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Evaluation of the antioxidant activities and nutritional properties of ten edible plant extracts and their application to fresh ground beef

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ABSTRACT

In this study, we assessed the antioxidant efficacy and nutritional value of 10 leafy edible plants and evaluated their potential as natural antioxidants for meat preservation. We measured total phenolic content, 2,2-diphenyl-1-picryl-hydrazil (DPPH) radical scavenging activity, and vitamin C, chlorophyll, and carotenoid contents of 70% ethanol and water extracts of the edible plants. Based on these results, we investigated the effects of butterbur and broccoli extracts on lipid oxidation in ground beef patties. Plant extracts and butylated hydroxytoluene (BHT) were individually added to patties at both 0.1% and 0.5% (w/w) concentrations. Thiobarbituric acid reactive substance (TBARS) values and color parameters were tested periodically during 12 days of refrigerated storage. TBARS levels were significantly lower ($p \leq 0.05$) in the samples containing plant extracts or BHT than the non-treated control. In addition, the beef patties formulated with the selected plant extracts showed significantly ($p \leq 0.05$) better color stability than those without antioxidants. These results indicate that edible plant extracts are promising sources of natural antioxidants and can potentially be used as functional preservatives in meat products.

1. Introduction

Lipid oxidation is a major cause of quality deterioration in meat and meat products because it leads to color alteration, off-flavor, and loss of nutrients, all of which are major determinants of meat quality (Chan, Decker, & Means, 1993). Lipid oxidation is promoted by diverse factors such as heat, light, metal ions, heme (in meat), oxygen, free radicals, and oxidative enzymes (Barbut, Josephson, & Mauer, 1985; Buckley et al., 1989). Color is another important factor that influences the quality and acceptability of meat. Color is regarded as an indicator of perceived quality and freshness of meat and is the first limiting factor in the shelf-life of meat (Smith, Belk, Sofos, Tatum, & Williams, 2000). Discoloration of meat is closely related to oxidative denaturation of meat pigments (Kanner & Harel, 1985).

One way to prevent oxidative deterioration is to use antioxidants in meat products (Lee et al., 2010). These antioxidants can retard lipid oxidation by acting as free radical scavengers, oxygen scavengers, and/or metal chelators (Teets & Were, 2008). Various synthetic antioxidants, such as BHT, butylated hydroxyanisole (BHA), and tertiary butyl hydroquinone (TBHQ), are used in the food industry to delay lipid oxidation (Mansour & Khalil, 2000). However, concerns have been raised about using synthetic antioxidants due to their possible side-effects, which has given impetus to finding alternative "natural" antioxidants (Sebranek, Sewalt, Robbins, & Houser, 2005).

Most natural antioxidants are obtained from plant resources including culinary herbs, spices, fruits, vegetables, and oilseed products (Shahidi & Zhong, 2010). Many previous studies have focused on herbs and spices consumed in Europe, Southern Asia, and Southeast Asia. Several herbs and spices, such as clove (Naveena, Muthukumar, Sen, Babji, & Murthy, 2006), rosemary (Georgantelis, Blekas, Katikou, Ambrosiadis, & Fletouris, 2007), and oregano and sage (Fasseas, Mountzouris, Tarantilis, Polissiou, & Zervas, 2007) have been reported to significantly improve the keeping quality of meat products, and are effective in delaying lipid oxidation. However, their strong flavor is unfamiliar to East Asians, which limits their applications to foods. Therefore, in this study, we attempted to explore new natural antioxidant sources suitable for East Asians. We selected several leafy, green, and edible plants commonly consumed in Northeast Asian regions. These culinary plants have been eaten for centuries and their safety and edibility are recognized by the Korean Food and Drug Administration (KFDA). They have a milder flavor than herbs and spices and are reported to be good for health. They are often used in salads or as side dishes, either fresh or blanched.

