UDC 547.97:634.73
ISSN 1330-9862
(FTB-1353)

scientific note

Antioxidant Activities of Total Pigment Extract from Blackberries

Zhonggao Jiao, Jiechao Liu and Sixin Wang*

Zhengzhou Fruit Research Institute, The Chinese Academy of Agricultural Sciences, 450009 Zhengzhou, Henan, P. R. China

Received**:** July 19, 2004
Accepted: November 22, 2004

Summary

Total pigment has been extracted from blackberries and its antioxidant activity against lipid peroxidation and scavenging capacities towards superoxide anion radicals, hydroxyl radicals and nitrite in different *in vitro* systems have been investigated. The total pigment extract from blackberries (TPEB) exhibited strong antioxidant activity against lipid peroxidation in a linoleic acid model system and scavenging capacities towards superoxide anion radicals, generated by a pyrogallol autoxidation system or by an illuminating riboflavin system, hydroxyl radicals generated by Fenton reaction, and nitrite. Furthermore, the antioxidant activities were correlated with the concentrations of the TPEB. In the test concentration range, the maximum inhibition percentage against linoleic acid peroxidation was 98.32 % after one week's incubation, and the maximum scavenging percentages for the free radicals and nitrite inhibition in the above reactive systems reached 90.48, 96.48, 93.58 and 98.94 %, respectively. The TPEB is a natural, edible colorant with excellent antioxidant activities and health benefits and it seems to be applicable in both healthy food and medicine.

Key words: antioxidant, blackberry, hydroxyl radical, nitrite, pigment, scavenging effect, superoxide anion radical

Introduction

Consumers all over the world are becoming more conscious of the nutrition value, health benefits and safety of their food and its ingredients. In addition, there is a preference for natural functional food ingredients that are believed to be safer, healthier and less subject to hazards than their artificial counterparts. Evaluation of the functional properties of naturally occurring substances, especially those that are present naturally in human diets, has been of interest in recent years (*1–6*).

Blackberries (*Rubus fruticosus*) contain large amounts of anthocyanins, and these flavonoid pigments give blackberries their characteristic red to blue color (*7*). The total pigment extract which is produced from blackberry fruits or the pomace after the extraction of juice has been widely used as a natural colorant in beverages, baked products, chewing gums, jellies and fruit wine making (*8*). Many studies have demonstrated the antioxidant activities and health benefits of the anthocyanins occurring in various fruits and vegetables (*9–13*). However, little about the health functional properties of the total pigment extract from blackberries has been reported so far.

This work aims to investigate the antioxidant activity against lipid peroxidation and scavenging capacities towards superoxide anion radicals, hydroxyl radicals and nitrite of the total pigment extract from blackberries

* Corresponding author; Phone: ++86 371 533 09 50; Fax: ++86 371 533 09 87; E-mail: jiaozhonggao@caas.net.cn

(TPEB) as a potential source of natural functional substances for use as dietary antioxidants.

Materials and Methods

Materials

Blackberries (*Rubus fruticosus*) growing in a forestry farm in Henan province of the P. R. China were picked at maturity and then stored at –18 °C. AB-8 resin was purchased from The Chemical Plant of NanKai University, linoleic acid and 2-deoxyribose were from Fluka Chemie, and other reagents were from China National Pharmaceutical Group Corporation.

Preparation of total pigment extract from blackberries (TPEB)

A mass of 100 g of blackberries was smashed and then extracted three times for 15 minutes at 45 °C, with 200 mL of distilled water acidified with 0.5 % of HCl. The combined extracts were filtered and then purified with an adsorbent resin (AB-8) column using aqueous ethanol (80 %) as solvent. The resulting solution was concentrated on a rotary evaporator at 40 °C under reduced pressure, and 1.02 g of total pigment extract from blackberries (TPEB) was obtained after residual water had been removed at 45 °C in a vacuum drier.

