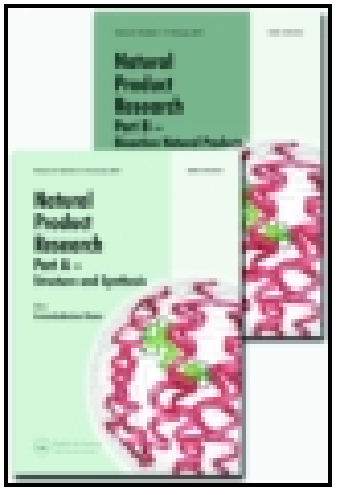


Natural Product Research: Formerly Natural Product Letters

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

Evaluation of chemical constitute, fatty acids and antioxidant activity of the fruit and seed of sea buckthorn (Hippophae rhamnoides L.) grown wild in Iran

Keramatollah Saeidi[a], Abolfazl Alirezalu[b] & Zahra Akbari[c]

[a] Department of Horticultural Science, Shahrekord University, Shahrekord, Iran

[b] Department of Medicinal and Industrial Plants, Institute of Biotechnology, Urmia University, Urmia, Iran

[c] Department of Horticulture, Science and Research Branch, Islamic Azad University, ,Tehran, Iran

Published online: 27 Jul 2015.

To cite this article: Keramatollah Saeidi, Abolfazl Alirezalu & Zahra Akbari (2015): Evaluation of chemical constitute, fatty acids and antioxidant activity of the fruit and seed of sea buckthorn (Hippophae rhamnoides L.) grown wild in Iran, Natural Product Research: Formerly Natural Product Letters, DOI: 10.1080/14786419.2015.1057728

To link to this article: http://dx.doi.org/10.1080/14786419.2015.1057728

Natural Product Research, 2015
http://dx.doi.org/10.1080/14786419.2015.1057728

Taylor & Francis
Taylor & Francis Group

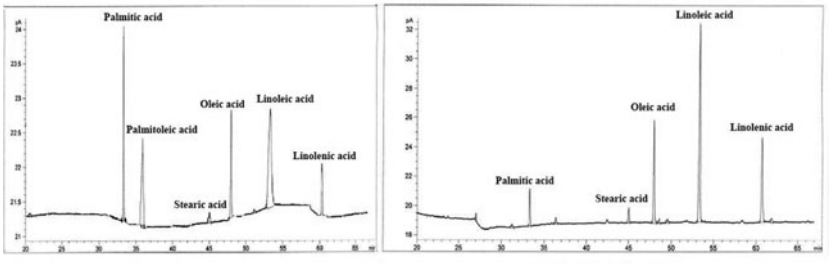
SHORT COMMUNICATION

Evaluation of chemical constitute, fatty acids and antioxidant activity of the fruit and seed of sea buckthorn (*Hippophae rhamnoides* L.) grown wild in Iran

Keramatollah Saeidi[a]*, Abolfazl Alirezalu[b] and Zahra Akbari[c]

[a]*Department of Horticultural Science, Shahrekord University, Shahrekord, Iran;* [b]*Department of Medicinal and Industrial Plants, Institute of Biotechnology, Urmia University, Urmia, Iran;* [c]*Department of Horticulture, Science and Research Branch, Islamic Azad University,Tehran, Iran*

(*Received 24 January 2015; final version received 30 May 2015*)



In this investigation, the chemical compositions of berries from sea buckthorn were studied. The amount of ascorbic acid and β-carotene determined by HPLC was 170 mg/100 g FW and 0.20 mg/g FW, respectively. Total phenols, anthocyanins, acidity and total soluble solids (TSS) contents were 247 mg GAE/100 g FW, 3 mg/L (cyanidin-3-glucoside), 5.32% and 13.8%, respectively. Fruit antioxidant activity determined by the ferric reducing ability of plasma (FRAP) method was 24.85 mM Fe/100 g FW. Results confirmed the presence of six dominant fatty acids (determined by GC) in fruit including linoleic (34.2%), palmitoleic (21.37%), palmitic (17.2%), oleic (12.8%), linolenic (5.37%) and stearic acid (1.67%). Five dominant fatty acids of the seeds were linoleic (42.36%), linolenic (21.27%), oleic (21.34%), palmitic (6.54%) and stearic acid (2.54%). The nitrogen content was 3.96%. The P, K, Ca, Mg, Fe, Zn, Mn, Cu, Cd and Cl contents of fruit were 491, 1674, 1290, 990, 291, 29.77, 108.37, 17.87, 0.021 and 2.18 mg/kg DW, respectively.