Phenolic compounds are the major constituents of plants that contribute to their antioxidant capacity. Phenolic compounds include phenolic acids, flavonoids, and tocopherols (Wong, Hashimoto, & Shibamoto, 1995). Several studies have demonstrated that phenolic compounds scavenge free radicals. Therefore, the total amount of

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phenolic compounds is one of the most important factors affecting antioxidant activity (Møller, Madsen, Aaltonen, & Skibsted, 1999).

There are also many other compounds that have functional and nutritional value in edible plants, such as ascorbic acid, nitrogen compounds (amino acids, amines, alkaloids, and chlorophyll derivatives), and carotenoids (Hall & Cuppett, 1997; Hudson 1990). These compounds play a role as nutrients, bioactive substances, as well as antioxidants. Ascorbic acid, also known as vitamin C, is a strong antioxidant found mainly in fresh fruits and vegetables (Gardner, White, Mcphail, & Duthie, 2000). Vitamin C retards free radical-induced cellular damage, and acts as a co-factor of several biosynthesis reactions in the human body (Chan, 1993). Chlorophylls and carotenoids are the most abundant plant pigments in nature, and have antioxidant activity due their singlet oxygen quenching properties (Hirayama, Nakamura, Hamada, & Kobsyasi, 1994; Tanielian & Wolff, 1988). Some carotenoids, such as α- and β-carotene, are precursors of vitamin A (Harrison, 2012). Chlorophyll and its derivatives may prevent certain types of cancers, aid in wound healing, and reduce inflammation in some cases (Morgan, Jackson, Zheng, Pandey, & Pandey, 2010; Smith & Livingston, 1945; Subramoniam et al., 2012).

The antioxidant activities of edible plants have been explored by several research groups. However, it is difficult to compare the antioxidant capacity of these plant materials between studies because of different methods of extraction and antioxidant activity determination. Moreover, the nutritional values of these plants were rarely evaluated in these previous studies. Our objectives in this study were therefore to (1) evaluate the antioxidant activities and nutritional properties of 70% ethanol and water extracts of 10 edible plants; and (2) to determine the effectiveness of these extracts in preventing or reducing lipid oxidation as well as color changes in ground beef patties during storage at a chilled temperature (4 °C).

2. Materials and methods

2.1. Edible plants

Fresh leaves of crown daisy (*Chrysanthemum coronarium* var. spatiosum), pumpkin (*Cucurbita moschata* Duch.), chamnamul (*Pimpinella brachycarpa* (Kom.) Nakai), fatsia (*Aralia elata*), leek (*Allium tuberosum*), bok choy (*Brassica campestris* var. chinensis), acanthopanax (*Acanthopanax sessiliflorum* Seeman), butterbur (*Petasites japonicus*), soybean (*Glycine max* L. Merr), and the flower heads of broccoli (*Brassica oleracea* L. var. *italica* Plenck) were purchased from local farms during the harvest season in April. Although broccoli is not a leafy edible plant, it was used for comparison with other plant materials because of its well-documented antioxidant activities (Aldrich et al., 2011). The edible plants were washed and dehydrated using an electric food dehydrator (Lequip Co., Hwaseong, Korea) at 65 °C for 24 h. Each edible plant was finely pulverized using an electric grinder (Daesung Artron Co., LTD., Seoul, Korea), weighed, and then stored at −20 °C until extraction.

2.2. Preparation of edible plant extracts

Extracts were prepared using 70% (v/v) ethanol and water as solvents. Ethanol 70% (v/v) was added to the finely ground plant powders at a ratio of 1:20 (w/v) and the mixture was stirred at 200 rpm using an overhead stirrer (Mtops Co., Seoul, Korea) for 24 h at room temperature. Water extracts were also prepared using distilled–deionized water (1:20, w/v) with stirring for 1 h at 95−100 °C. The extracts were then separated from the residue by filtration through Whatman no.1 filter paper (Whatman International Ltd., Maidstone, U.K.) and the filtrate was concentrated under reduced pressure at 55 °C using a rotary evaporator (Büchi Laboratory Equipment, Postfach, Switzerland). The extra solvent was removed by freeze-drying and the dried extract powder was stored at −20 °C until further analyses.

