African Journal of Biotechnology Vol. 10(51), pp. 10472-10477, 7 September, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1501
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Total phenolic, condensed tannin and antioxidant activity of four *Carya* species from China

Zhiping He[1,2], Maorun Fu[3] and Linchun Mao[1]*

[1]Department of Food Science and Nutrition, College of Biosystem Engineering and Food Science, Zhejiang University, Hangzhou 310029, China.
[2]Department of Food Science, Zhejiang Forestry College, Linan 311300, China.
[3]Shandong Analysis and Test Center, Shandong Academy of Sciences, Jinan 250014, China.

Accepted 14 April, 2011

Different species of functional agricultural crops may vary in antioxidant capacities. In this study, the antioxidant activities of methanol extracts from four species of *Carya* genus were compared by various antioxidant assays, including the reducing power, 1,1-diphenyl-2-pycrylhydrazyl (DPPH) radical scavenging activity and the superoxide anion scavenging activity. The reducing power of extracts from *Carya dabieshanensis, Carya cathayensis*, *Carya hunanensis* and *Carya illinoensis* were 0.246, 0.237, 0.22 and 0.073 at the concentration of 0.50 mg/ml, respectively. The scavenging effect on the DPPH radical (IC_{50}) were 1.140, 1.364, 1.437 and 3.682 mg/ml, respectively, while the scavenging effect on superoxide anion radical were 27.44, 22.80, 26.15, 1.99 mg AE/g, respectively. Among the four species, *C. dabieshanensis* possessed the highest antioxidant activity, while *C. illinoensis* was the lowest. The total phenolic (TP) contents and condensed tannins (CT) were determined in all samples spectrophotometrically. For all species, *C. dabieshanensis* possessed the highest TP content (80.54 mg GE/g defatted kernel) and *C. hunanensis* possessed the highest CT content (59.62 mg CE/g defatted kernel). In addition, strong correlations of total phenolic contents and condensed tannins contents with reducing powers, DPPH radical and superoxide anion scavenging activities were also found in this work.

Key words: *Carya*, antioxidant, phenolic compounds, condensed tannins.

INTRODUCTION

Tree nuts have long been considered as important components of the diet due to several bioactive and health-promoting components. Epidemiological evidence indicates that, the consumption of tree nuts may lower the risk of cardiovascular disease. The European prospective investigation into cancer and nutrition (EPIC) cohort study, conducted in ten European countries also showed that, women consuming more than 6.2 g per day of nuts and seeds reduced their risk of colon cancer by 31% (Jenab et al., 2004). The *Carya* genus (family *Juglandaceae*) comprises several species and is commercially cultivated in North America and east Asia for over 500 years. Recently, growing interest was developed in the exploitation of *Carya* (Taipina et al., 2009; Osorio et al., 2010). Pecan, belonging to the Carya genus, has been reported to possess the highest antioxidant capacity and the highest phenolic content among the common fruits and vegetables across the US (Wu et al., 2004). Villarreal-Lozoya et al. (2007) also found that, kernels from different pecan cultivars had high antioxidant capacity and total phenolic content.

Growing evidence suggests that, species and genotype would alter the antioxidant compositions and properties in a selected agricultural crop (Gursoy et al., 2009; Esmaeili et al., 2010). Thus, it is of paramount importance to examine species and genotype for their antioxidant activity, in order to find potential species rich in special healthy functions. The objective of the present study was to

*Corresponding author. E-mail: hzhping8@gmail.com or linchun@zju.edu.cn. Tel: +86 571 8827 3136. Fax: +86 571 8827 3009.

Abrreviations: DPPH, 1,1-Diphenyl-2-pycrylhydrazyl; **TP,** total phenolic; **CT,** condensed tannins; **NBT,** nitro blue tetrazolium; **BHA,** butylated hydroxyanisole; **IC_{50},** half maximum inhibitory concentration; **EC_{50}**, half maximum effective concentration.

characterize four different *Carya* species for their nutraceutical constituents, including total phenolic content, antioxidant activities and condensed tannins content.

