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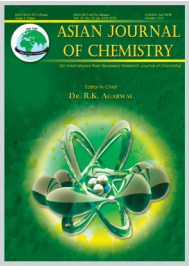

Changes of Phenolics, Condensed Tannins and Antioxidant Activity of Chinese Hickory (*Carya cathayensis Sarg*.) after Different Thermal Processing

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To select the proper thermal treatments, changes of phenolic compounds, condensed tannins and antioxidant activities of methanol extracts from Chinese hickory kernels after different thermal processing were investigated. Total phenolic and condensed tannins were determined in all samples spectrophotometrically. Comparing with the raw materials, total phenolic of samples were reduced by 4.47, 7.37, 47.90 and 66.73 % and condensed tannins reduced by 33.65, 4.79, 46.23 and 69.94 % after roasting, microwave baking, boiling and pressured-steam heating treatment, respectively. Antioxidant activities of Chinese hickory were obviously decreased after boiling and pressured-steam heating, while little influence after roasting and microwaving. A strong correlations between total phenolic and reducing power, DPPH radical scavenging activities and superoxide anion scavenging activities (R^2 = 0.9875, R^2 = 0.9933, R^2 = 0.8917, respectively) was observed. The results of this work indicated that Chinese hickory after roasting or microwave baking possesses higher functional benefit than other thermal processing to the view of nutrition.

Key Words: Chinese hickory, Thermal processing, Phenolic compound, Condensed tannin, Antioxidant activity.

INTRODUCTION

Chinese hickory (*Carya cathayensis Sarg*.) has been cultivated commercially in northwestern of Zhejiang province and southeastern of Anhui province in China for about 500 years. In these regions, about 50,000 hectares under cultivation yielded 22,000 metric tons Chinese hickory in 2010. The kernels listed high nutrition value and health benefit, the dried kernels contains 61.69-70.89 % unsaturated fatty acids, 7.8-9.6 % proteins and 22 minerals[1]. In addition, the kernels from Genus *Carya* are traditionally used to tonify the kidney, warm the lung, relax the bowels and prevent cancer, atherosclerosis, cardiovascular disease in China. Wu *et al.*[2] reported that pecan (*C. illinoinensis*) kernels had the highest antioxidant capacity and the highest phenolic content among the common fruits and vegetables across the US. Villarreal-Lozoya and Rosa also founded kernels from different pecan cultivars had high antioxidant capacity and total phenolic content[3-4].

Drying was necessary step for kernels after harvest, different thermal methods such as boiling, pressured-steam heating, roasting were used usually in the processing of Chinese hickory[5]. Many studies showed that thermal processing significantly alters phytochemical constituents and antioxidant capacity, although published data on the effects of thermal processing on antioxidant activity and antioxidant compounds were not consistent[6-8]. Thermal processing may lead to a decrease in total phenolics and antioxidant activities in grains and buckwheat flour[9,10]. However, an increase in the total phenolic content and antioxidant activities was obtained after cooking or roasting on barley[11], sweet corn[12] and beans[13]. Changes of the antioxidant activities and related compounds in the thermal processed products hold great significance in both dietary and nutritional values. However, there was no information available on their variations in kernels from Chinese hickory after processing.

In present, four thermal methods (boiling, pressured-steam heating, microwave baking and roasting) were employed during processing of Chinese hickory to identify the method with enhanced levels of antioxidant activity and related compounds targeting increased functional properties. Total phenolic and condensed tannin from Chinese hickory, as well as their antioxidant activities, were determined before and after the processing.

EXPERIMENTAL

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

Material and thermal processing: Chinese hickory (*C. cathayensis Sarg.*) seeds were mechanically harvested at Linan City, Zhejiang Province, China, in the early September. After the removal of green husks, the seeds were washed with excess water and then sun-dried at about 30 ºC for 3 days. Dried seeds were divided into five groups of 4 kg each. Group one was immersed in boiling water in the ratio of 1:10 (w/v) for 240 min (boiling). Group two was pressured-steam heated in autoclave at 121 ºC (MLS-3020, Sanyo, Japan) for 100 min (steaming). Group three was cooked in a microwave oven at 2450 MHz (Model WD 800 G, Galanz, Guangdong, China) for 2 min (microwave baking). Group four was put in a baking oven at 150 ºC for 270 min (roasting). Group five was not processed and served as control.

