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Article

Roasting Process Enhances Antioxidative Effect of Cinnamon (Cinnamomi Cortex) via Increase in Cinnamaldehyde Content

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Regarding chemical and biological activity changes in oriental drugs after food processing such as roasting, fermentation, and extrusion, fifty commonly-used medicinal plants were investigated. As a result, cinnamon (Cinnamomi Cortex) showed remarkably different HPLC profiles and *ca* 40% of increased antioxidative effect after being roasted. An increased peak was isolated by a reverse phase silica gel chromatography and identified as cinnamaldehyde by means of instrumental analysis including ^{1}H and ^{13}C NMR and MS. The enhanced antioxidative effect might originate from the increased content of cinnamaldehyde since this compound is one of the most well known natural antioxidants. The cinnamaldehyde content in cinnamon reached its maximum level after being roasted for 10 min at 120±2°C (108.42±0.26 mg/g extract, *ca* 137 times of increase over untreated control). Although there were no significant changes in *in vitro* biological activity such as anti-dementia, anti-hypertension or cytotoxicity, before and after the roasting process, the results suggested that simple heat treatment might improve the value of the above oriental drugs since cinnamaldehyde has been shown to possess various biological properties such as antibiotic, antioxidative, anti-diabetic, anti-influenza, and apoptosis-inducing properties.

Key words: *antioxidative effect, cinnamaldehyde, cinnamomi cortex, cinnamon, roasting process*

Food processing techniques have generally focused on promoting nutritional values and/or improving specific flavors or color in foodstuffs, changing the textures to improve palatability, sensory characteristics or organoleptic quality, and extending the storage period by inhibiting the microbiological or biochemical changes. Processing techniques are divided into two categories, thermal and non-thermal methods. Thermal processing methods include using steam or water (blanching, pasteurization, sterilization, evaporation, distillation, and extrusion), processing using hot air (dehydration and roasting or baking), processing using hot oils (frying), and processing by direct and radiated energy such as microwave, infrared, and ohmic heating [Fellows, 2000]. There is no difference between baking and roasting because roasting is bakeing and vice versa. Thermal treatment is extensively used for the preservation and preparation of food and leads to desirable changes such as protein coagulation, starch swelling, textural softening, and the formation of aroma components [Lewis, 2006]. However, heat processing also alters or destroys components responsible for their individual flavor, color, taste or texture, leads to biopolymer formation by thermal reaction, and lowers the quality and value of food [Ohlsson, 2000; Arnoldi, 2001]. The non-thermal methods include high pressure treatment, light such as ultraviolet and laser, ultrasound, and pulsed electric fields, and are used as alternatives to thermal processing [Ohlsson, 2000].

On the other hand, herbal processing techniques have a history that goes back as far as that of oriental herbal medicine. In the writings of Hwang Je Nae Gyung (The Yellow Emperor's Internal Classic, 475-221 B.C.), the processing of Pinelliae Rhizoma was already mentioned.

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Abbreviations: ACE, angiotensin-converting enzyme; APTT, activated partial thromboplastin times; BACE1, â-secretase; DPPH, 1,1-Diphenyl-2-picrylhydrazyl; HUVECs, human umbilical vein endothelial cell; NMR, nuclear magnetic resonance; PEP, prolyl endopeptidase.

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Herbal processing techniques have been used to reduce the toxicity or side effects, potentiate effects, change properties or functions, preserve active constituents, facilitate administration flavor and correct unpleasant tastes, and increase purity [Zhu, 1998]. The thermal processing methods for traditional herbal drugs were classified into several categories, that is, simple stir-roasting, stir-roasting with a solid adjuvant such as sand, soil and wheat bran or with a liquid (ginger, saline, honey or alcohol), calcining, steaming and wrap-up roasting [Lee and Kang, 1991]. The reasons and effects of processing, however, have not yet been fully elucidated chemically or pharmacologically, except in the case of a few herbal drugs.

Cinnamon (Cinnamomi Cortex) is mainly the stem bark of the *Cinnamomum cassia, C. obtssifolium,* and *C. zeylanicum* which belong to the genus Cinnamomum (Lauraceae). This medicinal food is commonly used in oriental traditional medicine for treating dyspepsia, gastritis, blood circulation disturbance, and inflammatory disease [Hsu *et al*., 1986]. From the C. Cortex, phenyl propanoids such as cinnamaldehyde and cinnamic acid, diterpenoids including cinnzeylanine, cinncassol A, B, derivatives of C and D, E and their glycosides, tannins such as procyanidin B-2, B-5, C-1 and polysaccharide, arabinoxylan had been isolated [Kimura *et al*., 1996].

