Available online at www.sciencedirect.com
SCIENCE DIRECT®

Food Chemistry 94 (2006) 19–25

Food Chemistry

www.elsevier.com/locate/foodchem

Radical scavenging activity of various extracts and fractions of sweet orange peel (*Citrus sinensis*)

Maria A. Anagnostopoulou [a,*,1], Panagiotis Kefalas [b], Vassilios P. Papageorgiou [a], Andreana N. Assimopoulou [a], Dimitrios Boskou [c]

[a] *Department of Chemical Engineering, Faculty of Engineering, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece*
[b] *Mediterranean Agronomic Institute of Chania, PO Box 85, GR-73100 Chania, Crete, Greece*
[c] *Department of Chemistry, Faculty of Science, Aristotle University of Thessalonica, Greece*

Received 31 May 2004; received in revised form 22 September 2004; accepted 22 September 2004

Dedicated to the memory of Dr. Panagiotis Routsis

Abstract

Seven different extracts, fractions and residues of Navel sweet orange (*Citrus sinensis*) peel were evaluated for their radical scavenging activity by the DPPH$^{\cdot}$ and luminol induced chemiluminescence methods. Also, the Folin–Ciocalteu method was used to determine the total phenolic content. High phenolic content and radical scavenging activities were found for the ethyl acetate fraction. Comparison was made with reference compounds, Trolox, ascorbic acid, quercetin, which are already known for their good antioxidant activity. The radical scavenging activity of the ethyl acetate fraction approached the activity of the standards.

Total phenolic content showed a small relation with radical scavenging activity. The radical scavenging activity examined with the DPPH method correlated well to values obtained by chemiluminescence.

The antioxidant activity found in the fractions of *Citrus sinensis*, should be attributed to the presence of flavonoids and other phenolic compounds. Among the various classes of flavonoids: flavanone glycosides, flavones and flavonols seem to prevail as indicated by two dimensional thin layer chromatography and color reactions. This information shows that ethyl acetate fraction of navel sweet orange peel can be used as antioxidant in food and medicinal preparations.
© 2005 Elsevier Ltd. All rights reserved.

Keywords: Total phenolic content; Sweet orange peel; Radical scavenging activity; DPPH; Chemiluminescence; *Citrus sinensis*

1. Introduction

Reactive species that initiate reactions that damage organic molecules of biological importance, are considered to be the cause of several health problems including cancer, heart diseases and possibly, the process of aging itself (Halliwell & Gutteridge, 1989). Also, lipid oxidation by radicals results in food deterioration, especially in high fat foods (Kanner et al., 1994). Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used as antioxidants. However, demand for natural antioxidants has been increased due to consumer concerns about the safety of synthetic antioxidants (Hudson, 1990).

Antioxidants, either as additives or as pharmaceutical supplements, can terminate radical reactions in vivo,

Abbreviations: DPPH, 1,1-diphenyl-2-picryl-hydrazyl; CL, chemiluminescence; Tro, Trolox; AscA, ascorbic acid; Que, quercetin; AA, antiradical activity; AE, antiradical efficiency; TPC, total phenol content; FRSA, free radical scavenging activity; HRSA, hydroxyl radical scavenging activity.

* Corresponding author. Tel.: +30 3231 081 0481.
E-mail address: managnos@auth.gr (M.A. Anagnostopoulou).
[1] Present address: 7 Megalou Alexandrou, 54640, Greece.

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.09.047

which can damage life essential molecules such as nucleic acids and proteins (Saez et al., 1994). Phenolic compounds, particularly flavonoids, have been shown to possess an important antioxidant activity towards these radicals, which is principally based on their structural characteristics (number and position of phenolic hydroxyls, other groups, conjugation) (Bors, Hellers, Michel, & Saran, 1990a, 1990b).