After processing, 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 moisture content of kernel powder was determined using an infrared moisture analyzer (Ohaus, MB45, Switzerland). The kernel powder was defatted with hexane at room temperature according to the method of Villarreal-Lozoya *et al.*[3], freeze-dried and stored at -20 ºC until analyses.

Preparation of methanol extracts: Extraction was prepared by macerating 1 g of defatted kernel powder with 40 mL of 70 % methanol. Mixture was kept in a rotary shaker overnight and centrifuged at 3000 g for 20 min. A working solution (2.5 µg defatted kernels/µL) was prepared by dissolving 1 mL of the supernatant in 10 mL of methanol.

Determination of reducing power: The reducing power of methanol extract was determined by the method of Oyaizu and Mao *et al.*[14,15]. The 1 mL of methanol extract (200 µg defatted kernels /mL) prepared from the 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 centrifugated for 10 min at 1,000 g (SCR20BC, Hitachi, Japan). The upper layer of 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 (UV-2100, Unico, Shanghai, China).

Determination of DPPH radical scavenging activity: Scavenging activity of DPPH free radical was measured based on Lee *et al.*[16]. Positive control was prepared by mixing 0.2 mL of ascorbic acid (0.5 mg/mL) and 3.8 mL of DPPH (0.04 mg/mL). Negative control was prepared by mixing 0.2 mL distilled water with 3.8 mL of DPPH. The 0.2 mL of the methanol extract (120 µg defatted kernels/mL) prepared from working solution was added to 3.8 mL DPPH. The mixture was gently homogenized and left to stand at room temperature for 0.5 h. Absorbance was read using a spectrophotometer at 517 nm. Activity of scavenging DPPH radicals was calculated using the equation: Scavenging activity (%) = $[(A_{(-)} - As)/(A_{(-)} - A_{(+)})] \times 100$ %, where, A is the absorbance of the sample, $A_{(-)}$ and $A_{(+)}$ are the absorbance values of negative and positive controls, respectively.

Determination of superoxide anion scavenging activity: The superoxide anion scavenging activity was measured using the xanthine/xanthine oxidase method[15-17]. Working solution of extract (120 µg defatted kernels/mL) was separately added to a 1 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 mL solution of xanthine oxidase (0.049 units/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 ºC for 40 min. The reaction was terminated by adding 2 mL of an aqueous solution of 69 mmol/L sodium dodecylsulphate (SDS) and the absorbance of nitro blue tetrazolium was measured at 560 nm. Activity to scavenge superoxide anion was calculated using the following equation: Scavenging activity (%) = $[1- (A_1/A_0)] \times 100$ %, where, A_0 is the absorbance of the blank and A_1 is the absorbance in the presence of the extract.

Determination of total phenolic and condensed tannin content: Total phenolic content was determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton[18] using gallic acid as standard. In a volumetric flask, 0.1 mL of the methanol extract (final concentrations were 30, 60, 120 µg defatted kernels/mL), 46 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. Total phenolic content was expressed as gallic acid equivalents. The final results were expressed as mg gallic acid equivalents/g of defatted kernels (mg GE/g).

Condensed tannin content was evaluated using the vanillin assay[19]. An aliquot of 0.5 g of defatted kernels was placed in a centrifuge tube 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 ºC with constant shaking for 20 min. After the 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. The 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 the samples and 4 % HCl in methanol to the blanks. After 20 min, the absorbance was measured at 500 nm by a spectrophotometer. The results were expressed as mg catechin equivalents/g of defatted kernels (mg CE/g).

Statistical analysis: Data were reported as mean ± SD for triplicate determinations. Analysis of variance and 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

Moisture content: The moisture contents in roasted, microwave baked, boiled, pressured-steam heated and control samples were 1.74 ± 0.29, 2.72 ± 0.61, 26.55 ± 0.55, 25.26 ± 1.24 and 23.03 ± 1.06 %, respectively. The increase of moisture

content of boiling and pressured-steam samples indicated that the mass transmission between water and Chinese hickory kernels is occurred during boiling and pressured-steam processing.

Total phenolic content: Phenolic compounds had attracted a great deal of public and scientific interest because of their health promoting effects as antioxidants. Recent studies had shown walnuts and pecans are good sources of antioxidant phenolic compounds[2,3,20]. In this study, the content of total phenolic in extracts of defatted kernels from raw Chinese hickory is 78.28 ± 0.378 mg GE/g, higher than the values in pecan kernels[3].