Keywords: ascorbic acid; fatty acid; fruit; sea buckthorn; seed

1. Introduction

Sea buckthorn (*Hippophae rhamnoides* L.) is a spiny shrub or tree belonging to the Elaeagnaceae family. This plant is beneficial for esophagitis, aphthous ulcers, acid reflux, peptic ulcers, cerebrovascular diseases regulate immunofunctions, attenuate inflammation and anti-carcinogenic (Zadernowski et al. 1997; Li & Beveridge 2003). The berries of sea buckthorn contain organic acids, phenols, carbohydrates, carotenoids, proteins, minerals and fatty acids (Kallio et al. 1999; Chauhan & Varshneya 2012; Yildiz et al. 2012; Pop et al. 2014). The characteristic property of sea buckthorn fruit/pulp lipid is the high content of palmitoleic acid.

*Corresponding author. Emails: saeidi@agr.sku.ac.ir; ka.saeedi@gmail.com

This high concentration of palmitoleic acid may have cholesterol and triglyceride lowering as well as stroke-suppressing effects (Yang et al. 2000; Yang & Kallio 2001). Palmitoleic acid and palmitic acid are the major fatty acids in the fruits. Oleic, palmitic, linoleic and linolenic acids are the major fatty acids in the seeds (Yang & Kallio 2001; Cakir 2004). The aim of this investigation was evaluation of some phytochemical constitute of fruit and seed of sea buckthorn. There was no data on the fruit and seed constitutes content of sea buckthorn grown in Iran.

2. Results and discussion

The amount of ascorbic acid in berries was 170 mg/100 g FW (Table S1). In previous studies, the ascorbic acid content of sea buckthorn berries ranged from 28 to 1330 mg/100 g (Yao et al. 1992; Jeppson & XiangQun 2000; Tang & Tigerstedt 2001). Origin, temperature, harvesting time, ripening and geographical factors affect the ascorbic acid content of sea buckthorn berries (Jeppson & XiangQun 2000; Yang 2009; Zheng et al. 2011). β-Carotene, total phenolics and total anthocyanin contents of berries were 0.20 mg/g FW, 247 mg GAE/100 g FW and 7.1 mg/L cyanidin-3-glucoside, respectively (Table S1). The total phenolic content for sea buckthorn fruit in this study was 1.5–3 times higher than that reported for this fruit in Europe (Gao et al. 2000). Yildiz et al. (2012) reported that the total phenolic content in fruit was ranged from 220 to 260 mg GAE/100g FW. The anthocyanin content of different sea buckthorn genotypes has previously been reported to be 0.5–25 mg/L (Sabir et al. 2005), that is in accordance with our results. Genetic and environmental conditions affect the total anthocyanins in plants (Naczk & Shahidi 2004).

The results showed that total antioxidant activity of the fruits was 24.85 mM/100 g FW, which was higher than previous study (Kruczek et al. 2012). The differences can be explained by differences in the environmental conditions of the regions under study which may affect the quality of the fruits. The N content of the fruits was 3.96%. The P, K, Ca and Mg contents were 491, 1674, 1290 and 990 mg/kg, respectively. The Fe, Zn, Mn, Cu, Cd and Cl contents were 291, 29.77, 108.37, 17.87, 0.021 and 2.18 mg/kg, respectively (Table S2). Fruit maturity and soil condition affects the level of minerals (Bounous & Zanini 1998). Difference between our results and other studies may be originating from the natural contents of elements in the soil.