Citrus processing byproducts represent a rich source of naturally occurring flavonoids (Horowitz, 1961). The peel which represents roughly half of the fruit mass, contains the highest concentrations of flavonoids in the *Citrus* fruit (Manthley & Grohmann, 1996, 2001). Many authors have found antioxidants in juice and edible parts of oranges of different origin and from different varieties (Miller & Rice-Evans, 1997; Rapisarda et al., 1999; Roberts & Gordon, 2002; Vinson, Su, Zubik, & Bose, 2001). As far as the peel is concerned, extracts from this part of the fruit were found to have a good total radical antioxidative potential (TRAP) (Gorinstein et al., 2001). Also, Larrauri, Ruperez, Bravo, and Saura-Calixto (1996) compared lime and orange peel fibre with α-tocopherol and BHA.

According to Wang, Cao, and Prior (1996), the different results observed for the antioxidant potential of juices are probably due to various factors, namely climate, soil, fruit variety and degree of maturation. So, in the present work, an effort was made to evaluate the radical scavenging activity of Greek navel sweet orange peel for the first time. Navel oranges have commercial importance and their byproducts should be thoroughly studied for possible utilization. Two assays were used to determine free and hydroxyl radical scavenging activity: (i) DPPH and (ii) luminol-enhanced chemiluminescence respectively. The Folin–Ciocalteu method was used to determine the total phenolic content of each one of the extracts, fractions and residues. Extracts and residues examined were prepared using successive solvents of varying polarity and by partitioning the methanol fraction with diethyl ether, ethyl acetate and *n*-butanol.

2. Materials and methods

2.1. Plant material

17.5 kg Greek oranges (*Citrus sinensis*), variety: Navel, provenance: Leonidi–Tripoleos (Pelloponisos, South Greece), season: Nov–Jan were bought from a local supermarket.

The oranges were examined for their anatomy and the variety was checked in the Laboratory of Arboriculture, Department of Agriculture, Aristotle University of Thessaloniki, Greece.

2.2. Sample preparation

Oranges were washed with tap water, peeled off sharply in order to collect only the flavedo part of the peel and air dried at room temperature (18–20 °C) and darkness for 12 days. The dried peels were crushed in a mixer and 277.4 g of them were put into a cartridge made of filter paper. The above amount of crushed peels was large enough to be used for the isolation procedure later on.

2.3. Chemicals and reagents

The solvents used in the present work were purchased from Merck (Germany), Riedel-deHaen (Germany) and Baker (Holland). Folin–Ciocalteu reagent, sodium carbonate, $CoCl_2 \cdot 6H_2O$ and perhydrol 30% H_2O_2 were purchased from Merck (Germany). Gallic acid 1-hydrate was purchased from Panreac (Spain). DPPH (1,1-diphenyl-2-picryl-hydrazyl 90%), EDTA, Luminol (3-aminophtahydrazine) and boric acid were from Sigma (Germany). Hesperidin was from Biochemika, Fluka (Germany). All solvents and reagents were of analytical grade.

Bradford cuvettes (22 × 22 mm) were used in the Folin–Ciocalteu and CL tests. Disposable cuvettes (1 cm × 1 cm × 4.5 cm) from Kartel (Italy) were used for visible absorbance measurements in the DPPH test.

2.4. Extraction procedure

The procedure followed was according to Mellidis, Papageorgiou, and Kokkalou (1993). The cartridge was put in a Soxhlet apparatus (1 l) and extracted successively with three solvents of increasing polarity ((a) toluene, (b) dichloromethane, (c) methanol) until decoloration (~3 times). The three extracts obtained were evaporated under vacuum to dryness. Their weights were 2.82 g for toluene extract, 2.80 g for dichloromethane extract and 136.41 g for methanol extract.