MATERIALS AND METHODS

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), nitro blue tetrazolium (NBT), ascorbic acid, xanthine and xanthine oxidase (0.5 units/mg protein) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Material and thermal processing

Carya cathayensis seeds were mechanically harvested in early September 2009 from Linan, Zhejiang Province, China. The seeds of *Carya dabieshanensis*, *C. cathayensis*, *Carya hunanensis* and *Carya illinoensis* were purchased from Hangzhou Donglin Co. Ltd. The seeds were washed with excess water and then sun-dried at about 30°C for three days. The kernels with the brown outer testa or pellicle were separated from the shell by cracking with a small hammer and were ground in a mortar. The kernel powder was defatted with hexane according to the method of Villarreal-Lozoya et al. (2007), then, samples were freeze-dried and stored at -20°C until analyses.

Preparation of methanol extracts

Extraction was done by macerating 1 g of defatted kernel powder with 40 ml of 70% methanol. The mixture was kept in a rotary shaker overnight and centrifuged at 3,000 g for 20 min (SCR20BC, Hitachi, Japan). A working solution (2.5 mg defatted kernels/ml) was prepared by dissolving 1 ml of the supernatant in 10 ml of methanol.

Determination of total phenolic and condensed tannin content

Total phenolic content was determined with Folin-Ciocalteu reagent according to Slinkard and Singleton (1977) using gallic acid as standard. In a volumetric flask, 0.1 ml of the methanol extract (final concentrations were 2.5 mg defatted kernels/ml), 0.9 ml of distilled water and 1 ml Folin-Ciocalteu reagent were mixed thoroughly. After 3 min, 3 ml of 0.188 mol/l Na_2CO_3 was added, then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer (UV-2100, Unico, Shanghai, China). The final results were expressed as milligram gallic acid equivalents per gram of defatted kernels (mg GE/g).

Condensed tannin (CT) content was evaluated using the vanillin assay (Price et al., 1978). An aliquot of 0.5 g of defatted kernels was placed in centrifuge tubes and 20 ml of 1% HCl in methanol was added to each sample. Each tube was vortexed every 10 min and placed in a water bath at 30°C with constant shaking for 20 min. After incubation, tubes were centrifuged and supernatants were extracted. Aliquots of the supernatants were placed in two separate assay tubes, one for the sample determination and the other for blank determination. Samples and blanks were incubated for exactly 20 min after adding 5 ml of the vanillin reagent (0.5 g of reagent and 200 ml of 4% HCl methanol) to samples and 4% HCl in methanol to the blanks. After 20 min, the absorbance was measured at 500 nm in a spectrophotometer. Results were expressed as milligram catechin equivalents per gram of defatted kernels (mg CE/g).

Determination of reducing power

The reducing power of methanol extract was determined by the method of Mao et al. (2006). The 1 ml of methanol extract (0.25, 0.5 and 0.75 mg defatted kernels) prepared from working solution were mixed with 2.5 ml of 0.2 mol/l phosphate buffer (pH 6.6) and 2.5 ml of 0.03 mol/l potassium ferricyanide ($K_3Fe(CN)_6$). Aliquots (2.5 ml) of 0.6 mol/l trichloroacetic acid were added to the mixture, which was then centrifuged for 10 min at 1,000 g. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.006 mol/l $FeCl_3$ and the absorbance was measured at 700 nm in a spectrophotometer.