Determination of anthocyanin and phenolic content in TPEB

The total content of monomeric anthocyanins in TPEB was determined using the pH-differential method (*14*). Absorbance was measured in a spectrophotometer at 510 and 700 nm, in buffers at pH=1.0 and 4.5, using $A = [(A_{510}–A_{700})_{pH=1.0} – (A_{510}–A_{700})_{pH=4.5}]$ with the molar absorption coefficient of cyanidin-3-glucoside of 29 600 mol^{-1} L cm^{-1}. The result was expressed as milligram of cyanidin-3-glucoside equivalents (CGE) per gram of TPEB. For total phenolic assay, Folin-Denise reagent was used according to the method described by Chavan *et al*. (*15*), with a minor modification, and chlorogenic acid was used as standard. A volume of 0.5 mL of Folin-Denis reagent was added to 0.5 mL of sample solution. After 3 minutes, 1 mL of saturated sodium carbonate and 8 mL of distilled water were added and mixed well. After exactly 30 minutes, the absorbance at 760 nm was measured. The result was calculated according to the standard and expressed as milligram of chlorogenic acid equivalents (CAE) per gram of TPEB.

Antioxidant activity in linoleic acid emulsion

The antioxidant activity of the total pigment extract from blackberries (TPEB) was determined using a linoleic acid model system, and the peroxide value was measured applying a thiocyanate method (*1*). Each sample of various concentrations was mixed with 5 mL of linoleic acid emulsion (25 g/L in ethanol) and 4 mL of phosphate buffer solution (0.05 mol/L, pH=7.0) in a test tube and placed in darkness at (40±1) °C to accelerate oxidation. The peroxide value was determined by reading the absorbance at 500 nm with a spectrophotometer after coloring with ferrous chloride and thiocyanate at intervals during incubation. The inhibition percentage was calculated according to the peroxide value determined a week later by the following formula:

$$\text{Inhibition percentage} = (A–A_1) \cdot 100/(A–A_2)$$

where: A is the absorbance of the control without a sample, A_1 is the absorbance after adding samples and A_2 is the absorbance of blank control of thiocyanate reagent.

Scavenging capacity towards superoxide anion radicals

For one superoxide anion radical assay, the superoxide anion radicals were generated by a pyrogallol autoxidation system (*16*). A volume of 9 mL of Tris-HCl buffer solution (50 mmol/L, pH=8.2) was added into a test tube, and the test tube was incubated in a water bath at 25 °C for 20 minutes. A volume of 40 μL of pyrogallol solution (45 mmol/L of pyrogallol in 10 mmol/L of HCl), which was also pre-incubated at 25 °C, was injected to the above test tube with a microlitre syringe and mixed up. The mixture was incubated at 25 °C for 3 minutes and then a drop of ascorbic acid was dripped into the mixture promptly to terminate the reaction. The absorbance at 420 nm marked as A_0 was measured 5 minutes later, and this A_0 denotes the speed of pyrogallol autoxidation. The A_1 autoxidation speed was obtained applying the above method and with the addition of a certain concentration of TPEB into the Tris-HCl buffer solution. Simultaneously, a blank control of reagent was obtained as A_2. The scavenging percentage was calculated according to the following formula:

$$\text{Scavenging percentage} = [A_0–(A_1–A_2)] \cdot 100/A_0.$$

For another assay of superoxide anion radicals, the superoxide anion radicals were generated by an illuminating riboflavin system (*17*). The reaction mixture contained riboflavin ($3.3 \cdot 10^{-6}$ mol/L), methionine (0.01 mol/L) and nitro blue tetrazolium chloride (NBT, $4.6 \cdot 10^{-5}$ mol/L), and the solvent of the above solutions was phosphate buffer solution (0.05 mol/L, pH=7.8). After adding the sample solution of a certain concentration, the reaction mixture was illuminated at 4000 lx and 25 °C for 30 minutes. Then the absorbance of the reaction mixture was measured at 560 nm with a spectrophotometer and the scavenging percentage was calculated according to the following formula:

$$\text{Scavenging percentage} = (A_0–A) \cdot 100/A_0$$

where: A_0 is the absorbance of the control without a sample and A is the absorbance with a sample.

Scavenging capacity towards hydroxyl radicals

Hydroxyl radicals were generated by a Fenton reaction system, and the scavenging capacity towards the hydroxyl radicals was measured by using a deoxyribose method (*18*). The reaction mixture contained 0.8 mL of phosphate buffer solution (50 mmol/L, pH=7.4), 0.2 mL of a sample of different concentrations, 0.2 mL of EDTA (1.04 mmol/L), 0.2 mL of $FeCl_3$ (1.0 mmol/L) and 0.2 mL of 2-deoxyribose (60 mmol/L). The mixtures were kept in a water bath at 37 °C and the reaction was started by adding 0.2 mL of ascorbic acid (2 mmol/L) and 0.2 mL