108 g of the methanol dry extract was dissolved in 2 l hot water. The water solution was filtered (residue 1: 2.68 g) and partitioned with diethyl ether, ethyl acetate and *n*-butanol (6 × 150 ml). Organic layers of each of the three solvents were dried with sodium sulphate anhydrous (Merck p.a.), filtered and evaporated under vacuum to dryness to give 1.02 g ether fraction, 1.39 g ethyl acetate fraction, 9.65 g butanol fraction and 71.0 g of the remaining water fraction. The insoluble white part of the aqueous methanolic extract after partition between the above three solvents was carefully collected, filtered, air-dried, weighed (residue 2: 0.53 g) and kept in the fridge (0 °C) together with the other extracts, under nitrogen atmosphere, until use.

2.5. Two dimensional thin layer chromatography

1 or 2 mg of each one of the seven samples and one standard, hesperidin, were dissolved into 3 ml of methanol and spotted onto cellulose plates (20×20 cm^2, 0.1 mm thick). The plates were developed in the first direction, using EAW as solvent (80:20:40, ethyl acetate:acetic acid:water) and after drying, they were developed in the second direction using 15% acetic acid (Mabry, Markham, & Thomas, 1970). Spots were observed under UV light with and without the presence of ammonia fumes.

2.6. Total phenolic content

The amount of total soluble phenolics was determined according to the Folin–Ciocalteu method (Gutfinger, 1981). The reaction mixture was consisted of 0.5 ml of the extract (1.5–15 mg/ml, depending on the activity), 5 ml of distilled water, 0.5 ml of the Folin–Ciocalteu's reagent. After a period of 3 min, 1 ml of saturated sodium carbonate solution was added. The 10 ml volumetric flasks were shaken and allowed to stand for 1 h. The absorbance was measured at 725 nm (each measurement repeated three times) in a Shimadzu UV–Vis-160A spectrophotometer (the same equipment was used in the DPPH test). The total phenolic content was expressed as mg gallic acid/g dry extract, mg gallic acid/100 g dry peel.

2.7. Free radical scavenging activity

Radical scavenging activity of the seven extracts was measured using the stable radical DPPH$^\bullet$. The procedure followed was according to Brand-Williams, Cuvelier, and Berset (1995) with some variations. For each extract, different concentrations were tested. At least 5 dilutions of each extract were prepared in methanol using a 10 ml volumetric flask (methanol for the control). Concentrations ranged from 48 to 1.51 mg/ml for less active extracts and from 10 to 0.33 mg/ml for more active ones. An aliquot of methanol (0.1 ml) solution containing different concentrations of orange peel extracts was added to 3.9 ml of DPPH$^\bullet$ (10^{-4} M). Absorbance was measured at 515 nm until the reaction reached a plateau (each measurement repeated twice). After preliminary experiments, the plateau was fixed at 4 h for all the extracts due to the slow kinetics of certain extracts. The absorbance of the DPPH solution, was measured daily. The DPPH$^\bullet$ concentration in the reaction medium was calculated from the following calibration curve, determined by linear regression:

$$A(515\ \text{nm}) = 26.501[\text{DPPH}^\bullet]_T - 0.0244,$$

where $[\text{DPPH}^\bullet]_T$ as mg/ml and $r^2 = 0.9992$.

The percentage of remaining DPPH was calculated as follows:

$$\%\ \text{DPPH}_{\text{REM}} = [\text{DPPH}^\bullet]_T/[\text{DPPH}^\bullet]_{T=0}.$$

The percentage of the % remaining DPPH$^\bullet$ against mg dry extract/mg DPPH$^\bullet$ was plotted to obtain the amount of antioxidant necessary to decrease the initial DPPH$^\bullet$ concentration by 50% (EC_{50}), using the exponential model: ln[% DPPH$^\bullet$ rem] = b[mg antioxidant/mg DPPH$^\bullet$] + a, where b is the slope and a is the intercept. For each of the extracts, the Antiradical Efficiency, $1/EC_{50}$was calculated. Values were also obtained for the three standards.