Determination of DPPH radical scavenging activity

The model of scavenging the 1,1-diphenyl-2-pycrylhydrazyl (DPPH) radical is a widely used method to evaluate the free radical scavenging ability of various samples (Ebrahimzadeh et al., 2009). Scavenging activity of DPPH free radical was measured based on Lee et al. (1996). Negative control was prepared by mixing 0.125 ml distilled water with 3.875 ml of 0.2 mol/l DPPH. The 0.125 ml of the methanol extract (0.5, 1.0, 1.5, 2.0 and 2.5 mg defatted kernels/ml) was added to 3.875 ml of 0.2mol/ l DPPH. The mixture was gently homogenized and left to stand at room temperature for 30 min. Absorbance was read using a spectrophotometer at 517 nm. The activity of scavenging DPPH radicals was calculated using the equation:

$$\text{Scavenging activity (\%)} = (A_{(-)} - As) / A_{(-)} \times 100\%$$

where, As is the absorbance of the sample, $A_{(-)}$ is the absorbance values of negative controls, respectively.

Determination of superoxide anion scavenging activity

The superoxide anion scavenging activity was measured using the xanthine/xanthine oxidase method Mao et al., 2006). Working solution of extract (1.25 mg defatted kernels/ml) was separately added to a 1.0 ml mixture of 0.4 mmol/l xanthine and 0.24 mmol/l nitro blue tetrazolium chloride (NBT) in 0.1 mol/l phosphate buffer (pH 8.0). A 1.0 ml solution of xanthine oxidase (0.049 unit/ml), diluted in 0.1 mol/l phosphate buffer (pH 8.0), was added and the resulting mixture incubated in a water bath at 37°C for 40 min. The reaction was terminated by adding 2.0 ml of an aqueous solution of 69 mmol/l sodium dodecylsulphate (SDS) and the absorbance of NBT was measured at 560 nm. A standard curve was prepared using ascorbic acid as reference reagent. Superoxide anion scavenging activity was expressed in milligram ascorbic acid equivalents per gram of defatted sample (mg AE/g).

Statistical analysis

The data were reported as mean ± SD for triplicate determinations. Analysis of variance and the least significant difference tests (SPSS for Windows, 1999, SPSS Inc., Chicago, IL) were conducted to identify differences among means. Statistical significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Total phenolic content

It is well known that phenolic compounds exist in many

Table 1. Total phenolic contents and condensed tannin contents of methanol extracts from defatted *Carya* kernels.

Carya species	*C. dabieshanensis*	*C. cathayensis*	*C. hunanensis*	*C. illinoensis*
Total phenolic content(mg GE/g)	80.54±2.55[a]	75.45±1.79[b]	71.94±3.49[b]	14.63±1.29[c]
Condensed tannin content (mg CE/g)	50.12±0.77[c]	55.67±1.91[b]	59.62±3.01[a]	13.21±1.28[d]

Each value represents mean ± standard deviation of three replicates. Different letters in the same line meant significant difference ($P < 0.05$).

plants, which have attracted a great deal of public and scientific interest because of the health promoting effects as antioxidants. Recent studies have shown that walnuts and hickories are good sources of antioxidant phenolic compounds. Kornsteiner et al. (2006) reported that, the mean content of total phenolics varied between 32 mg gallic acid equivalents/100 g (pines) and 1,625 mg (walnuts). Wu et al. (2004) stated that, pecan (*C. illinoinensis*) kernels had the highest phenolic content among the common fruits and vegetables across the US. Villarreal-Lozoya et al. (2007) also found that, kernels from different pecan cultivars had high antioxidant capacity and total phenolic content.

Table 1 shows the total phenolic (TP) contents of the *Carya* species, which were significantly ($p<0.05$) different. Total phenolic content ranged from 14.63 to 80.54 mg GE/g defatted kernel with *C. dabieshanensis* showing the highest TP value, while the *C. illinoensis* had the lowest. For all species, the following trend was found; *C. dabieshanensis* > *C. cathayensis* > *C. hunanensis* > *C. illinoensis* ($P < 0.05$). The TP value of *C. dabieshanensis*, *C. cathayensis* and *C. hunanensis* were at least 4.9 times higher than that of *C. illinoensis* ($P < 0.05$). The TP value of *C. illinoensis* in this study was in agreement with those of Wu et al. (2004) who found that, the TP value of *C. illinoensis* ranged from 12.84 to 20.16 mg GE/g.

