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Antioxidant activity of bovine and porcine meat treated with extracts from edible lotus (*Nelumbo nucifera*) rhizome knot and leaf

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ABSTRACT

Lotus (*Nelumbo nucifera*) is an extensively cultivated vegetable in eastern Asia, particularly in China. Both lotus rhizome knot (LRK) and lotus leaf (LL) are waste products of the lotus industry. Extracts from LRK and LL are proposed as antioxidants for meat. Porcine and bovine ground meat samples were subjected to three treatments: CONTROL (with no additives), LRK (lotus rhizomes knot extract 3% w/w), and LL (lotus leaf extract 3% w/w). Raw and cooked samples were stored at 4 °C and the antioxidant activity was determined at 1, 3, 6 and 10 days. Antioxidant activity was significantly increased in all meat samples with the addition of both LRK and LL, but LRK was more effective against lipid oxidation. The results show the potential for using LRK and LL extracts in the meat industry to prolong shelf life.

1. Introduction

Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone (TBHQ) have been widely used by the food industry because they are both powerful and inexpensive. However, BHA (Sherwin, 1990) and BHT (Chen, Pearson, & Gray, 1992; Sun & Fukuhara, 1997) are suspected carcinogens, and consumer concern has led to a decrease in their use in the food industry (Namiki, 1990). Vitamins in meat also play a role in preventing lipid oxidation. However, while vitamin oxidation may protect fatty acids from oxidation, the nutritional value of meat can be negatively affected by a general reduction in the availability of vitamins A, D, E and C (Madhavi, Deshpande, & Salunkhe, 1996).

For the food industry, the use of natural antioxidants can be advantageous because natural products do not require extensive safety testing prior to use. Examples of natural products include oregano, sage (Fasseas, Mountzouris, Tarantilis, Polissiou, & Zervas, 2007), rosemary (Sebranek, Sewalt, Robbins, & Houser, 2005), black pepper (Martínez, Cilla, Beltrán, & Roncalés, 2007), tea (Bañón, Díaz, Rodríguez, María, & Alejandra, 2007), and chickpea flour (Verma, Ledward, & Lawrie, 1984). However, natural products are often more expensive and less effective than synthetic antioxidants. As a result, special attention has been focused on the extraction of antioxidants from inexpensive or residual sources from agricultural industries, such as potato peel (Singh & Rajini, 2008), grape seeds (Brannan, 2009), citrus peel and seeds (Bampidis & Robinson, 2006), carrot waste (Eim, Simal, Rosselló, & Femenia, 2008) and tea leaves (Farhoosh, Golmovahhed, & Khodaparast, 2007).

Lotus (*Nelumbo nucifera*) is an ornamental plant that is also a dietary staple in eastern Asia, particularly in China. The rhizome (lotus roots) knot is a common vegetable used as a traditional herbal remedy to stop bleeding. Lotus rhizome knots (LRK) contain high levels of polyphenolic compounds and distinctive antioxidative properties in comparison with commonly used plant extracts (Hu & Skibsted, 2002). Lotus leaf (LL) contains several flavonoids (Mukherjee, Mukherjee, Maji, Rai, & Heinrich, 2009) and leaf extracts showed significant antioxidant effects in previous studies (Huang et al., 2010). Lotus leaves have long been used in China to package meat to prolong shelf life and improve taste.

Both LRK and LL are reported to possess significant antioxidant activity due to the presence of polyphenolic compounds (tannins and flavonoids) (Editor Committee of National Chinese Medical Manage Bureau, 1999; Liu, Hou, Jin, Guo, & Wang, 2009; Mukherjee et al., 2009). However, to the best of our knowledge, no investigation has addressed the use of LRK and LL as readily available and inexpensive antioxidants in meat and meat products. Thus, the objective of this work was to assess the antioxidant activity of LRK and LL in both raw and cooked porcine and bovine meat. In addition, the main polyphenolic compounds in the two extracts were investigated.

2. Materials and methods

2.1. Chemicals

Standards (i.e., gallic acid and rutin) were purchased from the National Institute for the Control of Pharmaceutical and Biological

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Products (Beijing, PR. China). It was reported that tannins and flavonoids are the main polyphenolic compounds in LRK and LL, respectively. (Editor Committee of National Chinese Medical Manage Bureau, 1999; Liu et al., 2009; Mukherjee et al., 2009). Gallic acid was used as a standard for the determination of total tannins and rutin was used as a standard for the determination of total flavonoids. α,α-diphenyl-β-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid (ABTS) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were 'AnalaR' grade, obtained from China Medicine (Group) Shanghai Chemical Reagent Corporation (Shanghai, PR. China).

2.2. Preparation of the extracts

Minced fresh rhizome knots and dried leaves of *N. nucifera* were extracted with distilled water at room temperature overnight. The extracts were filtered, and the solvent removed by rotary evaporation at 35 °C. The residues were evaporated to dryness *in vacuo* and the resulting dry powder was stored at 4 °C. The yield of LRK and LL extracts were 6.6 ± 0.3% and 10.3 ± 0.6%, respectively.

2.3. Determination of total phenolics, tannins, and flavonoids in the extracts

Total phenolic content (TPC) of LRK and LL extracts (0.1 g for each) were determined using the Folin–Ciocalteau assay (Zoecklin, Fugel-sang, Gump, & Nury, 1995). Results were expressed as grams of gallic acid equivalents per 100 g of dry extract.