2.8. Hydroxyl radical scavenging activity

Co (II)/EDTA-induced luminol chemiluminescence measurements were carried out on a model 6200 Fluorimeter, JENWAY (Jenway Gransmore Green Felsted Dunmow Essex CM6 3 LB), keeping the lamp off and using only the photo multiplier of the apparatus. The procedure used is the one described by Parejo, Codina, Petrakis, and Kefalas (2000) with some variations.

1 ml boric acid buffer solution (0.05 M, adjusted to pH 9 with NaOH 1 M) containing 1 mg/ml EDTA and 0.2 mg/ml $CoCl_2 \cdot 6H_2O$ was vortexed for 15 s with 100 μl of luminol solution (5.6×10^{-4} M) in boric acid (0.05 M, adjusted to pH 9 with 1 M NaOH). Then, 25 μl of H_2O_2 aqueous solution (5.4×10^{-3} M) were added and the mixture was vortexed again for 30 s and fast taken into a glass cuvette. The CL intensity (I_0) was recorded when the plateau was reached (the lifetime of the plateau is 30 s). Immediately afterwards, 25 μl of the sample solution were added with a Pasteur pipette for thorough mixing and the instantaneous decrease of the plateau was recorded (I) (each measurement repeated three times).

The instantaneous reduction in luminol intensity elicited by the addition of the sample extract was symbolized as I. The light intensity in the absence of the sample was symbolized as I_0.

The ratio I_0/I was plotted against μg dry extract/ml and a linear regression was established $I_0/I = a$[μg dry extract/ml] + b, where a is the slope and b is the intercept, in order to calculate the IC_{50}, which is the amount of sample necessary to decrease by 50% the initial CL intensity. Again, the Antiradical Efficiency was calculated which is the $1/IC_{50}$.

Comparison was made with the three standards, which have already been mentioned in the DPPH test.

2.9. Statistical analysis

Values shown in tables and graphs was the mean of at least two determinations ± SD. Discrepancies among determinations of each sample was tested with the

criterion Q (Hajioannou et al., 1997), and found to be $Q_{exp} < Q_{cal}$ for all the samples which means that all determinations for each sample are acceptable (Q_{cal} is taken from statistical tables).

3. Results and discussion

3.1. Two dimensional thin layer chromatography (preliminary test)

TLC plates checked under UV with and without the presence of ammonia, showed yellow, orange, violet and deep purple spots. According to Mabry et al. (1970), deep purple or violet spots turning to yellow in the presence of NH_3 fumes indicate the presence of flavones with 5-OH and 4′-OH or 3-OH substituted flavonols with 5-OH and 4′-OH and some 5-OH flavanones and 4′-OH chalcones lacking B-ring hydroxyl groups. Deep purple color spots not changing in the presence of NH_3 fumes show the presence of 5-OH flavones or flavonols in the molecule of which 4′-OH is absent or substituted.

Table 1
Total phenol content of the seven samples as determined by the colorimetric Folin–Ciocalteu method

Sample	Sample no.	mg GA/g dry extract	mg GA/100 g dry peel
TPC			
Dichloromethane extract	1	10.0 ± 1.1	10.1
Methanolic extract			
Diethyl ether fraction	2	17.2 ± 0.8	8.0
Ethyl acetate fraction	3	105 ± 10	**66.9**
n-butanol fraction	4	42.7 ± 1.8	188
Water fraction	5	7.9 ± 1.6	254
Residue 1	6	3.0 ± 0.1	3.63
Residue 2	7	4.3 ± 0.2	1.05

Values in the first column are expressed as a mean of three determinations ± SD.

In most of the extracts examined by TLC and UV light, deep purple or violet colours appeared which indicate the presence of flavanones, flavones and flavonols. These compounds are in the form of diglycosides as demonstrated by the R_f values in the two-dimensional TLC using hesperidin (flavanone diglycoside) as a standard. This is clear in the extracts obtained by ethyl acetate and *n*-butanol.