of H_2O_2 (10 mmol/L). After incubation at 37 °C for 1 hour, 2 mL of cold thiobarbituric acid (10 g/L) were added into the reaction mixture followed by 2 mL of HCl (25 %). The mixtures were heated at 100 °C for 15 minutes and then cooled down with water. The absorbance of the solution was measured at 532 nm with a spectrophotometer. Hydroxyl radical scavenging capacity was evaluated with the inhibition percentage of 2-deoxyribose oxidation by hydroxyl radicals. The scavenging percentage was calculated according to the following formula:

$$\text{Scavenging percentage} = [A_0-(A_1-A_2)]\cdot 100/A_0$$

where: A_0 is the absorbance of the control without a sample, A_1 is the absorbance after adding the sample and deoxyribose and A_2 is the absorbance of the sample without deoxyribose.

Scavenging capacity towards nitrite

Each sample of different concentrations was mixed with 1 mL of standard solution of sodium nitrite (5.0 μg/mL) in a colorimetric tube of 25 mL. The tubes were incubated in a water bath at 37 °C for 30 minutes. Then the quantity of residual sodium nitrite was determined with a diazo-coupling colorimetric method (*19*) and the scavenging percentage was calculated according to the following formula:

Scavenging percentage for sodium nitrite = (quantity of the standard solution of sodium nitrite-quantity of the residual sodium nitrite)·100/quantity of the standard solution of sodium nitrite.

Results and Discussion

Characterization of the total pigment extract from blackberries (TPEB)

The total monomeric anthocyanin and phenolic content in TPEB amounted to CGE 49.74 mg/g and CAE 576.7 mg/g, respectively. As is well known, many phenolic compounds including anthocyanins have strong antioxidant activity *(2–6,10,12,13)*. The TPEB may have some antioxidant activities and health benefits related to its high anthocyanin and phenolic content. That is why some procedures for the measurement of the antioxidant activity of TPEB should be undergone to confirm them.

Antioxidant activity in linoleic acid emulsion

The antioxidant effect on the peroxidation of linoleic acid was investigated to evaluate the *in vitro* activity of the total pigment extract from blackberries (TPEB) at the initiation stage of lipid peroxidation. As shown in Fig. 1, the peroxidation of linoleic acid was accelerated notably when incubated at 40 °C. However, the peroxidation was inhibited after adding antioxidants, including TPEB of different concentrations, and butyl hydroxy toluene (BHT). The inhibition percentages of various concentrations of TPEB against linoleic acid peroxidation after one week's incubation are shown in Fig. 2. The inhibition effects of TPEB at 0.075–0.5 mg/mL were identical to that of BHT at 0.1 mg/mL, and superior to that of ascorbic acid at 0.5 mg/mL. In the test concentration range from 0.05 to 0.5 mg/mL, the antioxidant activity of TPEB increased depending on the sample concentration and the maximum inhibition percentage against linoleic acid peroxidation was 98.32 % after one week's incubation.

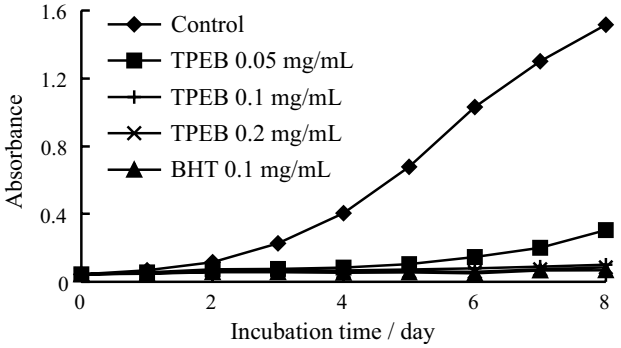

Fig. 1. Peroxidation of linoleic acid and the inhibition effects of antioxidants

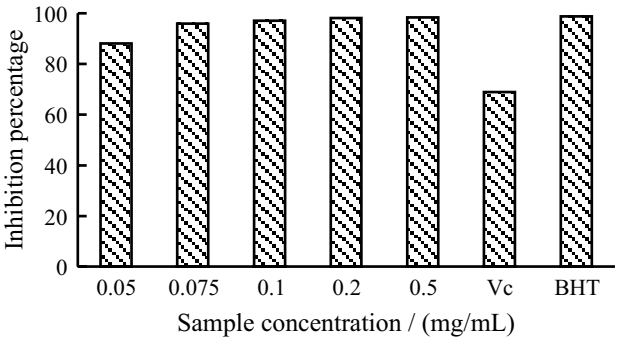

Fig. 2. Inhibition percentages of different concentrations of TPEB against linoleic acid peroxidation. The concentrations of ascorbic acid (Vc) and BHT were 0.5 and 0.1 mg/mL, respectively