Our results confirmed that linoleic (34.2%), palmitoleic (21.37%), palmitic (17.2%), oleic (12%), linolenic (5.37%) and stearic acid (1.67%) were dominant fatty acids in fruit (Table S3). Macadamia and sea buckthorn oil are botanical sources of palmitoleic acid with high concentrations (Li & Beveridge 2003). The sea buckthorn fruit/pulp has high content of palmitoleic acid (Yang & Kallio 2001). Palmitoleic acid is low in seed oils, but is characteristic of the oil in the fruit pulp (Gao et al. 2000). Result about fruit fatty acids in this study was almost same as the previous studies. A little difference between our findings and other studies could be the results of growth conditions and environmental factors. The fatty acid composition of sea buckthorn fruit oil depends on the climatic and environmental conditions where is grown (Li & Beveridge 2003). Linoleic acid was the most abundant fatty acid in seeds. Concentration of five dominant fatty acids of seed including linoleic, linolenic, oleic, palmitic and stearic acid were 42.36%, 21.27%, 20.34%, 5.54% and 2.54%, respectively (Table S3). In this study more than 80% of the fatty acids in seeds were unsaturated. The seed oil comprises two essential fatty acids including linolenic and linoleic acids. In general, the pulp oil contains more saturated fatty acids than the seed oil (Kallio et al. 1999).

3. Conclusion

Results showed that sea buckthorn fruit is a rich source of vitamin C, citric acid, phenols, carbohydrates, beta carotene, fatty acid and minerals. Moreover, the seed was rich in unsaturated fatty acid acids.

Supplementary material

Experimental details relating to this article are available online, alongside Tables S1–S3.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Bounous G, Zianin E. 1998. The variability of some components and biometric characteristics of fruit of six tree and shrub species. Hort Abstr. 60:4153.

Cakir A. 2004. Essential oil and fatty acid composition of the fruits of *Hippophae rhamnoides* L. (sea buckthorn) and *Myrtus communis* L. from Turkey. Biochem Sys Ecol. 32:809–816. doi:10.1016/j.bse.2003.11.010.

Chauhan S, Varshneya C. 2012. The profile of bioactive compounds in seabuckthorn: berries and seed oil. Int J Theor Appl Sci. 4:216–220.

Gao X, Ohlander M, Jeppsson N, Björk L, Trajkovski V. 2000. Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. J Agric Food Chem. 48:1485–1490. doi:10.1021/jf991072g.

Jeppson N, XiangQun G. 2000. Changes in the contents of kaempherol, quercetin and L-ascorbic acid in sea buckthorn berries during maturation. Agri Food Sci (Finland). 9:17–22.

Kallio K, Yang BR, Tahvonen R, Hakala M. 1999. Composition of sea buckthorn berries of various origins. International Symposium on Sea Buckthorn; Beijing, China.

Kruczek M, Swiderski A, Mech-Nowak A, Krol K. 2012. Antioxidant capacity of crude extracts containing carotenoids from the berries of various cultivars of sea buckthorn (*Hippophae rhamnoides* L.). Acta Biochim Pol. 59:135–137.

Li TSC, Beveridge THJ. 2003. Sea buckthorn (*Hippophae rhamnoides* L.): production and utilization. Ottawa, ON: NRC Research Press.

Naczk M, Shahidi F. 2004. Extraction and analysis of phenolics in food. J Chromatogr A. 1054:95–111. doi:10.1016/j.chroma.2004.08.059.

Pop RM, Weesepoel Y, Socaciu C, Pintea A, Vincken JP, Gruppen H. 2014. Carotenoid composition of berries and leaves from six Romanian sea buckthorn (*Hippophae rhamnoides* L.) varieties. Food Chem. 147:1–9. doi:10.1016/j.foodchem.2013.09.083.

Sabir SM, Maqsood H, Ahmed SD, Shah AH, Khan MQ. 2005. Chemical and nutritional constituents of sea buckthorn (*Hippophae rhamnoides* ssp. *turkestanica*) berries from Pakistan. Ital J Food Sci. 17:455–462.

Tang X, Tigerstedt PMA. 2001. Variation of physical and chemical characters within an elite sea buckthorn (*Hippophae rhamnoides* L.) breeding population. Sci Hortic. 88:203–214. doi:10.1016/S0304-4238(00)00208-9.