Condensed tannin content

Most phenolic compounds commonly identified in walnut and hickory are phenolic acids and condensed tannins (Fukuda et al., 2003; Ito et al., 2007; Zhang et al., 2009). The condensed tannins (CT) content evaluated with the vanillin assay showed differences among *Carya* species, ranging from 13.21 to 59.62 mg CE/g defatted kernel which was similar to the values found by Polles et al. (1981). Also, Prado et al. (2009a) reported that, the CT content of pecan nut (*C. illinoinensis* (Wangenh.) C. Koch) shell infusion and acetone extracts of kernel cake were 43 ± 7 and 16.4 ± 4.2 mg CE/g, respectively. The CT content of defatted kernel from different pecan cultivars ranged from 23 to 47 mg CE/g (Villarreal-Lozoya et al., 2007). Among the four species, *C. hunanensis* presented the highest CT values, while *C. illinoensis,* had the lowest (Table 1). *Carya* species showed the following in a descending order: *C. hunanensis* > *C. cathayensis* > *C. dabieshanensis* > *C. illinoensis* ($P < 0.05$).

Scavenging effect on DPPH radical

1,1-Diphenyl-2-pycrylhydrazyl (DPPH) is a stable nitrogen-centered free radical whose color changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore, radical scavengers (Brand-Williams et al., 1995). The scavenging effects of extracts from *Carya* kernels tested on the DPPH radical were measured as shown in Figure 1. The scavenging activity of extracts on inhibition of the DPPH radical was related to the concentration of extracts added, the activity increased as a result of increasing concentration for each species. The scavenging effect of extracts from four species of *Carya* kernels on the DPPH radical followed the order: *C. dabieshanensis* > *C. cathayensis* > *C. hunanensis* > *C. illinoensis* ($P < 0.05$) and the half maximum inhibitory concentration (IC_{50}) were 1.140, 1.364, 1.437 and 3.682 mg/ml, respectively, which was low, compared to that reported by Zhu et al. (2008), who stated that, the percentage of DPPH radical scavenging activity at 200 µl volume of Chinese Hickory (*C. cathayensis*) kernel ethanol extracts, α-tocopherol and butylated hydroxyanisole (BHA) were 87.8, 82.4 and 87.8%, respectively. Villarreal-Lozoya et al. (2007) reported that, the mean value of the scavenging effect on DPPH radical of different pecan cultivars was 487 ± 42 µg Trolox equivalents per gram of defatted sample. The scavenging effect on DPPH radical of acetone extracts of kernel cake from Pecan nut were 68.0 ± 21.0 mg Trolox equivalent antioxidant capacity per gram of defatted sample (Prado et al., 2009b). Futher study on the milligram Trolox equivalent antioxidant capacity per gram of four species in this study should be done.

Reducing capacity

The Fe^{3+}-Fe^{2+} transformation was determined as the reducing capacity in this study. The presence of reductants (antioxidants) in the samples would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability (Ebrahimzadeh et al., 2010). The reducing capacity of extracts from the four species of