Scavenging capacity towards superoxide anion radicals

Superoxide anion is an initial free radical and plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical, or singlet oxygen in living systems (*20*). It can also react with nitric oxide and form peroxynitrite, which can generate toxic compounds such as hydroxyl radical and nitric dioxide *(21)*. We evaluated the scavenging capacity of TPEB towards superoxide anion radicals by using a pyrogallol autoxidation system and an illuminating riboflavin system, respectively.

Pyrogallol can autoxidate fast in alkali conditions and release superoxide anions, and, in return, the superoxide anions can accelerate the autoxidation. However, the superoxide anions can be scavenged by adding some scavenger or antioxidant, the autoxidation will thus be depressed. As shown in Fig. 3, the inhibition effects of TPEB on the autoxidation of pyrogallol were relatively feeble at lower concentrations, but the TPEB exhibited strong inhibition activities at higher concentrations. In the range of test concentrations from 0.05 to 1.25 mg/mL, the maximum inhibition percentage was 90.48 %. This indicates that TPEB has a strong inhibition effect on the

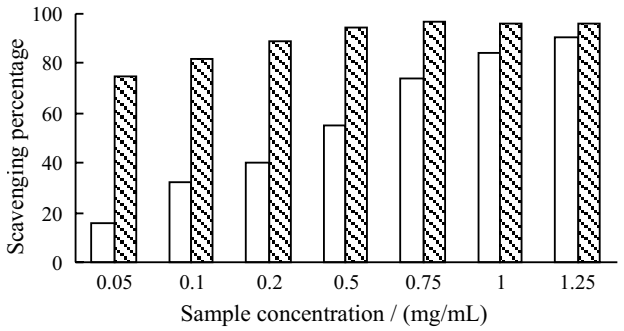

Fig. 3. Dose dependency of the scavenging effects of TPEB on superoxide anion radicals
□ pyrogallol autoxidation system ▨ illuminating riboflavin system

autoxidation of pyrogallol. In other words, it can scavenge the superoxide anion radicals generated by the pyrogallol autoxidation system effectively.

While in illuminating riboflavin system, TPEB of different concentrations all exhibited strong scavenging activities towards the superoxide anion radicals, and the scavenging effects showed a dose–dependent fashion at concentrations from 0.1 to 0.75 mg/mL. The scavenging percentage was 81.71 % at concentration of 0.1 mg/mL and 94.32 % at concentration of 0.5 mg/mL. However, it had no significant effect on the scavenging capacity towards the superoxide anion radicals with a further increase of the concentration.

The above test systems both originate from the measurement of superoxide dismutase, but they have different mechanisms for generating superoxide anion radicals and this difference may account for the different results, when applied to evaluate the antioxidant activity of TPEB. As mentioned above, the superoxide anions were generated by the oxidation of pyrogallol and the scavenging effects were expressed as the inhibition of pyrogallol autoxidation, so any substance existing in the reaction system that might have effects on the oxidation of pyrogallol might affect the test results. Since the TPEB was a crude extract, there might be some substance that could enhance the oxidation of pyrogallol and thereby offset some inhibition effects. While in the illuminating riboflavin reaction system the superoxide anions were generated and not influenced by the components of the reaction system, the scavenging percentage of TPEB in it might be higher than in pyrogallol autoxidation system at the same concentration. This difference had also been found in the evaluation of the radical scavenging effects of tea polyphenol and its oxidant (22). Furthermore, Zhang and Ge *(23)* also found there was no result when applying pyrogallol autoxidation system to measure the superoxide dismutase in crude corn extract due to the complexity of composition, while the illuminating riboflavin system had higher specificity than pyrogallol autoxidation system, when applied to determine the superoxide dismutase or superoxide anions.