This study investigated the chemical and biological activity changes in oriental drugs after processing, fifty commonly-used medicinal plants were investigated. As a result, dramatic changes were observed in HPLC chromatogram and antioxidative effect of the roasted C. Cortex compared with an unroasted sample. In this study, the compound that increased due to processing was isolated and its structure was elucidated. Several kinds of *in vitro* biological activity including DPPH assay were also compared before and after the roasting treatment.

Materials and Methods

Materials and experimental apparatus. C. Cortex purchased from a herbal medicine supplier in Daegu, Korea, was identified by Dr. Jong Hwan Kwak of Sung Kyun Kwan University, Suwon, Korea. The voucher specimen (KNUNPC-CC-08-02) is deposited at The Innovative Laboratory of Natural Products Medicine, Kyungpook National University, Daegu, Korea. Column chromatography resin (Kieselgel 60, Art. 7734) and pre-coated silica gel TLC plates (Kieselgel 60 F254, Art. 5715 and RP-18, Art. 1.15685) were purchased from Merck (Darmstadt, Germany). The MPLC columns were ODS-S-50A (11×300 mm) and ODS-S-50A (26×300 mm) purchased from Yamazen, Japan. HPLC was performed on a Jasco (Tokyo, Japan) system equipped with a PU2080 pump and a MD2010 photodiode array detector. Gemini 5 μ C18 (4.6×30 mm and 4.6×250 mm, Phenomenex, Torrance, CA) and Eclipse XDB-C 18 (4.6×250 mm, Agilent, Canta Clara, CA) were used as stationary phases. ESI-MS was estimated with API 2000 (Applied Biosystems, Foster City, CA). ^{1}H- and ^{13}C-NMR were measured on a Brucker Avance Digital 400 (400 and 100 MHz, respectively) and chemical shifts were given as δ (ppm) from an internal standard tetramethylsilane (TMS).

Roasting process. C. Cortex (1 kg) was roasted for 30 min at 190°C with a self-made roasting apparatus. The EtOH extract of roasted and unroasted C. Cortex were compared by HPLC. In order to investigate an optimal roasting condition, each 10 g sample was roasted for different times (5-60 min) at different temperatures (90-225±2°C).

Standard curve of cinnamaldehyde. The cinnamaldehyde used in this work (more than 99% under UV 280 nm in HPLC analysis) was purchased from Sigma (St. Louis, MO). The standard curve was made by taking the cinnamaldehyde concentration and the corresponding peak area in the HPLC chromatogram as functions. The calibration curve was Y=0.3189X−0.0004 (r^2=0.9953), where Y represents the peak area and X is the weight in mg. An HPLC analysis was performed on a Gemini 5 mC_{18} (4.6×250 mm) column with a gradient solvent system, by varying the proportion of the solvent A (water 99%-acetic acid 1%) to solvent B (acetonitrile 99%-acetic acid 1%). Solvent B increased 0 to 100% in 60 min at a flow rate of 0.8 mL/min. Detection was carried out under UV 280 nm. All data is presented as the mean value °æ standard deviation of triplicate experiments.

Extraction, isolation, and structure determination. To isolate an increased compound, the roasted C. Cortex (1 kg) was extracted with 95% EtOH (2×, 3 h) and the extract was evaporated to one-tenth its original mass. The concentrate was then applied to an MPLC (ODS-S-50, 26×300 mm, 50 to 100% MeCN) to obtain a pale brown sticky oil (compound **1**, 23.35 g). MS and NMR data of compound **1** was summarized as follows.

Compound **1**. ESI-MS (positive) *m/z* 133 $[M+1]^+$; ^{1}H-NMR (400 MHz, methanol-d_4) δ 9.66 (1H, *d*, *J*=8.0 Hz), 7.32 (1H, *d*, *J*=18.0 Hz), 6.16 (1H, *dd*, *J*=8.0, 18.0 Hz), 7.1-7.7 (5H, *m*); ^{13}C-NMR (100 MHz, methanol-d_4) δ 196.5, 155.5, 135.2, 132.8, 130.1, 128.2.

