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COMPARATIVE STUDY ON ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL FROM WHITE AND BLACK PEPPER

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ABSTRACT: *In this paper, the antioxidant activities of black and white pepper (Piper nigrum L.) essential oils (BEO and WEO) were evaluated and compared based on radical scavenging activities, reducing power, and DNA oxidative damage protection assays. The results showed that EOs exhibited antioxidant activities, and DNA damage protective effect to various extents. For BEO and WEO, they showed a concentration-dependent antioxidant activity at some concentrations. On the whole, WEO exhibited higher antioxidant activity than BEO. These results suggest that the essential oil of white pepper and black pepper may be a new potential source as a natural antioxidant.*

KEYWORDS: Antioxidant activity, Damage protective effect, Essential oil, Pepper

INTRODUCTION

Protection of food commodities during transportation, storage and processing from the moulds and their toxic metabolites as well as from oxidative degradation is a challenging issue for food industries. Therefore, a natural antioxidant is an urgent need to research and development. Different plant products have been used traditionally in preserved food and food-related, such products are included in frontier areas of research in food security and green consumerism programmes (da Silveira *et al*., 2014). These products are cost-effective and have low toxicity to human and live stocks (Burt 2004). Among natural products, essential oils (EOs) obtained from the aromatic plants have a wide variety of biological properties, including positive effects on cardiovascular diseases, inflammatory processes, and oxidative diseases (Calsamiglia *et al*., 2007), analgesic and anti-inflammatory activities (Tasleem *et al*., 2014) and have ability to scavenge free radicals, inhibit lipid peroxidation and stimulate the activity of antioxidant enzymes (Prakash *et al*., 2014). Most of the EOs having potential to inhibit lipid peroxidation would prevent the undesirable deleterious changes, loss of flavour, colour, nutrient value in food items and also the formation of toxic free radicals having undesirable effects on consumer's health (Prakash *et al*., 2011, Prakash *et al*., 2012).

Piper nigrum L. is an aromatic plant that belongs to the Piperaceae family. This plant is currently cultivated in tropical regions such as central and northern South America, and the Asia Pacific region. Among the spices, *P. nigrum* L. has been nicknamed as 'black gold' and the 'king of spices' which is the most important and most extensively consumed spice in the world. It is used in food industry as a taste and flavor enhancing agent. *P. nigrum* L. is known

and prized for its pungency and flavor which is attributed to the alkaloid piperine and the essential oil respectively (Ravindran & Kallupurackal 2004). Harvesting commences based mainly on the visual appearance of the berries. When a couple of berries in a spike turn orange or red in color, the immature spikes harvested tend to produce black pepper. At the same time white pepper is a major pepper product preferred by consumers all over the world. Soaking ripe berries is done to make white pepper. The purpose of this study was to utilize different assays to investigate and compare comprehensively and systematically the profiles of antioxidant activities, and DNA damage protective effect of WEO and BEO.

MATERIALS AND METHODS

Materials and Regents: The black and white pepper (*Piper nigrum* L.) was purchased from the local market. 2, 2´-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid diammonium salt (ABTS), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), and 1,1-diphenyl-2-picrylhydrazyl (DPPH), and were purchased from Sigma Chemical Co. Other chemicals used were all of analytical grade.

Extraction of the Essential Oil: Dried black piper and white piper were grounded and passed through a 40 mesh screen respectively, adding a certain mount of water to soak them fully, then essential oil was extracted by hydrodistillation process. The extracted essential oil was was separated from water and dried over anhydrous sodium sulfate, and stored in tightly closed dark vials at 4 °C until use.

DPPH Radicals Scavenging Activity Assay: DPPH radical scavenging activity was determined according to the method of Xu et al. (2012). The 2.5 mL of 0.6 mM DPPH ethanol solution was added to 0.5 mL of sample solution of different concentrations to make the test solutions; The negative control (blank) consisted of 2.5 mL of DPPH solution plus 0.5 mL of ethanol. These solutions were allowed to react at room temperature for 30 min in the dark, and the absorbance was measured at 517 nm. The scavenging rate of DPPH radical was calculated according to the formula given below: DPPH radical scavenging ability (%) = $[A_0-(A_1-A_2)]/A_0\times100$, where A_0 is the absorbance of the control in which ethanol substitutes extract and A_1 is the result of the mixture of the extract and DPPH radicals while A_2 is the absorbance of the mixture of the extract and 2.5 mL ethanol to eliminate the color effect of the extract. The DPPH radical scavenging activity of was measured by IC_{50} value which represents the effective concentration of the extract at which DPPH radical scavenging ability up to 50%.