Scavenging capacity towards hydroxyl radicals

Hydroxyl radical is the most reactive free radical and can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions such as copper or iron. Hydroxyl radical has the highest 1-electron reduction potential (2310 mV) and can react with anything in living organisms at the second order rate constants of 10^9 to 10^{10} mol L^{-1} s^{-1} (*24*). It can react with lipids, polypeptides, saccharides, nucleotides and organic acids, especially thiamine and guanosine and thereby cause cell damage (*25*). Hydroxyl radical can be generated by the reaction of hydrogen peroxide with Fe^{2+} or Cu^{2+}. To test the scavenging capacity of TPEB towards hydroxyl radicals, we used the Fenton reagent (Fe^{2+} + $H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$) as a source of hydroxyl radicals. As shown in Fig. 4, TPEB exhibited strong scavenging capacity towards the hydroxyl radicals generated by Fenton reaction in the test concentration range and the scavenging effects were dependent on concentration from 0.05 to 1.25 mg/mL. The scavenging percentage achieved 93.58 % at the concentration of 1.25 mg/mL.

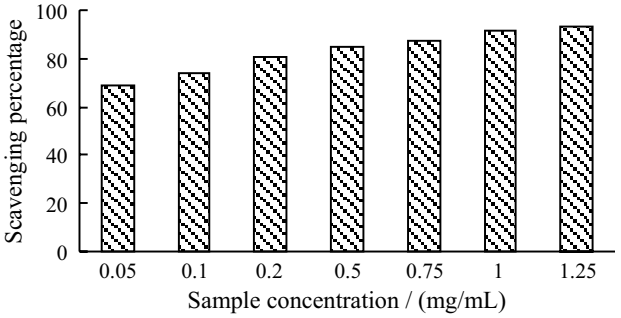

Fig. 4. Dose dependency of the scavenging effects of TPEB on hydroxyl radicals

Scavenging capacity towards nitrite

Nitrite has been applied in the processing of meat products as a preservation agent and color agent for a long time. The nitrate occurring in many vegetables can also be transformed to nitrite by reduction reactions with the action of bacteria in human bodies. These nitrites may be transformed into nitrosamines combining with secondary and tertiary amines in human bodies. These nitrosamines are procarcinogenic substances *(26)*. Thus, if some substance can scavenge nitrosamine or its precursor substance such as nitrite, it probably has protective functions from cancer. As shown in Fig. 5, though the scavenging capacities of TPEB towards nitrite were

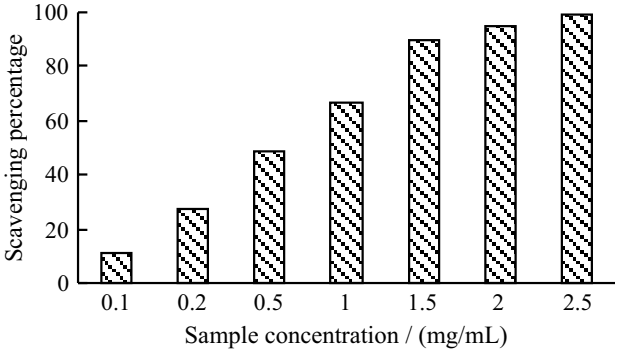

Fig. 5. Dose dependency of the scavenging effects of TPEB on nitrite

relatively feeble at lower concentrations, the scavenging activities became obviously stronger with an increasing concentration and showed a dose-dependent manner in the concentration range from 0.1 to 1.5 mg/mL. In the range of test concentration from 0.1 to 2.5 mg/mL, the maximum scavenging percentage was 98.94 %. Many substances have been demonstrated to scavenge nitrite and block the nitrosation reaction, but the scavenging effects differed due to the different reaction conditions *(27–29)*. Therefore, a standard substance should be used as a control for comparison with the sample. Ascorbic acid, a well-known nitrite scavenger, was used as control in our test. Results showed that the nitrite scavenging effect of ascorbic acid of 0.5 mg/mL was equivalent to that of TPEB of 1.0 mg/mL. This indicates that TPEB is also an excellent nitrite scavenger.

Conclusions

Lipid oxidation is an old but still very important topic in food stability and in human health. Oxidative damage to cellular components such as lipids and cell membranes by free radicals and other reactive oxygen species is believed to be associated with the development of a range of degenerative diseases including heart disease, cancer, inflammation, arthritis, immune system decline, brain dysfunction and cataracts *(21,30–33)*. This research has demonstrated that the total pigment extract from blackberries (TPEB), which is commonly used as a natural food colorant, is also an excellent natural antioxidant and free radical scavenger. Thus the TPEB may play an important role in the prevention of human diseases related to oxidative damage. Furthermore, the TPEB has also exhibited strong scavenging activity towards nitrite and thereby prevents the formation of nitrosamine and reduces the carcinogenesis induced by nitrosamines. Therefore, the TPEB is a natural, edible colorant with excellent antioxidant properties and health benefits and it seems applicable in both health food and medicine.