Studies in vegetables confirm that the thermal processing significantly alters the physical and bio-chemical composition. Blanching and boiling significantly ($P < 0.05$) reduced total phenolic contents in red cabbage by 43 % and 16 %, respectively[21]. Zhang and Hamauzu found a 72 % reduction in total phenolic in broccoli florets boiled for 5 min[22]. In this study, total phenolic content was significantly affected by thermal processing. The phenolic contents in roasted, microwave baked, boiled and pressured-steam heated samples were 74.78 ± 3.18, 72.51 ± 2.48, 40.78 ± 1.77 and 26.05 ± 1.48 mg GE/g, respectively. Comparing with the control, total phenolic content was reduced by 4.47, 7.37, 47.90 and 66.73 % after roasting, microwave baking, boiling and pressured-steam heating treatment, respectively. Barba *et al.*[23] also found that microwave baking treatment provoked a lower loss of phenolics than boiling in potatoes.

The results showed that the reduction of total phenolic content after boiling and pressured-steam heating processing are higher than the samples after roasting and microwave baking processing. This may be carefully interpreted that the mass transmission is occurred between water and Chinese hickory during boiling and pressured-steam heating processing and those water soluble phenolic compounds consequently leach into the liquid medium. The total phenolic loss of pressured-steam heating samples is higher than boiling samples mainly due to the acceleration of mass transmission caused by pressure. These results agree with those of Dini *et al.*[24] who found a significant decrease of phenolics content in bitter quinoa and sweet quinoa seeds during boiling treatment due to the loss of phenolic in cooking water. The higher loss of total phenolic was also found by Volden *et al*.[21] in boiled red cabbage due to the exposure to water. It was also reported that the thermal processing conditions may result in the loss of natural antioxidants because heat may accelerate the oxidation and other degradation reactions[25,26]. This may be the main reason for the low loss of phenolic compounds after roasting, microwave baking.

Condensed tannin content: The slightly astringent flavour of walnut fruit and pecan has been associated with the presence of phenolic compounds[27]. Most phenolic compounds commonly identified in walnut are phenolic acids and condensed tannins[28,29]. In this study, the content of condensed tannins in Chinese hickory is 44.67 mg CE/g, higher than the values in pecan[3].

In this work, all treatments conducted in this work caused a significant decrease in the tannin contents of all investigated samples (Fig. 1). The highest reduction was caused by pressured-steam heating followed by that of boiling and roasting. However, the lowest reductions were those of microwave baked Chinese hickory. Comparing with the raw samples, the condensed tannin content was reduced by 69.94, 46.23, 33.65 and 4.79 %, respectively.

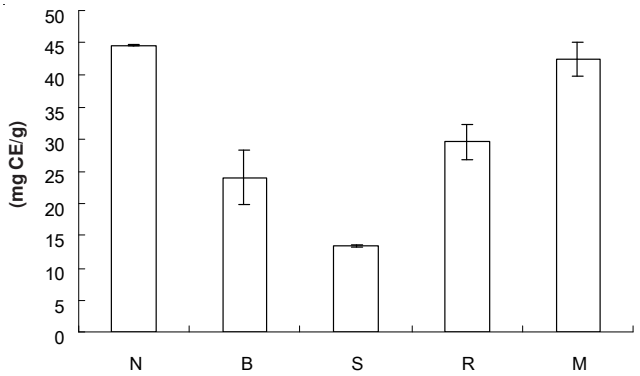

Fig. 1. Condensed tannin contents in Chinese hickory seeds processed by boiling, pressured-steam heating, microwave baking and roasting. Control seeds were not processed. Each value represents mean ± standard deviation of three replicates. The values represented by the same letters are not significantly different at $P > 0.05$ compared with each other among the same component. Symbols are: N, raw samples; B, boiling; S, pressured-steam heating; R, roasting; M, microwave baking. Thermal processing- N: 44.67 ± 0.16; B: 24.02 ± 4.18; S: 13.43 ± 0.26; R: 29.64 ± 2.72; M: 42.53 ± 2.56 CT(mgCE/g)

These results was in agreement with those of Udensi *et al.*[30] who found that tannin content of vegetable cowpea was reduced by 37.00, 12.50 and 37.00 % after water boiling for 45 min, roasting at 120 ºC for 30 min and autoclaving at 120 ºC for 15 min, respectively. These results come also in harmony with those of Rehman and Shah who stated that tannin content of black grams, red kidney bean and white kidney bean was significantly reduced after ordinary cooking and pressure cooking[31].