Yang B. 2009. Sugars, acids, ethyl β-D-glucopyranose and a methyl inositol in sea buckthorn (*Hippophaë rhamnoides*) berries. Food Chem. 112:89–97. doi:10.1016/j.foodchem.2008.05.042.

Yang B, Kalimo KO, Tahvonen RL, Mattila LM, Katajisto JK, Kallio HP. 2000. Effect of dietary supplementation with sea buckthorn (*Hippophaë rhamnoides*) seed and pulp oils on the fatty acid composition of skin glycerophospholipids of patients with atopic dermatitis. J Nutr Biochem. 11:338–340. doi:10.1016/S0955-2863(00)00088-7.

Yang B, Kallio HP. 2001. Fatty acid composition of lipids in sea buckthorn (*Hippophaë rhamnoides* L.) berries of different origins. J Agric Food Chem. 49:1939–1947. doi:10.1021/jf001059s.

Yao Y, Tigerstedt PMA, Joy P. 1992. Variation of vitamin C concentration and character correlation between and within natural sea buckthorn (*Hippophae rhamnoides* L.) populations. Acta Agric Scand. 42:12–17.

Yildiz H, Sengul M, Celik F, Ercisli S, Duralija B. 2012. Bioactive content of sea buckthorn (*Hippophae rhamnoides* L.) berries from Turkey. Agric Cons Sci. 77:53–55.

Zadernowski R, Nowak-Polakowska H, Lossow B, Nesterowicz J. 1997. Sea-buckthorn lipids. J Food Lipids. 4:165–172. doi:10.1111/j.1745-4522.1997.tb00090.x.

Zheng J, Kallio H, Linderborg K, Yang B. 2011. Sugars, sugar alcohols, fruit acids, and ascorbic acid in wild Chinese sea buckthorn (*Hippophaë rhamnoides* ssp. *sinensis*) with special reference to influence of latitude and altitude. Food Res Int. 44:2018–2026. doi:10.1016/j.foodres.2010.10.007.

SUPPLEMENTARY MATERIAL

Evaluation of chemical constitute, fatty acids and antioxidant activity of the fruit and seed of sea buckthorn (*Hippophae rhamnoides* L.) grown wild in Iran

Keramatollah Saeidi[a*], Abolfazl Alirezalu[b] & Zahra Akbari[c]

[a] *Department of Horticultural Science, Shahrekord University, Shahrekord, Iran. Tel: +984424401-8 Fax: +984424401-8*

[b] *Department of Medicinal & Industrial plants, Institute of Biotechnology, Urmia University, Urmia, Iran.*

[c] *Department of Horticulture, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[*] Corresponding author: E-mail addresses: saeidi@agr.sku.ac.ir & Ka.saeedi@gmail.com

Abstract

In this investigation the chemical compositions of berries from sea buckthorn were studied. The amount of ascorbic acid and β-carotene determined by HPLC were 170 mg/100 g FW and 0.20 mg/g FW, respectively. Total phenols, anthocyanins, acidity and TSS contents were 247 mg GAE/100g FW, 3 mg/L (cyanidin-3-glucoside), 5.32% and 13.8%, respectively. Fruit antioxidant activity determined by FRAP method was 24.85 mM Fe /100g FW. Results confirmed the presence of six dominant fatty acids (determined by GC) in fruit including linoleic (34.2%), palmitoleic (21.37%), palmitic (17.2%), oleic (12.8%), linolenic (5.37%) and stearic acid (1.67%). Five dominant fatty acids of the seeds were linoleic (42.36%), linolenic (21.27%), oleic (21.34%), palmitic (6.54%) and stearic acid (2.54%). The nitrogen content was 3.96%. The P, K, Ca, Mg, Fe, Zn, Mn, Cu, Cd and Cl contents of fruit were 491, 1674, 1290, 990, 291, 29.77, 108.37, 17.87, 0.021 and 2.18 mg/kg DW, respectively.

Keywords: Ascorbic acid, Fatty acid, Fruit, Sea buckthorn, Seed.