References

1. G.C. Yen, H.Y. Chen, Antioxidant activity of various tea extracts in relation to their antimutagency, *J. Agric. Food Chem. 43* (1995) 27–32.
2. T. Kanda, H. Akiyama, A. Yanagida, M. Tanabe, Y. Goda, M. Toyoda, R. Teshima, Y. Saito, Ihhibitory effects of apple polyphenol on induced histamine release from RBL-2H3 cells and rat mast cells, *Biosci. Biotechnol. Biochem. 62* (1998) 1284–1289.
3. O.S. Yi, A.S. Meyer, E.N. Frankel, Antioxidant activity of grape extracts in a lecithin liposome system, *J. Am. Oil Chem. Soc. 74* (1997) 1301–1307.
4. P.L. Teissedre, E N. Frankel, A.L. Waterhouse, H. Peleg, J.B. German, Inhibition of *in vitro* human LDL oxidation by phenolic antioxidant from grapes and wines, *J. Sci. Food Agric. 70* (1996) 55–61.
5. E.N. Frankel, J. Kanner, J.B. German, E. Parks, J.E. Kinsella, Inhibition of oxidation of human low density lipoprotein by phenolic substances in red wine, *Lancet*, *341* (1993) 454–457.
6. E.N. Frankel, A.L. Waterhouse, P.L. Teissedre, Principle phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low density lipoproteins, *J. Agric. Food. Chem. 43* (1995) 890–894.
7. C. Felgines, O. Texier, C. Besson, D. Fraisse, J.L. Lamaison, C. Rémésy, Blackberry anthocyanins are slightly bioavailable in rats, *J. Nutr. 132* (2002) 1249–1253.
8. G.T. Ling: *Handbook of Natural Food Additives*, Chemical Industry Press, Beijing (2000) pp. 226–228.
9. M.P. Kahkonen, A.I. Hopia, M. Heinonen, Berry phenolics and their antioxidant activity, *J. Agric. Food Chem. 49* (2001) 4076–4082.
10. M.K. Ehlenfeldt, R.L. Prior, Oxygen radical absorbance capacity (OROC) and phenolic and anthocyanin concentrations in fruit and leaf tissues of highbush blueberry, *J. Agric. Food Chem. 49* (2001) 2222–2227.
11. H. Xue, R.M. Aziz, N. Sun, J.M. Cassady, L.M. Kamendulis, Y. Xu, G.D. Stoner, J.E. Klaunig, Inhibition of cellular transformation by berry extracts, *Carcinogenesis, 22* (2001) 351–356.
12. H. Wang, M.G. Nair, G.M. Strasburg, Y.C. Chang, A.M. Booren, J.I. Gray, D.L. DeWitt, Antioxidant and anti-inflammatory activities of anthocyanins and their aglycone from tart cherries, *J. Nat. Prod. 62* (1999) 294–296.
13. F.C. Stintzing, A.S. Stintzing, R. Carle, B. Frei, R.E. Wrolstad, Color and antioxidant properties of cyanidin-based anthocyanin pigments, *J. Agric. Food Chem. 50* (2002) 6172–6181.
14. M.M. Giusti, R.E. Wrolstad: Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. In: *Current Protocols in Food Analytical Chemistry*, R.E. Wrolstad (Ed.), John Wiley & Sons, New York (2001) pp. 1–13.
15. U.D. Chavan, D.B. Mckenzie, R. Amarowicz, F. Shahidi, Phytochemical components of beach pea (*Lathyrus maritimus* L.), *Food Chem. 81* (2003) 61–71.
16. T.Y. Jing, X.Y. Zhao, The improved pyrogallol method by using terminating agent for superoxide dismutase measurement, *Progr. Biochem. Biophys. 22* (1995) 84–86.
17. C. Beauchamp, I. Fridovich, Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels, *Anal. Biochem. 44* (1971) 276–287.
18. B. Halliwell, J.M.C. Gutteridge, O.I. Aruoma, The deoxyribose method: A simple »test tube« assay for determination of rate constants for reactions of hydroxyl radicals, *Anal. Biochem. 165* (1987) 215–219.
19. F.L. Liu, X.J. Dai: *Analytical Methods in Food Physics and Chemistry*, Chinese Light Industry Press, Beijing (1987) pp. 177–183.
20. T.W. Stief, The physiology and pharmacology of singlet oxygen, *Med. Hypotheses, 60* (2003) 567–572.
21. B. Halliwell, Antioxidants and human disease: A general introduction, *Nutr. Rev. 55* (1997) 44–49.
22. C.M. Li, B.J. Xie, Investigation with spectrophotomeric method on the radical scavenging effect of tea polyphenol and its oxidant, *Fine Chemicals, 17* (2000) 241–244.
23. W.J. Zhang, C. Ge, Comparation of pyrogallol autoxidation and NBT photoreduction assay in detecting activity of SOD, *J. Hebei Vocation-Technical Teachers College, 14* (2000) 68–70.
24. M.B. Korycka-Dahl, T. Richardson, Activated oxygen species and oxidation of food constituents, *Crit. Rev. Food Sci. Nutr. 10* (1978) 209–241.
25. B. Ashok, R. Ali, The aging paradox: Free radical theory of aging, *Exp. Gerontol. 34* (1999) 293–303.
26. H. Bartsch, R. Montesano, Relevance of nitrosamines to human cancer, *Carcinogenesis, 5* (1984) 1381–1393.
27. T. Rundlof, E. Olsson, A. Weirnik, S. Back, M. Aune, L. Johansson, I. Wahlberg, Potential nitrite scavengers as inhibitors of the formation of N-nitrosamines in solution and tabacco matrix systems, *J. Agric. Food Chem. 48* (2000) 4381–4388.

