). Garlic: a review of its medicinal effects and indicated active compounds. In L. D. Lawson, & R. Bauer (Eds.), *Phytomedicines of Europe: chemistry and biological activity, ACS Symposium Series, No 691* (pp. 176–209). Washington, DC: American Chemical Society.

Lindros, K. O., & Penttilä, K. E. (1985). Digitonin-collagenase perfusion for efficient separation of periportal or perivenous hepatocytes. *Biochemical Journal*, *228*, 757–760.

Malterud, K. E., Diep, O. H., & Sund, R. B. (1996). C-Methylated dihydrochalcones from *Myrica gale L.*: effects as antioxidants and as scavengers of 1,1-diphenyl-2-picrylhydrazyl. *Pharmacology & Toxicology*, *78*, 111–116.

Malterud, K. E., Farbrot, T. L., Huse, A. E., & Sund, R. B. (1993). Antioxidant and radical scavenging effects of anthraquinones and anthrones. *Pharmacology*, *47*, 77–85.

Miguez, M. P., Anundi, I., Sainz-Pardo, L. A., & Lindros, K. O. (1994). Hepatoprotective mechanism of silymarin: no evidence for involvement of cytochrome P450 2E1. *Chemico-Biological Interactions*, *91*, 51–63.

Miller, H. E., Rigelhof, F., Marquart, L., Prakash, A., & Kanter, M. (2000). Antioxidant content of whole grain breakfast cereals, fruits and vegetables. *Journal of the American College of Nutrition*, *19*, 1–8.

Nuutila, A. M., Kammiovirta, K., & Oksman-Caldentey, K.-M. (2002). Comparison of methods for the hydrolysis of flavonoids and phenolic acids from onion and spinach for HPLC analysis. *Food Chemistry*, *76*, 519–525.

Patil, B. S., & Pike, L. M. (1995). Distribution of quercetin content in different rings of various coloured onion (*Allium cepa* L.) cultivars. *Journal of Horticultural Science*, *70*, 643–650.

Price, K. R., & Rhodes, M. J. C. (1997). Analysis of the major flavonol glycosides present in four varieties of onion (*Allium cepa*) and changes in composition resulting from autolysis. *Journal of the Science of Food and Agriculture*, *74*, 331–339.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology & Medicine*, *20*, 933–956.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144–158.

Yamaguchi, T., Takamura, H., Matoba, T., & Terao, J. (1998). HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Bioscience, Biotechnology, and Biochemistry*, *62*, 1201–1204.

Yin, M., & Cheng, W. (1998). Antioxidant activity of several *Allium* members. *Journal of Agricultural and Food Chemistry*, *46*, 4097–4101.