Experimental

Collection of fruit sample

Fruits collected at the ripening stage from Talegan region (semi-wet and cold climate , 36° 09' N, 50° 42' E and 1720 m elevation) on September 2013. Voucher specimen (number MPH-2237) has been deposited at the Herbarium of the Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran. The samples kept in the cooled bags for transferring to the laboratory and then stored in polyethylene bags at -20 $^{\circ}$C prior to analysis.

Extraction and Determination of ascorbic acid and β-carotene

Extraction and determination of ascorbic acid and beta-carotene content was carried out by high performance liquid chromatography (HPLC, Knaure, Germany) using the method described by Daood et al. (1994) and Hudsan et al. (1997), respectively. C18 sep-pak cartridge (Merck) was used for ascorbic acid purification before injection to HPLC. Ascorbic acid analyses were performed by HPLC using Vertex Column, Prontosil 120- 3 C18 AQ (250×46 mm; dp=3 µm), Knauer, Germany. The mobile phase was 15 mM KH_2PO_4 /Methanol (95/5 pH=2) at a flow rate of 1 ml/min, and the injection volume was 20 µL. Separation was carried out at room temperature by UV–VIS detector (Knauer, model K2500) at 242 nm for ascorbic acid (calibration equation of standard for ascorbic acid was Y=0.043x-1.718, R^2=0.999).

β-carotene analyses were performed by using of Vertex Column, Eurospher 100-5 C18, (250 × 4 mm; dp = 3 µm), Knauer, Germany. The mobile phase was acetonitrile/methanol/dichloromethane/n-hexane (50:40:5:5 V:V:V:V) at a flow rate of 1 ml/min. The injection volume was 20 µL. Separation was carried out at room temperature by UV–VIS detector (Knauer, model K2500) at 450 nm (calibration equation of standard for β-carotene was Y= 0.1568x + 0.057, R2=0.997).

Determination of total phenolic content, total anthocyanins, total acidity and TSS.

Total phenolic content of fruit was determined using the method described by Meda et al. (2005). The fruits were packaged in polypropylene plastic containers, and frozen at -20 °C for 24 h. The frozen samples were put in the freeze-drier (Scanvac, Denmark) for three days until they were completely dried (temperature < 0.01 °C and pressure < 4.6 mmHg). Briefly, 0.1 g of lyophilized powder of sample was dissolved in 1 ml of deionized water. The solution (0.1 ml) was mixed with 2.8 ml of deionized water, 2 ml of 2% sodium carbonate (Na_2CO_3), and 0.1 ml of 50% Folin-Ciocalteu reagent. After incubation at room temperature for 30 min, absorbance of the mixture was measured at 750 nm against a deionized water blank on a spectrophotometer (Jenway, 6505). Gallic acid was chosen as a standard. Results expressed as mg gallic acid equivalents (GAE) g^{-1} FW. For extraction of anthocyanin, samples were extracted using 1% (v/v) HCl in methanol and shaking in a shaker at 4 ℃ for 24 hours, and total anthocyanin content measured using the pH differential method. Absorbance of the extracts was measured at 510 nm and 700 nm in buffers at pH 1.0 and 4.5 with a molar extinction coefficient of cyanidine-3-glucoside. Results were expressed as mg L^{-1} cyanidin 3-glucoside (Lee et al. 2005). Total acidity measured by titration method. Total soluble solid of the samples (TSS) were determined by a digital refractometer (KRUSS Co. Germany, HR Series) at 22℃ (AOAC 1995).

Antioxidant activity

Reagents: Solution (A): 0.3 M acetate buffer, pH 3.6 (3.1 g sodium acetate 3 H_2O and 16 ml acetic acid in 1000 ml buffer solution. Solution (B): 0.01 M 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCL. Solution (C): 0.02 M $FeCl_3$-6 H_2O in distilled water.

FRAP working solution: 25 ml of solution (A), 2.5 ml of solution (B) and 2.5 ml of solution (C). The working solution was prepared freshly. For calibration, the aqueous solution of known amount of Fe (II) was used.