3.2. Total phenolic content

The TPC values of the successive orange peel extracts ranged from 105 to 3 mg GA/g dry extract (see Table 1). The phenolic content of the seven samples decreased in the following order: ethyl acetate fraction > butanolic fraction > ether fraction > dichloromethane extract > water fraction > residue 2 > residue 1. As it can be seen, the methanolic fractions have the highest phenolic content. Ethyl acetate seems to be the solvent, that concentrates best phenolic substances of intermediate polarity. This is in accordance with findings by Chung et al. (1999), Parejo et al. (2002).

3.3. Radical scavenging activity (DPPH, CL)

Free radical scavenging activity expressed as EC_{50} ranged from 0.5 to 8.9 mg dry extract/mg DPPH (see Table 2). Values varied in the following order: ethyl acetate fraction < ether fraction < butanolic fraction < dichloromethane extract < water fraction < residue 2 < residue 1.

Correlation coefficients in CL method ranged from 1 to 0.877. IC_{50} values of the hydroxyl radical scavenging activity ranged from 9.7 to 275 μg dry extract/ml (see Table 2). The order was: ethyl acetate fraction < ether fraction < butanolic fraction < dichloromethane extract < water fraction. As it is observed from the above results, the most active fraction is ethyl acetate fraction in both tests.

Table 2
Scavenging capacity of orange peel extracts expressed as EC_{50}/IC_{50} and AE

Sample	Sample no	(mg dry ext/mg DPPH)		(μg dryext/ml)	
		EC50 ± SD	AE ± SD	IC50 ± SD	AE ± SD
Dichloromethane extract	1	3.0 ± 0.01	0.3 ± 0.002	173 ± 29	0.006 ± 0.001
Methanolic extract					
Diethyl ether fraction	2	0.7 ± 0.02	1.4 ± 0.04	14.0±2	0.08 ± 0.01
Ethyl acetate fraction	3	0.5 ± 0.003	2.0±0.01	9.7±3.8	0.1 ± 0.04
n-butanol fraction	4	1.1 ± 0.02	0.9 ± 0.02	25.0±6.2	0.04 ± 0.01
Water fraction	5	6.1 ± 0.2	0.2 ± 0.01	275 ± 29	0.004 ± 0.0002
Residue 1	6	9.0 ± 0.4	0.1 ± 0.01	NE	NE
Residue 2	7	6.8 ± 0.1	0.1 ± 0.003	NE	NE

The values of the DPPH are the mean of two determinations ± SD.
The values of the CL are the mean of three determinations ± SD.
NE = not examined due to lack of quantity.

3.4. Total phenolic content versus radical scavenging activity

Among the seven samples analyzed, the three methanolic fractions showed a significant phenolic content and radical scavenging activity. In general, extracts or fractions with a high radical scavenging activity showed a high phenolic content as well, but good correlations could not be found among them (see Fig. 1). A direct correlation between radical scavenging activity and phenolic content of the samples was demonstrated by linear regression analysis. The correlation coefficient between total phenolics and DPPH and between total phenolics and CL found to be 0.425 and 0.375, respectively. In general, the ethyl acetate fraction showed the highest TPC and the highest radical scavenging activity in both the tests of DPPH and CL.

3.5. Comparison between the two methods of radical scavenging activity

The magnitude of values for the successive extracts, fractions and residues showed a similar trend both in the free radical and hydroxyl radical scavenging activities. (see Fig. 2 and Table 2). A direct correlation between the two methods of radical scavenging activity was demonstrated by linear regression analysis. The two methods showed a high correlation coefficient (0.9662).

3.6. Extracts and fractions

As shown in Table 1, the three methanolic fractions obtained by diethyl ether, ethyl acetate and *n*-butanol had the highest total phenol content and exhibited the best radical scavenging capacity among the others (Tables 2 and 3). The ether fraction exhibited the second highest AE values in both RSA tests after the ethyl acetate fraction, but in the TPC test, exhibited the third highest value. The second highest value in TPC test after the ethyl acetate fraction was possessed by the butanol fraction, which exhibited the third highest AE values in both RSA tests. Dichloromethane extract exhibited the fourth highest TPC and AE value in both RSA tests.