28. K. Kikugawa, T. Hakamada, M. Hasunuma, T. Kurechi, Reaction of *p*-hydroxycinnamic acid derivatives with nitrite and its relevance to nitrosamine formation, *J. Agric. Food Chem. 31* (1983) 780–785.

29. D. Lathia, A. Braasch, U. Theissen, Role of vitamin E as nitrite scavenger and N-nitrosamine inhibitor: A review, *Int. J. Vitam. Nutr. Res. 59* (1989) 430–438.

30. O.I. Aruoma, Free radicals, oxidative stress, and antioxidants in human health and disease, *J. Am. Oil Chem. Soc. 75* (1998) 199–212.

31. G. Block, L. Langseth, Antioxidant vitamins and disease prevention, *Food Technol. 7* (1994) 80–84

32. M.G.L. Hertog, E.J.M. Feskens, P.C.H. Hollman, M.B. Katan, D. Kromhout, Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen elderly study, *Lancet, 342* (1993) 1007–1011.

33. J. Lee, N. Koo, D.B. Min, Reactive oxygen species, aging, and antioxidative nutraceuticals, *Compr. Rev. Food Sci. Food Saf. 3* (2004) 21–33.

Antioksidativna aktivnost ekstrakta ukupnoga pigmenta kupine

Sažetak

Iz kupine je ekstrahiran ukupni pigment te je ispitana njegova antioksidativna aktivnost prema peroksidaciji lipida i sposobnost uklanjanja radikala superoksid aniona, hidroksilnih radikala i nitrita u raznim sustavima *in vitro.* Ekstrakt ukupnoga pigmenta kupine (TPEB) pokazao je jako antioksidativno djelovanje pri peroksidaciji lipida u modelnom sustavu s linolnom kiselinom i sposobnost uklanjanja radikala superoksid aniona (dobivenih u sustavu autooksidacije pirogalola ili iluminacijom sustava riboflavina), te hidroksilnih radikala (dobivenih Fentonovom reakcijom) i nitrita. Nadalje, antioksidativne aktivnosti uspoređene su s koncentracijama TPEB. Unutar ispitanog područja koncentracije ustanovljen je maksimalni postotak inhibicije peroksidacije linolne kiseline, i to 98,32 % nakon jednotjedne inkubacije, a maksimalni postotak uklanjanja slobodnih radikala i nitrita u prije navedenim reakcijskim sustavima iznosio je 90,48, 96,48, 93,58 i 98,94 %. TPEB je prirodno jestivo bojilo s izvrsnim antioksidativnim svojstvima te je primjenjiv u zdravoj hrani i medicini.