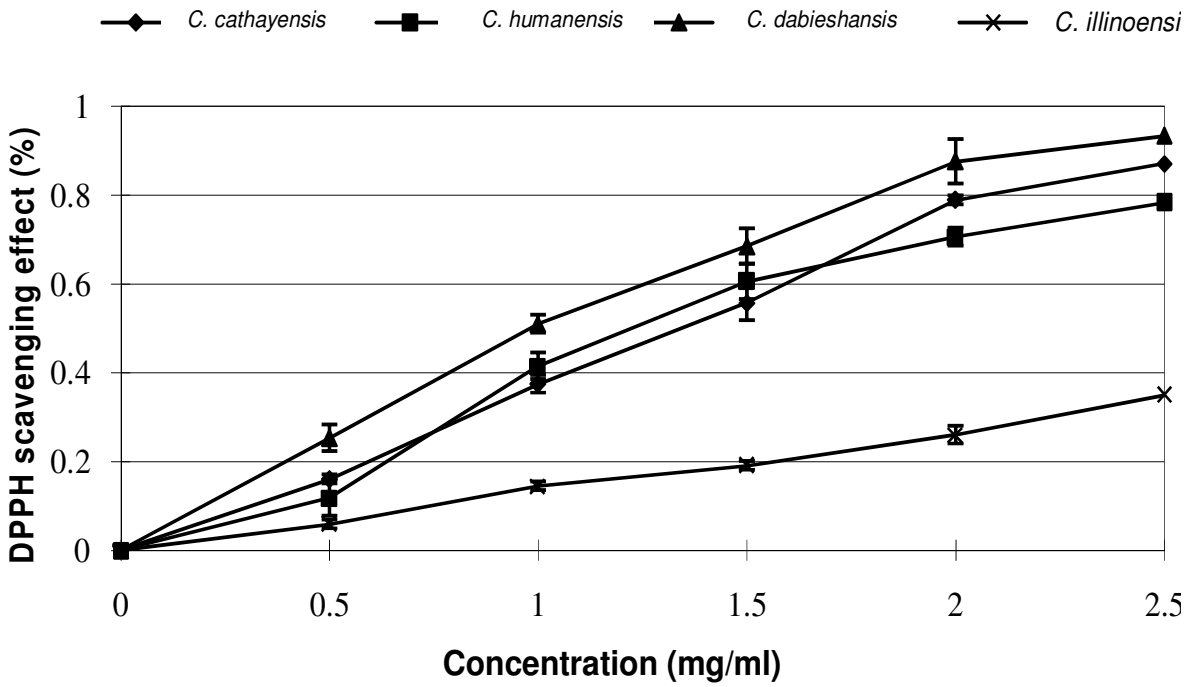

Figure 1. DPPH radical scavenging effect of methanol extracts from defatted *Carya* kernels . Each value represents mean ± standard deviation of three replicates.

Carya kernels also increased with increasing amount of the extracts and decreased in the order: BHA > *C. dabieshanensis* > *C. cathayensis* > *C. hunanensis* > *C. illinoensis* ($P < 0.05$) and the absorbances were 1.19, 0.246, 0.237, 0.22 and 0.073 at the concentration of 0.50 mg/ml, respectively (Figure 2). Zhu et al. (2008) reported that the reducing capacity of Chinese Hickory (*C. cathayensis*) kernel ethanol extracts, α-tocopherol and BHA at 700 nm was 0.76, 0.64, 1.08, at 200 µl volume, respectively. It was found that, the reducing capacity of four species of *Carya* kernels was correlated with the phenolic compounds from the correlation analysis.

Superoxide anion scavenging activity

Compared with other oxygen radicals, the superoxide anion ($O_2^{\cdot-}$) has a longer lifetime, can move to to a target cell at a longer distance and thus, is more dangerous. Therefore, it is important to study the ability of the extract to scavenge superoxide anion. Recent researches showed that, scavention of superoxide anion radicals is of importance for protection against early events in oxidative damage (Hu and Skibsted, 2002). In this study, the scavenging effect of extracts from four species of *Carya* kernels on superoxide anion radical followed the order: *C. dabieshanensis* > *C. hunanensis* > *C. cathayensis* > *C. illinoensis* ($P < 0.05$) and were 27.44, 26.15, 22.80 and 1.99 mg AE/g, respectively (Figure 3). Sun et al. (2004) reported that, the scavenging effect on superoxide was related to the number of active hydroxyl groups in the molecules. Therefore, the strong scavenging effect on superoxide by *C. dabieshanensis, C. hunanensis* and *C. cathayensis* may be due to the abundant phenolic hydroxyl groups.