3.7. Extracts/fractions and reference antioxidants

Three different standards known for their good antioxidant activity, trolox, ascorbic acid and quercetin were used. These standards were suggested by Parejo et al. (2000) who used them to evaluate scavenging activity by luminol chemiluminescence and DPPH assays. The radical scavenging activity of the standards increased in the following order: Ascorbic acid < Trolox < Quercetin.

Trolox was found to be 7.2 times more active than ethyl acetate fraction, ascorbic acid was 9.1 times more active and quercetin 10.9 times more active. Further, trolox was found to be 9.9 times more active than the ether fraction, ascorbic acid 12.5 times and quercetin

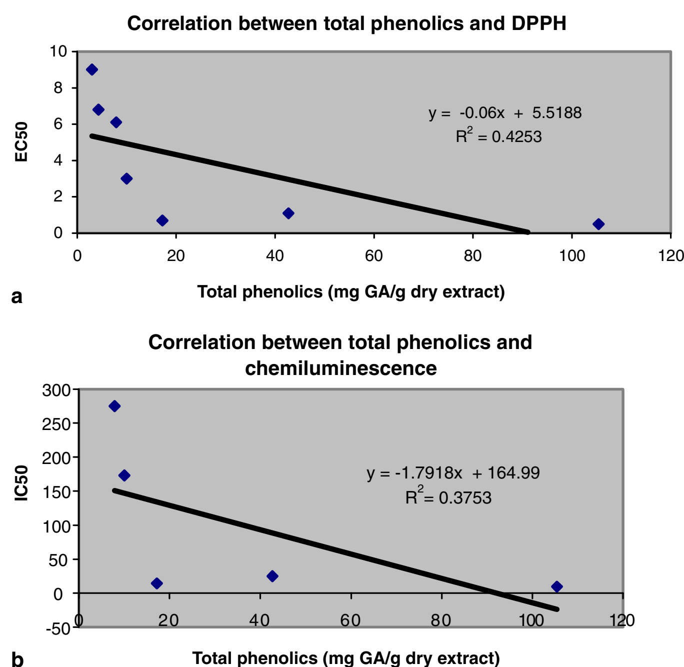

Fig. 1. Correlation of total phenol content and (a) EC_{50} values (DPPH), (b) IC_{50} (CL) for extracts/fractions/residues obtained from orange peel. TPC and CL (IC_{50}) values are the mean of three determinations ± SD. DPPH (EC_{50}) values are the mean of two determinations ± SD.

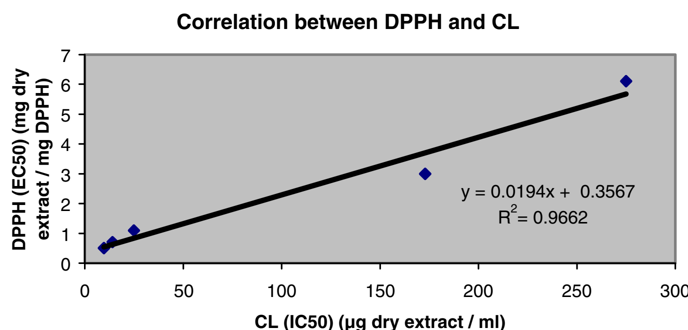

Fig. 2. Correlation of EC_{50} values (DPPH) and IC_{50} values (CL).