The reduction of tannins after boiling and pressured-steam heating processing is mainly due to the fact that those compounds, in addition to their predominance in seed coats[32], were water soluble[33] and consequently leach into the liquid medium. The decrease of tannins after thermal processes could also be related to the fact that condensed tannin were heat labile and degrade during heat treatment. A decrease of tannin content was found in thermally treated oak acorns kernels due to thermally degradation[34]. Cheng *et al.*[35] also found conjugated polyphenolics such as tannins in wheat were degraded at high temperatures to simple phenols.

Antioxidant activity: In this study, antioxidant activities of Chinese hickory were obviously decreased after boiling and pressured-steam heating, while little influence after roasting and microwave baking (Table-1). The highest decrease was in pressured-steam heating processing samples and was reduced by 53.9, 74.8 and 21.8 % in reducing capacity, DPPH scavenging activity and superoxide anion radicals scavenging activity, respectively. The follow processing is boiling and were reduced by 39.7,47.2 and 17.8 %, respectively. The lowest decrease was in roasting and microwaving processing and were reduced less than 9.7 %. Furthermore, even a small increase was found in superoxide anion radicals scavenging activity. The results of antioxidant activity assay indicated that Chinese hickory after roasting and microwave baking possess higher functional benefit than other thermal processing in view of nutrition.

TABLE-1
EFFECT OF THERMAL PROCESSES ON THE ANTIOXIDANT CAPACITY OF CHINESE HICKORY[a]

Thermal processes	Reducing capacity	DPPH scavenging activity (%)	Superoxide anion radicals scavenging activity (%)
Boiling	0.162 ± 0.007a	33.20 ± 0.59a	49.81 ± 0.71a
Pressured-steam heating	0.124 ±0.01b	15.80% ± 0.98b	47.41 ± 2.77a
Microwave baking	0.255 ± 0.012cd	59.40 ± 0.62c	65.93 ± 1.519b
Roasting	0.243 ± 0.005c	60.13 ± 2.49cd	63.45 ± 1.82bc
Raw sample	0.269 ± 0.012d	62.82 ± 1.95d	60.64 ± 0.63c

Results were expressed as means ± standard error of the mean (n = 3). Mean values represented by the same letters within the same column are not significantly different at P > 0.05. Antioxidant analysis of methanol extracts from defatted Chinese hickory included reducing power, DPPH radical scavenging activity and superoxide anion scavenging activity were determined at the concentration of 200 µg defatted kernels/mL, 120 µg/mL and 120 µg/mL, respectively

Corelation between total phenol, condensed tannin and antioxidant activity: A strong correlation of total phenolic content with reducing power, DPPH radical scavenging activities and superoxide anion scavenging activity was found in this work (R^2 = 0.9875, R^2 = 0.9933, R^2 = 0.8917, respectively). This may be explained that phenolics were the main substance contributed to reducing power, DPPH radical and superoxide anion scavenging activity of Chinese hickory. Therefore, the decrease of antioxidant activities of Chinese hickory is mainly due to the loss of phenolics compounds after thermal processes. Goh and Barlow[36] also found around 40 % of the antioxidant activity was lost when the Ginkgo biloba nuts were cooked for 4 h due to the transfer of non-ascorbic acid antioxidant compounds from the nuts to the leachate. Data also showed a strong correlation of total phenolic content with condensed tannin (R^2 = 0.8081). The reaction of polyphenols with Folin-Ciocalteau reagent is non-specific. The condensed tannin was also determined as a phenolic fraction of total phenolic by Folin-Ciocalteau assay. Therefore, the loss of condensed tannin was a fraction of the loss of total phenol. The data of this work also implied that there are some other phenolic compounds in Chinese hickory kernels and were decreased after thermal processing.

Conclusion

Significant variation in antioxidant properties, total phenolic content, condensed tannin content after different thermal processes of Chinese hickory was observed in this study. The antioxidant activity and total phenolic content of methanol extracts of defatted kernels after roasting and microwave baking process is higher than boiling, pressured-steam heating treated ones. Therefore, in order to obtain health-promoting Chinese hickory products, microwave baking and roasting processing may be promising thermal methods because of the lower loss of antioxidant properties and phenolic compounds.

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