Correlation between total phenolic content and antioxidant activity

It is important to examine the correlation between the content of the total polyphenols and antioxidant potential because some authors have reported that, there is no correlation between the content of these main antioxidant compounds and the radical scavenging capacity (Yu et al., 2002). The results obtained by us do not support these claims. In the present study, there is a strong correlation between total phenolic content and reducing power capacity ($R^2 = 0.9985$). In addition, the content of phenolic compounds was also highly correlated with 1,1–diphenyl-2-pycrylhydrazyl radical scavenging capacity ($R^2 = 0.9675$) and superoxide anion scavenging capacity ($R^2 = 0.9739$). These data are in accordance with others, who have shown that high total phenol content increases the antioxidant activity (Villarreal-Lozoya et al., 2007; Prado et al., 2009b). Therefore, the phenolics of *Carya* kernels may be responsible for its antioxidant properties, but further studies are warranted for the isolation and identification of individual phenolic compounds and also *in vivo* studies

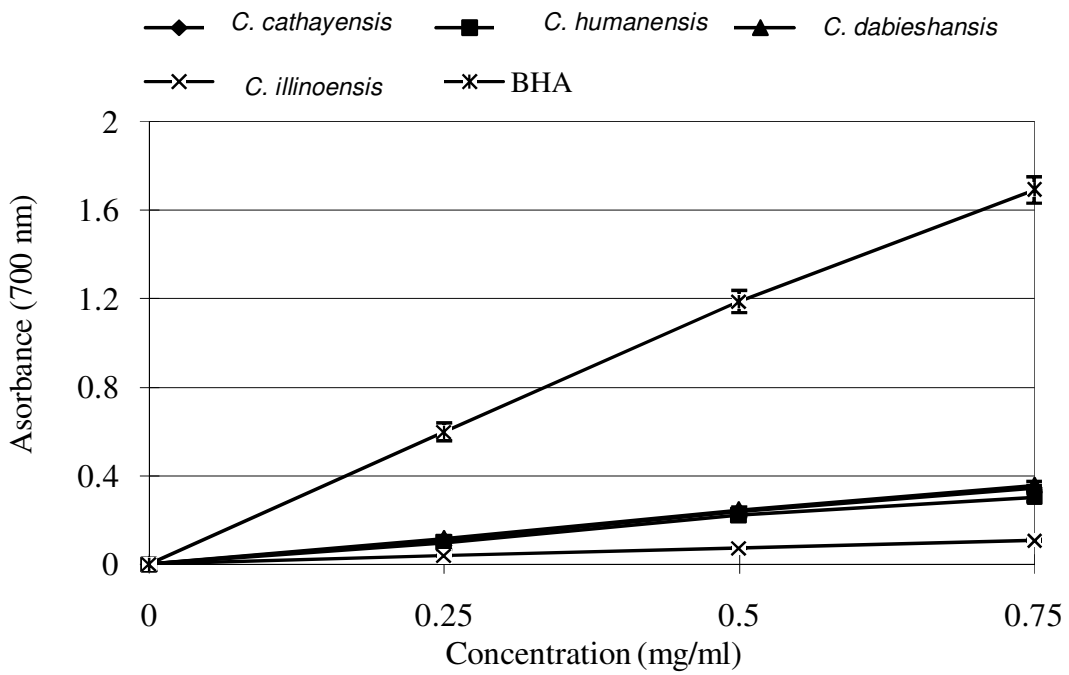

Figure 2. Reducing capapcity of methanol extracts from defatted *Carya* kernels. Each value represents mean ± standard deviation of three replicates.