Table 3
Comparison of samples with the three reference compounds: trolox, ascorbic acid, quercetin

Sample no.	g dry ext/g Trolox	g dry ext/g Asc. acid	g dry ext/g Quercetin
DPPH			
1	41.8	52.5	62.8
2	9.9	12.5	15.0
3	7.2	9.1	10.9
4	15.8	19.8	23.7
5	86.3	108.4	129.8
6	127.0	159.7	191.1
7	96.0	120.6	144.3
	µg dry ext/µg Trolox	µg dry ext/µg Asc. acid	µg dry ext/µg Quercetin
CL			
1	69.1	41.4	636
2	5.6	3.4	51.5
3	3.9	2.3	35.5
4	10.0	6.0	92
5	110	65.9	1011

The values are the quotient EC_{50} (g dry ext./mol DPPH)/EC_{50}(g reference compound/mol DPPH) in the DPPH test and the quotient IC_{50} (µg dry ext./ml)/IC_{50} (µg reference compound/ml) in the CL test.

15 times. Another relatively active fraction was the butanol fraction. Trolox was found to be 15.8 times more active than butanol fraction, ascorbic acid 19.8 times more active and quercetin 23.7.

Concerning of hydroxyl radical scavenging activity, Trolox was found to be 3.9 times more active than ethyl acetate fraction, ascorbic acid was 2.31 times and quercetin 35.5 times. Further, trolox was found to be 5.6 times more active than the ether fraction, ascorbic acid 3 times and quercetin 51.5 times more active than the pre-mentioned fraction. The third more active sample in CL was the butanol fraction. Trolox was found to be 10 times more active than butanol fraction, ascorbic acid was 6 times more active and quercetin was 92 times more active than the pre-mentioned fraction.

As it can be seen, the radical scavenging activity of ethyl acetate fraction approaches the activity of the standards better than the rest of the extracts, fractions and residues.

4. Conclusion

Extracts, fractions and residues of Greek navel sweet orange peel (*Citrus sinensis*) were examined by DPPH and luminol enhanced chemiluminescence methods for their free radical and hydroxyl radical scavenging activity respectively, as well as for their total phenolic content by the Folin–Ciocalteu test. Results showed that the methanolic fractions possessed significant radical scavenging activity approaching the activity of three standards examined by the same tests. More specifically, the fraction of ethyl acetate exhibited the best radical scavenging activity and total phenolic content among the others. It is interesting that the total phenolic content did not correlate well with the two tests of radical scavenging activity because they follow different mechanisms. In contrast, the two radical scavenging tests are compatible due to their similar mechanism of radical scavenging.

According to indications from TLC which are confirmed by HPLC-DAD-MS analysis of the extracts, fractions and residues of navel sweet orange peel (Anagnostopoulou, Kefalas, Kokkalou, Assimopoulou, & Papageorgiou, 2005), the radical scavenging activity of the samples is owed to flavonoids, phenolic acids and their derivatives. These information will probably be useful for the utilization of navel sweet orange peel as antioxidant in food and medicinal preparations.

Acknowledgements

Many thanks to MAICh for giving us the opportunity to use the apparatus for CL test. Also, thanks to Ph.D. student George Kalajakis, Department of Chemistry, Aristotle university of Thessaloniki, for his assistance.

References

Anagnostopoulou, M.A., Kefalas, P., Kokkalou, E., Assimopoulou, A.N., & Papageorgiou, V.P. (2005). Analysis of antioxidant

compounds in sweet orange peel by HPLC-DAD-MS (ESI+). *Biomedical chromatography*, in press.

Bors, W., Hellers, W., Michel, C., & Saran, M. (1990a). Radical chemistry of flavonoids antioxidant. In Emerit (Ed.). *Antioxidants in therapy and preventive medicine* (Vol. 1, pp. 165–170). New York: Plenum press.

Bors, W., Hellers, W., Michel, C., & Saran, M. (1990b). Flavonoids as antioxidants: determination of radical-scavenging efficiencies. In L. Packer & A. N. Glazer (Eds.). *Methods in enzymology* (Vol. 186, pp. 343–355). San Diego, CA: Academic press.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wiss und-Technology, 28*, 25–30.

Chung, H. S., Chang, L. C., Lee, S. K., Shamon, L. A., Van Breemen, R. B., Mehta, R. G., et al. (1999). Flavonoid constituents of chorizanthe diffusa with potential cancer chemopreventive activity. *Journal of Agriculture and Food Chemistry, 47*, 36–41.