300 ml freshly prepared FRAP reagent was warmed at 37 ˚C and a reagent blank reading was taken at 593 nm; 10 ml of sample was then added, along with 30 ml H_2O. The reaction was monitored up to 5 min at 594 nm using a spectrophotometer (UV-VIS Biorad, USA), at 37 ˚C. The Fe (II) standard solution was tested in a parallel process (200 to 2000 µmol L^{-1}). Calculations were made by a calibration curve (Benzie and Strain 1996).

Determination of nitrogen and mineral elements

Total N was determined by the Kjeldahl method. To determine the mineral composition of the fruits, samples were burned with a nitric acid solution, on a hot plate, at 200 °C. Then, the absorbance of the extract was measured by an atomic absorption spectrophotometer (Shimadzu, aa-670). Phosphorus content was analyzed by determining the absorbance of yellow color, obtained from the Barton reaction, using a spectrophotometer (Jenway, 6505) at 680 nm, and comparing the results to standard curve (James 1995).

Oil Extraction

Oil extracted from 20 g seeds and fruits using hexane solvent in a Soxhlet apparatus for 6-7 h then solvent removed by a rotary evaporator (Heidolph, Hei-VAP Value; pressure <10 mb bar and temperature 40 °C) (AOAC 1989).

Determination of fatty acid composition by gas chromatography

The extracted seeds and fruits oil were methylated in the common procedure by refluxing with a methanolic sodium hydroxide solution, boron trifluoride reagent and heptane (AOAC 1995). The fatty acid composition of the esterified oil was characterized and quantified using Agilent 7890A Chromatograph. For this purpose BPX 70 (120m×0.25 mm) column was used. The injection volume was 10 µl. The injector and detector temperature were 220 °C and 250 °C, respectively.

Results

Table S1. Some chemical and mineral composition of sea buckthorn fruit

Ascorbic acid (mg/100 ml)	Beta-carotene (mg/g FW)	Total phenols content (mg GAE/g DW)	Total anthocyanin content (mg/L cyanidin-3-glucoside)	Antioxidant activity (mmol Fe^{2+}/100g)	TSS (Brix)	Total acidity (%)
170 ± 1.3	0.20 ± 0.2	247 ± 1.91	7.1 ± 0.1	24.85 ± 0.35	13.8 ± 0.3	5.32 ± 0.09

Table S2. Mineral content of sea buckthorn fruit

N (%)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Cd (mg/kg)	Cl (mg/kg)
3.96 ± 0.07	491 ± 4.8	1674 ± 10.1	1290 ± 7.4	990 ± 4.93	291 ± 1.8	29.77 ± 0.8	108.37 ± 1.41	17.87 ± 0.6	0.021 ± 0.01	2.18 ± 0.11

Table S3. Fatty acid composition of sea buckthorn fruit and seed

Sample	Palmitic (%)	Palmitoleic (%)	Stearic (%)	Oleic (%)	Linoleic (%)	Linolenic (%)
Fruit	17.2 ± 0.2	21.37 ± 0.6	1.67 ± 0.03	12.8 ± 0.09	34.2 ± 0.9	5.37 ± 0.31
Seed	6.54 ± 0.1	-	2.54 ± 0.05	21.34 ± 0.4	42.36 ± 0.9	21.28 ± 0.7

References

AOAC. 1995. Official methods of analysis, Association of Official Analytical Chemists.

AOAC.1989. Official methods and recommended practices of the American Oil Chemist's Society. Champaign: American Oil Chemist's Society.

Benzie IF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal biochem. 239: 70-76.

Daood HG, Biacs PA, Dakar MA, Hajdu F. 1994. Ion-pair chromatography and photodiode-array detection of vitamin C and organic acids. J Chromatogr Sci. 32: 481-487.

Hudsan T, Socaciu C, Ropan I, Neamtu G. 1997. Carotenoid composition of *Rosa canina* fruits determined by thin-layer chromatography and high-performance liquid chromatography. J Pharmaceut Biomed. 16: 521-528.

James GS. 1995. Analytical chemistry of foods. Blackie Academic and Professional: London.

Lee J, Durst RW, Wrolstad RE. 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collabo-rative study. J AOAC Int. 88: 1269-1278.

Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. 2005. Determination of the total phenolic, flavonoids and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 91: 571-577.