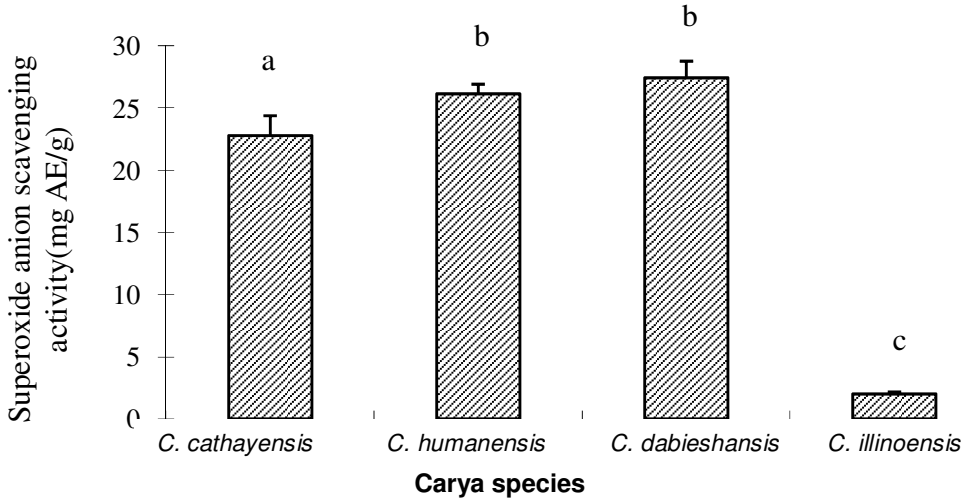

Figure 3. Superoxide anion scavenging activity(mg AE/g) of methanol extracts from defatted *Carya* kernels. Each value represents mean ± standard deviation of three replicates. Different letters (a.b,c) meant significant difference ($P < 0.05$).

are needed for a better understanding of their mechanism of action as an antioxidant.

Tannins are water-soluble polyphenols that are present in many plant foods. In this work, data also showed a strong correlation of condensed tannin with reducing power ($R^2 = 0.9049$), DPPH radical scavenging capacities ($R^2 = 0.8544$) and superoxide anion scavenging capacity ($R^2 = 0.9195$). This may be interpreted that, the tannins were the main polyphenolics of *Carya* kernels. A remarkable radical scavenging effect against DPPH (EC_{50} = 0.34-4.72 μM) of tannins from the n-butanol (n-BuOH) extract of walnuts (the seeds of *Juglans regia* L.) were also measured by Fukuda et al. (2003).

Conclusions

The results from various free radical scavenging systems

revealed that, the four species of *Carya* kernels have significant antioxidant activity. The significant variation in antioxidant properties, total phenolic content, condensed tannin content of different species of *Carya* was observed in this study. *C. dabieshanensis* possess the highest antioxidant activity and *C. illinoensis* had the lowest. A correlation between total phenolic content, condensed tannins and the antioxidant properties was also observed. This study can be used as a basis for future breeding programs aiming to develop *Carya* kernels with improved nutritional profile and health benefits.

ACKNOWLEDGEMENT

This work was supported by Natural Science Foundation of Zhejiang province (y3090428 and Y3100422), the Analysis and Testing Foundation of Zhejiang Province (2011C37067), and Science and Technology Project of Zhejiang Province (2009C12031)

REFERENCES

Brand-Williams W, Cuvelier M, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28: 25-30.

Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Eslami B (2009). Free radical scavenging ability of methanolic extract of *Hyoscyamus squarrosus* leaves. *Pharmacologyonline*, 2: 796-802.

Ebrahimzadeh MA, Nabavi SF, Nabavi SM, Eslami B (2010). Antihypoxic and antioxidant activity of Hibiscus esculentus seeds. Grasas Aceites, 61(1): 30-36.

Esmaeili MA, Sonboli A, Noushabadi MA (2010). Antioxidant and protective properties of six Tanacetum species against hydrogen peroxide-induced oxidative stress in K562 cell line: A comparative study. Food Chem. 121: 148-155.

Fukuda T, Ito H, Yoshida T (2003). Antioxidative polyphenols from walnuts (*Juglans regia L.*). Phytochemistry. 63: 795-801.

Gursoy N, Sarikurkcu C, Cengiz M, Solak MH (2009). Antioxidant activities, metal contents, total phenolics and flavonoids of seven Morchella species. Food Chem. Toxicol. 47: 2381-2388.