Gorinstein, S., Martin-Belloso, O., Park, Y., Haruenkit, R., Lojek, A., Ciz, M., et al. (2001). Comparison of some biochemical characteristics of different citrus fruits. *Food Chemistry, 74*, 309–315.

Gutfinger, T. (1981). Polyphenols in olive oils. *Journal of American Oil Chemical Society*, 966–968.

Hajioannou, T. P., & Kouppari, M. A. (1997). *Instrumental analysis* (pp. 34–35). Athens: Maurommati D. press.

Halliwell, B., & Gutteridge, J. M. C. (1989). *Free radicals in biology and medicine* (pp. 416–494). Oxford: Clarenson press.

Horowitz, R. M. (1961). The citrus flavonoids. In W. B. Sinclair (Ed.), *The orange. Its biochemistry and physiology* (pp. 334–372). Los Angeles, CA: University of California, Division of Agricultural Science.

Hudson, B. J. F. (1990). *Food antioxidants*. London: Elsevier applied science.

Kanner, J. (1994). Oxidative processes in meat and meat products: quality implications. *Meat Science, 36*, 169–189.

Larrauri, J. A., Ruperez, P., Bravo, L., & Saura-Calixto, F. (1996). High dietary fibre powders from orange and lime peels: associated polyphenols and antioxidant capacity. *Food Research International, 29*(8), 757–762.

Mabry, T. J., Markham, K. R., & Thomas, M. B. (1970). *The systematic identification of flavonoids* (pp. 4–13). Berlin, Heidelberg, NY: Springer-Verlag.

Manthley, J. A., & Grohmann, K. (1996). Concentrations of hesperidin and other orange peel flavonoids in citrus processing byproducts. *Journal of Agricultural and Food Chemistry, 44*, 811–814.

Manthley, J. A., & Grohmann, K. (2001). Phenols in citrus peel byproducts. Concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. *Journal of Agricultural and Food Chemistry, 49*, 3268–3273.

Mellidis, A. S., Papageorgiou, V. P., & Kokkalou, E. (1993). Phenolic constituents from *Onosma heterophylla*. *Journal of Natural Products, 56*(6), 949–952.

Miller, N. J., & Rice-Evans, C. A. (1997). The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. *Food Chemistry, 60*(3), 331–337.

Parejo, I., Codina, C., Petrakis, C., & Kefalas, P. (2000). Evaluation of scavenging activity assessed by Co (II)/EDTA-induced luminol chemiluminescence and DPPH free radical assay. *Journal of Pharmacological and Toxicological Methods, 44*, 507–512.

Parejo, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Flerlage, N., Burillo, J., et al. (2002). Comparison between the radical scavenging activity and antioxidant activity of six distilled and nondistilled Mediterranean herbs and aromatic plants. *Journal of Agricultural and Food Chemistry, 50*, 6882–6890.

Rapisarda, P., Tomaino, A., Lo Cascio, R., Bonina, F., De Pasquale, A., & Saija, A. (1999). Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *Journal of Agricultural and Food Chemistry, 47*, 4718–4723.

Roberts, W. G., & Gordon, M. H. (2002). Determination of the total antioxidant activity of fruits and vegetables by a liposome assay. *Journal of Agricultural and Food Chemistry*.

Saez Tormo, G., Oliva, M. R., Muniz, P., Valls, V., Iradi, A., Ramos, M., et al. (1994). Oxidative stress and genetic damage. In *Health and orange* (pp. 51–60). Valencia, Spain: Fundacion Valenciana de Estudios Avanzados.

Vinson, J. A., Su, X., Zubik, L., & Bose, P. (2001). Phenol antioxidant quantity and quality in foods: fruits. *Journal of Agricultural and Food Chemistry, 49*, 5315–5321.

Wang, H., Cao, G., & Prior, R. L. (1996). Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry, 44*, 701–705.