2.3. Antioxidant content and antioxidant activity

2.3.1. Total phenolic content

The content of total phenolics was measured spectrophotometrically using the Folin–Ciocalteu colorimetric method (Dewanto, Wu, Adom, & Liu, 2002). All plant extracts were diluted with extraction solvent (70% ethanol or distilled–deionized water) to obtain readings within the standard curve range of 0.0−0.8 mg gallic acid/mL. Briefly, 100 μL of diluted plant extract or gallic acid standard solution was mixed well with 2 mL of 2% (w/v) Na_2CO_3 solution. The mixture was then left to stand for 3 min, after which 100 μL of Folin–Ciocalteu reagent was added. After letting the mixture sit for 30 min at room temperature for color development, the absorbance was measured at 750 nm using a UV–visible spectrophotometer. Results are expressed as mg gallic acid equivalent (GAE)/g dried plant.

2.3.2. DPPH radical scavenging activity

The free radical scavenging activity of extracts was measured by the DPPH method as proposed by Brand-Williams et al. (1995). A solution of DPPH in methanol (0.078 mg/mL) was prepared and 0.25 mL of this radical solution was added to 0.05 mL of sample solution. The mixture was incubated for 30 min in the dark at room temperature and then the absorbance was measured at 517 nm with a spectrophotometer. Ascorbic acid solutions in the concentration range of 0.02−0.08 mg/ml were used to establish a standard curve. DPPH radical scavenging activity was expressed as mg ascorbic acid equivalent (AAE)/g dried plant.

2.4. Nutritional value

2.4.1. Vitamin C content

Vitamin C content was determined by the titrimetric method as described in the Association of Official Analytical Chemists (AOAC, 1995) method no. 967.21 (1995). Dried plant powder (2 g) was magnetically stirred in 25 mL of extract solution (metaphosphoric acid: acetic acid = 1:5). The homogenate was centrifuged at 12,000 rpm for 5 min, and the supernatant was filtered through Whatman no. 4 filter paper. Aliquots (2 mL) were then placed in test tubes, after which 200 μL of indophenol and 2 mL of thiourea metaphosphoric solution were added. After that, 1 mL of 2,4-dinitrophenyl hydrazine (DNP) solution was mixed with the sample solution and the mixture was allowed to stand at 37 °C for 3 h and then cooled on ice. Next, 5 mL of 85% H_2SO_4 solution was added, and the resulting mixture was incubated in the dark for 30 min at room temperature. Absorbance was measured at 540 nm and vitamin C content was expressed as mg ascorbic acid (AAE) equivalent/g of plant on a dry weight basis.

2.4.2. Total chlorophyll and total carotenoid contents

The content of chlorophylls *a* and *b*, as well as that of total carotenoids, was spectrophotometrically determined using the method of Lichtenthaler (1987). Dried edible plant samples (1 g) were stirred in 50 mL of 80% acetone (v/v) solution in the dark for 24 h at room temperature. After filtration (Whatman no. 4 filter paper), the filtrate volume was adjusted to 100 mL with 80% acetone (v/v). Absorbance was read at 662, 644, and 470 nm to measure the content of chlorophyll *a*, chlorophyll *b*, and carotenoids, respectively. Total chlorophyll was calculated as the sum of chlorophylls *a* and *b*. Total chlorophyll and total carotenoid contents were expressed as mg/100 g of plant on a dry weight basis.

2.5. Effects on lipid oxidation and color of beef patties

2.5.1. Determination of fat content in ground beef

Fresh raw beef was obtained from a local supermarket and prepared using separable lean from top round roasts. On the day of purchase, the roasts were trimmed of all separable fat and connective