Hu M, Skibsted LH (2002). Antioxidative capacity of rhizome extract and rhizome knot extract of edible lotus (*Nelumbo nuficera*). Food Chem. 76: 327-333.

Ito H, Okuda T, Fukuda T, Hatano T, Yoshida T (2007). Two novel dicarboxylic acid derivatives and new dimeric hydrolysable tannin from walnuts. J. Agric. Food Chem. 55: 672-679.

Jenab M, Ferrari P, Slimani N (2004). Association of nut and seed intake with colorectal cancer risk in the European Prospective investigation into cancer and nutrition. Cancer Epidem. Biomar. Prev. 13: 1595-1603.

Kornsteiner M, Wagner K, Elmadfa I (2006). *Tocopherols* and total phenolics in 10 different nut types. Food Chem. 98: 381-387.

Lee JH, Park JHC, Choi JS (1996). The antioxidant activity of *Ecklonia stolonifera*. Arch. Pharm. Res. 19: 223-227.

Mao L, Pan X, Que F, Fang X (2006). Antioxidant properties of water and ethanol extract from hot air-dried and freeze-dried daylily flowers. Eur. Food Res.Technol. 222: 236-241.

Osorio E, Flores M, Hernández D, Ventura J, Rodríguez R, Aguilar CN (2010). Biological efficiency of polyphenolic extracts from pecan nuts shell (*Carya Illinoensis*), pomegranate husk (*Punica granatum*) and creosote bush leaves (*Larrea tridentata Cov.)* against plant pathogenic fungi. Ind. Crop Prod. 31: 153-157.

Polles SG, Hanny BW, Harvey AJ (1981). Condensed tannins in kernels of pecan [*Carya illinoensis* (Wangenh.) K. Koch] cultivars. J. Agric. Food Chem. 29: 196-197.

Prado AC, Aragão AM, Fett R, Block JM (2009a). Antioxidant properties of Pecan nut [*Carya illinoinensis*(Wangenh.) C. Koch] Shell infusion. Grasas Aceites. 60 (4): 330-335.

Prado AC, Aragão AM, Fett R, Block JM (2009b). Phenolic compounds and antioxidant activity of Pecan [*Carya illinoinensis*(Wangenh.) C. Koch] kernel cake extracts. Grasas Aceites. 60(5): 458-467.

Price M L, Vanscoyoc S, Butler LG (1978). Critical evaluation of vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem. 26: 1214-1218.

Slinkard K, Singleton VL (1977). Total phenol analyses: automation and comparison with manual methods. Am. J. Enol. Viticult. 28: 49-55.

Sun T, Xie W, Xu P (2004).Superoxide anion scavenging activity of graft chitosan derivatives. Carbohydr. Polym. 58: 379-382.

Taipina MS, Lamardo LC, Rodas MA, Mastro NL (2009). The effects of gamma irradiation on the vitamin E content and sensory qualities of pecan nuts (*Carya illinoensis*). Radiat. Phys. Chem. 78: 611-613.

Villarreal-Lozoya JE, Lombardini L, Cisneros-Zevallos L (2007). Phytochemical constituents and antioxidant capacity of different pecan [*Carya illinoinensis* (Wangenh.) K. Koch] cultivars. Food Chem. 102: 1241-1249.

Wu XL, Beecher GR, Holden J, Haytowitz DB, Gebhardt SE, Prior RL (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. J. Agric. Food Chem. 52: 4026-4037.

Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M (2002). Free radical scavenging properties of wheat extracts. J. Agric. Food Chem. 50: 1619-1624.

Zhang Z, Liao L, Moore J, Wu T, Wang Z (2009). Antioxidant phenolic compounds from walnut kernels (*Juglans regia L.*). Food Chem. 113: 160-165.

Zhu C, Deng X, Shi F (2008). Evaluation of the antioxidant activity of Chinese Hickory (*Carya cathayensis*) kernel ethanol extraction. Afr. J. Biotechnol. 7: 2169-2173.