ABTS Free Radicals Scavenging Activity Assay: This assay based on the inhibitionby antioxidants of the absorbance of the free radical cation from ABTS. ABTS was incubated with potassium persulfate in order to produce the free radical cation (ABTS). In brief, ABTS was dissolved in deionized water to make a 7 mmol/L concentration solution. ABTS was produced by mixing ABTS stock solution with 2.45 mmol/L potassium persulfate (final concentration) and the mixture was allowed to stand in the dark at room temperature for 12-16 h before use. In our study, the ABTS solution was diluted with ethanol, to an absorbance of 0.70 (±0.02) at 734 nm. After addition of 3.8 mL of diluted ABTS to 100 µL of

essential oil, the absorbance reading was taken exactly 6 min after initial mixing. Ethanol blank were run in each assay. Radical scavenging activity was expressed as the percentage of inhibition. IC_{50} was the effective concentration at which 50% of ABTS was scavenged.

Reducing Power Assay: The reducing powers of the samples were determined according to the method described by Chung et al. (2005). A 0.1 mL aliquot samples are mixed with an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide, and then incubated at 50 ^{o}C for 20 min. 0.25 mL of 1% trichloroacetic acid was added to the mixture to stop the reaction, and then the mixture was centrifuged at 3000 g for 10 min. The supernatant (0.25 mL) was mixed with 0.25 mL distilled water and 0.1% $FeCl_3$ (0.5 mL) and then the absorbance was measured at 700 nm. The reducing powers of the tested samples increased with the absorbance values.

DNA Damage Protective Effect Assay: The ability of samples to protect supercoiled pBR322 plasmid DNA against H_2O_2 was estimated with the DNA nicking assay as described by Xu et al. (2012). The reaction mixtures (15 μL) contained 5 μL of phosphate buffer saline (PBS, 10 mM, pH 7.4), 1 μL of plasmid DNA (0.5 μg), 5 μL of the samples, 2 μL of 1 mM $FeSO_4$, and 2 μL of 1 mM H_2O_2 and were incubated at 37 °C for 30 min. After incubation, 2 μL of a loading buffer [50% glycerol (v/v), 40 mM EDTA, and 0.05% bromophenol blue] was added to stop the reaction, and the reaction mixtures were electrophoresed (DYCP-31A Agarose Electrophoresis Instrument, Beijing Liuyi Instrument Factory, Beijing, China) on 1% agarose gel containing 0.5 μg/mL ethidium bromide in Tris/acetate/EDTA gel buffer for 60 min (60 V), and the DNA in the gel was visualized and photographed under ultraviolet light. The protective effect was expressed as a percentage content of the supercoiled form of plasmid DNA treated with samples in untreated plasmid DNA.

Statistical Analysis. All experiments were conducted three times independently. Correlation coefficient, regression analyzes, one-way analysis of variance (ANOVA) and a Duncan's test that is used for determine significant differences ($p < 0.05$) between the means were carried out by Data Processing System (DPS, version 7.05) and EXCEL program.

RESULTS AND DISCUSSION

DPPH Radical Scavenging Activity: The DPPH method is faster than other method and it can be helpful in investigation of novel antioxidants for a rapid estimation and preliminary information of radical scavenging abilities. The method is sensitive and requires small sample amounts. The scavenging activity assayed herein on DPPH radicals is shown in Table 1. The IC_{50} values of BEO and WEO were estimated at 1335.8 and 10.9 mg/mL respectively, indicating that the DPPH radicals scavenging activity of WEO were much higher than that of BEO. For BEO and WEO, they showed a concentration-dependent scavenging of the DPPH radicals at some concentrations, which was also consistent with the previous reports (Gabriel *et al*., 2006; Wang *et al*., 2009).

Table 1. DPPH and ABTS Radicals Scavenging Activity of BEO and WEO

	DPPH		ABTS	
sample	regression equation	IC_{50} (mg/mL)	regression equation	IC_{50} (mg/mL)
BEO	y=0.0232x+19.01 R^2=0.9939	1335.8±26.1 a	y=0.0795x+32.195 R^2 = 0.9945	223.8±14.2 a
WEO	y=1.5397x+33.224 R^2 = 0.9934	10.9±0.3 b	y=1.0441x+19.828 R^2 = 0.9911	28.9±2.1 b

Values represent means of three independent replicates ± SD. R^2 refer to the regression coefficients. Different letters within a column indicate statistically significant differences between the means ($p < 0.05$) for BEO and WEO.

ABTS Radicals Scavenging Activity: The scavenging activity of BEO and WEO on ABTS radicals is shown in Table 1. The profile of scavenging activity of Eos on ABTS was similar to the result of the scavenging activity on DPPH radicals. Somewhat differently, the IC_{50} values on scavenging ABTS radicals were 223.8, 28.9 mg/mL for BEO and WEO, respectively, and the scavenging activity of WEO was significantly higher than that of BEO. Similarly, ABTS radicals scavenging activity of BEO and WEO increased dose-dependently at concentrations, which may be attributable to its hydrogen-donating ability. These differences in data between DPPH and ABTS assays were likely due to different experimental conditions.

