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Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation

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ABSTRACT

Leafy green vegetables (LGV) are rich in phenolic compounds that have a wide range of biological functions, including antioxidant and antimicrobial activities. Our first goal was to evaluate the antioxidant and antimicrobial activities of 70% ethanolic extracts of ten LGV commonly consumed in East Asia. To determine antioxidant activity, we measured total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, 2,2-azinobis-3 ethyl benxothiazoline-6-sulphonic acid (ABTS) cation decolorization activity, and reducing power. For the analysis of antimicrobial activity, the inhibitory effects of the extracts against *Escherichia coli*, *Salmonella enterica*, *Shigella flexneri*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus subtilis* were evaluated using agar well diffusion and broth-microdilution tests. Among all LGV, extracts of chamnamul (*Pimpinella brachycarpa*) and fatsia (*Aralia elata*) exhibited outstanding antioxidant and antimicrobial properties, and we thus investigated the influences of these extracts on lipid oxidation and microbial criteria in raw beef patties. The extracts and butylated hydroxytoluene (BHT, a positive control) were individually added to patties at both 0.1% and 0.5% (w/w) concentrations and the patties were stored at 4 °C for 12 days. The color parameters and thiobarbituric acid reactive substances (TBARS) values were monitored periodically, and a microbial analysis was performed. The addition of extracts and BHT resulted in concentration-dependent decreases in TBARS values and in the number of microorganisms in the beef patties and also improved meat color stability. The fatsia extract had more effective antioxidant and antimicrobial activities than the chamnamul. We conclude that extracts of LGV, especially fatsia, have potential as natural preservatives for meat products.

1. Introduction

LGV are widely consumed in East Asia and are prepared using various cooking methods. These vegetables have been eaten for centuries and are classified as GRAS (Generally Recognized As Safe). An abundance of research has shown that fresh LGV contain important functional food components, such as β-carotene, ascorbic acid, riboflavin, and folic acid, as well as minerals (Grusak & DellaPenna, 1999). In particular, these foods contain a large amount of polyphenols (e.g., phenolic acids, flavonoids, and aromatic compounds), the most abundant phytochemicals in the human diet. LGV are also known for their characteristic color, flavor, and therapeutic value (Faller & Fialho, 2009; Gupta, Jyothi Lakshmi, Manjunath, & Prakash, 2005). Their bioactive substances and phytonutrients have a wide range of biological functions, including antioxidant and antimicrobial activities (Burt, 2004; Gutierrez, Barry-Ryan, & Bourke, 2008). Some epidemiological evidence has shown that consumption of fruits and vegetables with high levels of natural antioxidants helps to prevent chronic diseases, such as cardiovascular diseases and cancer (Alia, Horcajo, Bravo, & Goya, 2003). Consequently, LGV have received substantial attention from researchers in recent years as a potential source of natural antimicrobial and antioxidant agents.

Fresh meat products are usually marketed at refrigerated temperatures (2–5 °C). Meat products typically spoil during refrigeration due to two major causes: microbial growth and oxidative rancidity (Sebranek, Sewalt, Robbins, & Houser, 2005). Lipid oxidation is initiated by the abstraction of a hydrogen atom in unsaturated fatty acids and propagated as a radical-mediated chain reaction (Vayalil, 2002). The grinding of meat disrupts the integrity of muscle membranes and exposes lipid membranes to metal ions, which facilitates interactions between prooxidants and unsaturated fatty acids. Furthermore, meats can be contaminated with microorganisms during the butchering or manufacturing process,

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though the tissues of healthy animals should be sterile at the time of slaughter (Gill, 1979). These microorganisms bring about undesirable quality changes in meats, especially with respect to lactic acid bacteria, a major bacterial group associated with meat spoilage (Egan, 1983). *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica* and some of *Escherichia coli* are known as common foodborne pathogenic bacteria and are frequently isolated from meat and meat products (Borch & Arinder, 2002).

Lipid oxidation and microbial growth during storage can be reduced by applying antioxidant and antimicrobial agents to the meat products, leading to a retardation of spoilage, extension of shelf-life, and maintenance of quality and safety (Devatkal & Naveena, 2010). Many synthetic preservatives, such as BHT, butylated hydroxyanisole (BHA) and propyl gallate (PG), are typically used to protect foods from spoilage, although their use is restricted due to possible carcinogenic effects. Therefore, there has been increasing interest in alternative additives from natural sources, which has gradually provided impetus to eliminating synthetic preservatives in food (Parke & Lewis, 1992; Shan, Cai, Brooks, & Corke, 2009).

As opposed to synthetic compounds, natural preservatives obtained from edible plants are rich in phenolic compounds, as mentioned above, and can enhance overall quality through decreases in lipid oxidation and microbial growth. However, many LGV are only available seasonally, and the scientific literature contains little information regarding their nutrient contents and bioactivities.

The objective of this study was to evaluate the antioxidant and antimicrobial activity of ten LGV extracts *in vitro*. Based on these *in vitro* results, some extracts with higher antioxidant and antimicrobial ability were selected and applied to raw beef patties. Then, we aimed at determining the reduction of microbial growth in the meat products, monitoring the color changes of the extract-treated patties, and analyzing the antioxidant activities of the plant extracts by TBARS assay during storage.