Total tannin content (TTC) of LRK and LL extracts (0.1 g for each) were estimated gravimetrically as the difference between total phenolics and phenolics remaining after binding the tannins with polyvinyl polypyrrolidone (PVPP) according to Makkar, Blummel, Borrowy, and Becker (1993). The concentrations of total tannins were expressed as grams of gallic acid equivalents per 100 g of dry extract.

Total flavonoid content (TFC) of LRK and LL extracts (0.1 g for each) was determined by colorimetric assay (Moreno, Isla, & Sampietro, 2000). A calibration curve was generated with rutin as the reference compound, and the results were expressed as grams of rutin equivalents per 100 g of dry extract.

2.4. Chemical composition analysis by HPLC-DAD

HPLC analysis of LRK and LL extracts (0.1 g for each) was accomplished using an Agilent-1100 HPLC system with a diode array detector (Agilent Technologies, Waldbronn, Germany), equipped with a quaternary pump, vacuum degasser, autosampler, and column heater-cooler (Agilent Technologies, Waldbronn, Germany). Spectroscopic data from all peaks were collected in the range of 200–400 nm, and chromatograms were recorded at 265 nm and 350 nm. The chromatographic separation was performed on a reversed-phase C18 analytical column (3 μm, 150 mm × 4.6 mm i.d., Advanced Chromatography Technologies, Aberdeen, U.K.) with the column temperature set at 45 °C. A linear gradient elution of water, containing 1% acetic acid (A) and MeCN (B) was used (0–25 min, solvent A was decreased from 100% to 0%, then solvent A was increased to 100% and held constant for 5 min). The flow rate was 0.4 ml/min, and the injection volume was 20 μl.

2.5. Preparation of meat samples

Fresh porcine (*Biceps femoris*) and bovine (*Biceps femoris*) samples (1 kg) from one pig and cattle were obtained from the local market and visible fat was removed. Samples from each meat were then divided into three groups and were homogenized with 3% w/w of either LRK or LL in a multi-functional food blender (BSS260-D6, Baoshi Electrical Co., Ltd., Foshan, China). No extract was added to the control samples (CONTROL). All samples (3 g, 5 cm diameter and 0.7 cm thick) were packed in polyethylene film from a local market and stored at 4 °C in darkness for 24 h and then tested for antioxidant activity (day 1). After testing, each sample was split into two. One portion remained in the raw state and the other was cooked at 85 °C in an oven (DGX-9143-B-1, Shanghai, China) for 30 min (Fasseas et al., 2007). Both raw and cooked samples were assayed for antioxidant activity as described below at 3, 6 and 10 days of storage at 4 °C in darkness, in a polyethylene film.

2.6. Assessment of lipid oxidation

The TBA assay followed the method of Fasseas et al. (2007) with slight modification. In brief, 0.03 g of sample was mixed with 0.6 ml deionised H_2O, 0.9 ml of phosphoric acid (pH 2.0) and 0.9 ml 0.8% (w/w) of thiobarbituric acid (TBA) in 1.1% (w/w) sodium dodecyl-sulfate (SDS) in a test tube, and then vortexed and heated at 100 °C for 60 min in a water bath. After cooling, butan-1-ol (3 ml) was added and the solution mixed. Samples were then centrifuged at 8960 × g for 10 min (TGL-16C, Anting Scientific Instrument Factory, Shanghai, China). The absorbance of the upper layer was determined at 532 nm (UV-1240, Shimadzu, Japan). Butan-1-ol was used as blank. The results were expressed as TBA values (the relative absorbance of each sample on day 1).

2.7. DPPH free radical scavenging activity

The hydrogen atom or electron-donating ability of the meat samples was measured from the bleaching of a purple-colored methanolic solution of DPPH (Gulluce et al., 2007). Free radical scavenging activity was evaluated by the DPPH assay using the method of Tepe et al. (2005). Samples (0.03 g) were constantly mixed with 3 ml of 0.004% DPPH in methanol in a test tube at room temperature for 30 min. The samples were centrifuged at 1430 × g for 10 min (TGL-16C, Anting Scientific Instrument Factory, Shanghai, China). Absorbance of the supernatants was measured at 517 nm (UV-1240, Shimadzu, Japan), using methanol as blank. Measurements were expressed as absorbance and decreasing values indicated increasing antioxidant activity.

2.8. ABTS free radical scavenging activity

Antioxidant activity of the samples was also measured using an improved ABTS (azinobisethylbenzthiazolinesulfonic acid) procedure (Re et al., 1999). The ABTS radical cation ($ABTS^+$) solution was prepared through the reaction of 7 mM ABTS and 2.45 mM potassium persulphate, after incubation at room temperature, in the dark for 16 h. The $ABTS^+$ solution was then diluted with 80% ethanol to obtain an absorbance of 0.700 ± 0.005 at 734 nm. $ABTS^+$ solution (3 ml; absorbance 0.700 ± 0.005) was added to 0.03 g of the samples and mixed vigorously (TS-1, Kylin-Bell Lab Instruments Co.,Ltd., Shanghai, China). The reaction mixture was allowed to stand at 30 °C for 30 min before centrifugation at 1430 × g for 10 min (TGL-16C, Anting Scientific Instrument Factory, Shanghai, China). The supernatant's absorbance at 734 nm (UV-1240, Shimadzu, Japan) was immediately recorded. Lower absorbance levels indicated higher antioxidant activity.

2.9. Reducing power assay

The potassium ferric cyanide reduction method measures the color change of the test solution from yellow to various shades of green and blue depending upon the reducing power of each sample. Meat samples (0.03 g) were mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferric cyanide. The mixture was incubated at 50 °C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid (w/v) was added and the mixture was centrifuged