Reducing Power Assay: The reducing power assay of EOs, In fact, the presence of reductants in samples causes the reduction of Fe^{3+}/ferracyanide complex to ferrous form (Yildirim *et al*., 2000). Therefore Fe^{2+} complex can be monitored by measuring the formation of perl's percussion blue at 700 nm (Chung *et al*., 2002). Figure. 1 revealed that the reducing power ability of the EOs at various concentrations. It can be observed that their reducing power increased with the increasing of the concentration of EOs (20-100 mg/mL). A difference in the reducing power is observed between the BEO and WEO at same concentration, which implied the WEO possessed a higher antioxidant activity at the same concentration.

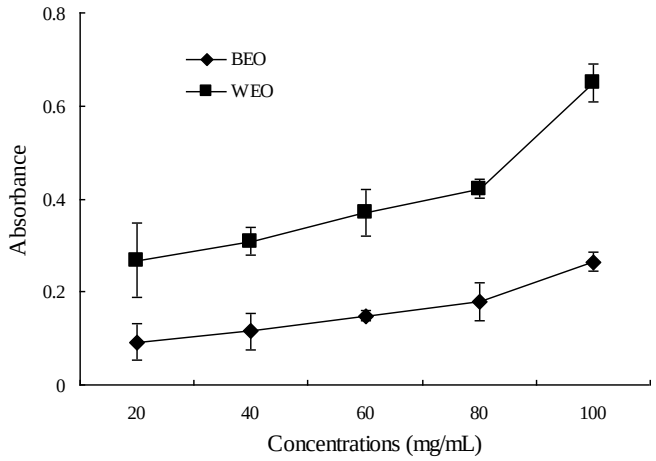

Figure 1. The reducing power of WEO (■)and BEO (♦)

DNA Damage Protective Effect: The efficiency of different EOs in preventing oxidative damage of DNA induced by H_2O_2 was also evaluated, and the result is shown in Figure 2. The formation of circular form of DNA is indicative of single strand breaks, and the formation of linear form of DNA is indicative of double strand breaks (Singh *et al*., 2009). The plasmid DNA was mainly of the supercoiled form in the absence of Fe^{2+} and H_2O_2 (Figure 2, lane 1). During the addition of Fe^{2+} and H_2O_2, the supercoiled form of DNA converted into the open circular and linear forms (Figure 2, lane 2), indicating that hydroxy radicals generated from iron-mediated decomposition of H_2O_2 produced both single strand and double strand DNA breaks. From the gel analysis, the protection offered against DNA damaged by EOs was concentration dependent. At some concentration (6.25-100 mg/mL), the DNA damage protective effect of WEO ranged from 8.1% to 65.6%. However, the BEO ranged from 62.5-1000 mg/mL this is quite achievable, which indicated the WEO had stronger protective effect on DNA damage than BEO. These results showed that EO from pepper might prevent the reaction of Fe ions with H_2O_2, on the other hand, and it probably quenched hydroxy radicals by donating hydrogen atoms or electrons, therefore protecting the supercoiled plasmid DNA from hydroxy radical-dependent strand breaks (Singh *et al*., 2009).

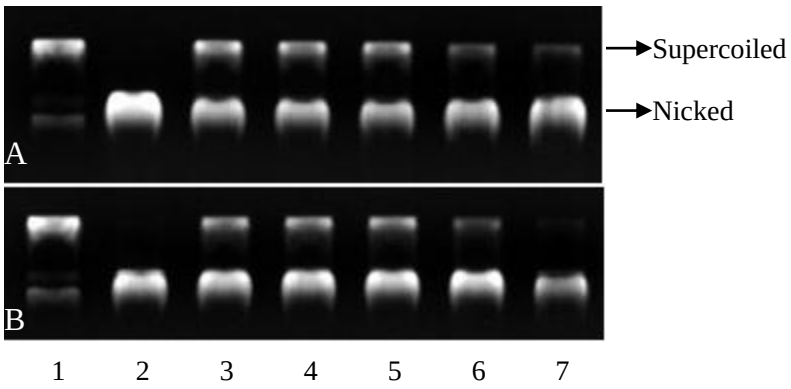

Figure 2. DNA damage protective effect of WEO (A) and BEO (B). Lane 1, the native DNA; lane 2, the DNA treated with 1 mM $FeSO_4$ and 1mM H_2O_2; Figure 2A: lanes 3−7, the DNA

treated with 1 mM $FeSO_4$ and 1 mM H_2O_2 and treated with 100, 50, 25, 12.5, 6.25 mg/mL respectively; Figure 2B: lanes 3−7, the DNA treated with 1 mM $FeSO_4$ and 1 mM H_2O_2 and treated with 1000, 500, 250, 125, 62.5 mg/mL respectively.

CONCLUSIONS

In conclusion, this work showed that the WEO and BEO studied exhibited antioxidant activities and DNA damage protective effect to various extents. On the whole, WEO possessed better antioxidative activities, which was caused by differences in EOs compositions, indicating that processing and maturity of pepper had great influences on the EOs from pepper.

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