2. Materials and methods

2.1. Plant materials

Fresh leaves of butterbur (*Petasites japonicus* Maxim), chamnamul (*Pimpinella brachycarpa* (Kom.) Nakai), bok choy (*Brassica campestris* L. ssp. *chinensis*), Chinese chives (*Allium tuberosum* Rottler ex Spreng), crown daisy (*Chrysanthemum coronarium* L.), fatsia (*Aralia elata* Seem), pumpkin (*Curcubita moschata* Duch.), sesame (*Perilla frutescens* var. *japonica* Hara), stonecrop (*Sedum sarmentosum* Bunge), and flowering head of broccoli (*Brassia oleracea* L. var. *italica* Plenk) were purchased from local farms during the harvest season in April–June in Suwon, South Korea. Although broccoli is not a LGV, it was used as a positive control due to its reported antioxidant activity. The plant stems from all plants were removed, and the leaves were washed and dehydrated in a dry oven at 65 °C for 24 h. The dried plants were then finely pulverized using an electric grinder (World Mix model DA 280-S, Daesung Artron Co., Ltd, Seoul, South Korea) and stored at −20 °C until extracted.

2.2. Chemicals and reagents

ABTS was purchased from Sigma Life Science (St. Louis, MO, USA), and DPPH and trolox were obtained from Aldrich-Chemistry (St. Louis, MO, USA). We used Folin-Ciocalteu solution and gallic acid from Sigma–Aldrich (St. Louis, MO, USA). BHT, PG, EDTA (ethylenediaminetetraacetic acid), and hydrochloric acid were obtained from DAE JUNG (Siheung, Korea). Thiobarbituric acid (TBA) was purchased from Alfa Aesar (St. Ward Hill, MA, USA).

We used tryptic soy broth (TSB) and agar; nutrient broth (NB) and agar (NA); brain heart infusion (BHI) broth and agar; plate count agar (PCA); Man, Rogosa and Sharpe (MRS) agar; violet red bile (VRB) agar; and potato dextrose agar (PDA) from Difco (Sparks, MD, USA).

2.3. Preparation of vegetable extracts

Fifty grams of each vegetable powder were soaked in 1 L of 70% (v/v) ethanol and mixed for 12 h at room temperature using an overhead stirrer (Lab Stirrer MS-280, Misung Co., Ltd, Seoul, Korea). After filtering (Whatman No. 2), the obtained extract was concentrated under reduced pressure in a water bath set at 60 °C using a rotary evaporator (Rotavapor RE121, Büchi, Fawil, Switzerland). The extra solvent was eliminated by a freeze-dryer (Heto FD 3, Heto Lap Equipment, Holten, Denmark), the residual was weighed, and the extraction yield of each plant material was calculated. The dried powder of vegetable extract was then stored at −20 °C.

2.4. TPC and antioxidant activities of vegetable extracts

2.4.1. TPC

TPC was analyzed using the Folin-Ciocalteau method of Dewanto, Wu, Adom, and Liu (2002) with some modifications. Each vegetable ethanol extract was diluted with 70% ethanol. Then, 2 ml of 2% Na_2CO_3 was added to 100 μl of the appropriately diluted sample and a standard solution of gallic acid. The solutions were left at room temperature for 5 min, after which 100 μl of 50% Folin-Ciocalteau phenol reagent was added and vortexed well. After incubation for 30 min, the absorbance was measured at 750 nm. The TPC was expressed as mg gallic acid equivalents (GAE) per g dried vegetable.

2.4.2. DPPH radical scavenging activity

Free radical scavenging activity was determined with a DPPH radical assay, performed according to the modified method described by Cheung, Cheung, and Ooi (2003). A DPPH radical solution was dissolved thoroughly with anhydrous ethanol. One ml of 0.2 mM DPPH radical solution was added to a 200 μl aliquot of sample and standard solution (5:1, v/v). After incubation in the dark at room temperature for 30 min, changes in the absorbance of the samples were analyzed at 517 nm. DPPH radical scavenging activity was expressed as mg ascorbic acid equivalents (AAE) per g dried vegetable and can estimate the abilities of several free radical scavenger molecules.

2.4.3. ABTS cation decolorization assay

ABTS radical assay was used to evaluate the ability to scavenge free ABTS radicals, based on the protocol of Re et al. (1999) with some modifications. ABTS radical cations ($ABTS^{\bullet+}$) were prepared by reacting a 7.4 mM ABTS stock solution with 2.45 mM potassium persulphate (1:1, v/v), after which the mixture was kept overnight (12–16 h) in the dark at room temperature. The ABTS radical solution was diluted with distilled water to an absorbance of 1.0–1.2. A 1 ml aliquot of the diluted sample was added to 50 μl of each of the above-prepared radical solutions and protected from light for 60 min. Absorbance readings were measured using a spectrophotometer at 734 nm. Results were expressed as mg trolox equivalents (TE) per g dried vegetable.

2.4.4. Reducing power

A 250 μl aliquot of sample and blank (control) were mixed with 250 μl of sodium phosphate buffer (200 mM, pH 6.6) and 250 μl of 1% potassium ferricyanide. The mixtures were left in a water bath at 50 °C for 20 min. The reaction was terminated by adding 250 μl of