Bioassays. DPPH radical scavenging and anti-coagulating activity (APTT; activated partial thromboplastin times) were tested according to the reported methods [Morin and Willoughby, 1975; Blois, 1985]. The inhibitory activities toward prolyl endopeptidase, β-secretase, and angiotensin-

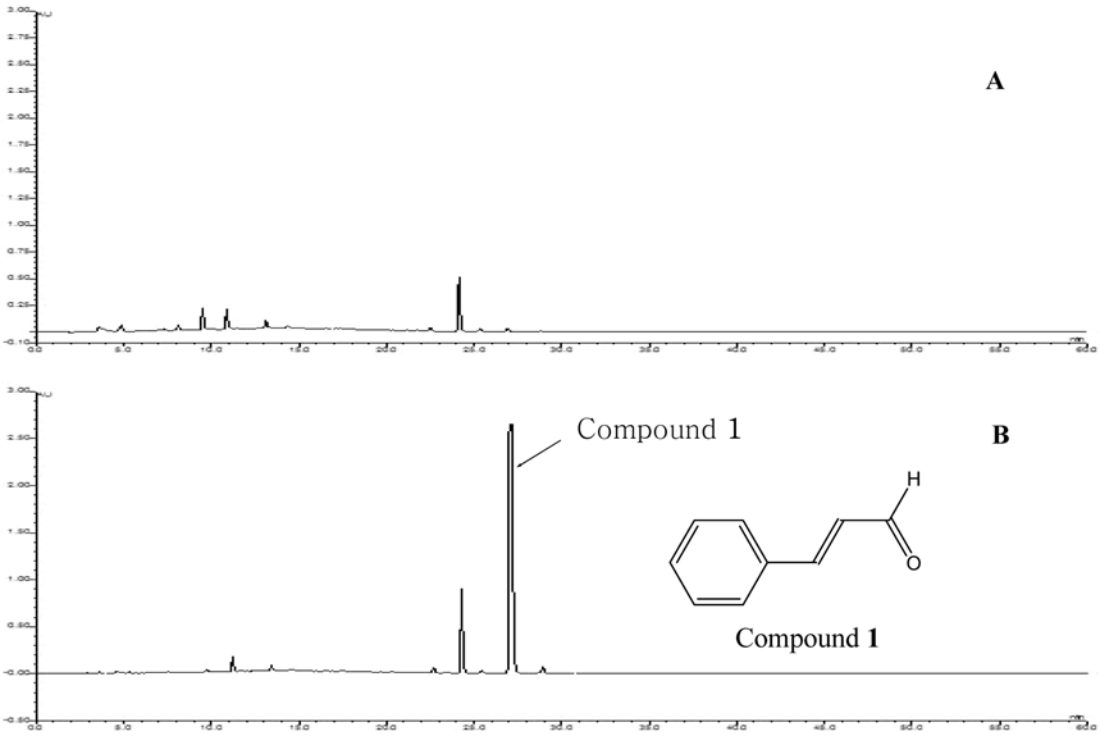

Fig. 1. HPLC profiles of EtOH extracts of unroasted (A) and roasted (B) Cinnamomi Cortex. Compound **1** is cinnamaldehyde. All data are presented as the mean value±standard deviation of triplicate experiments.

converting enzyme (ACE) were evaluated by previous reports [Cushman *et al*. 1983; Jeon *et al*. 2003; Lee *et al*. 2004b]. Cytotoxic effects toward human tumor and normal cells were studied with NIH-3T3 and HUVECs (human umbilical vein endothelial cell), respectively according to reported methods [Kanamaru and Yoshida, 1989; Lin *et al*., 1993].

Results and Discussion

After being roasted, an increased peak was found in the HPLC chromatogram (Fig. 1). The increased compound was purified by ODS column chromatography. The molecular weight of the compound was shown to be 132 from the ESI-MS analysis. In the ^{1}H-NMR spectrum, a doublet proton at δ 9.66 (J=8.0 Hz) was characteristic to aldehyde having one vicinal proton. The signals at δ 6.16 (1H, *dd*, J=8.0 and 18.0 Hz) and δ 7.32 (1H, *d*, J=18.0 Hz) were indicative to phenylpropenes with *trans*-configuration. From this data the increased compound was assumed to be *trans*-cinnamaldehyde and confirmed by comparing its NMR data with those in the reference [Lee *et al*., 2004a].

No significant changes in anti-dementia (PEP and β-secretase inhibition), antihypertension (ACE inhibition), and cytotoxicity (both in tumor and normal cells), were observed between the sample put through the roasting treatment and the control; however, an increase of about 40% in the antioxidative effect was observed when compared to the unroasted control (Table 1). Cinnamaldehyde is a major constituent of C. Cortex and its oils, and it provides

Table 1. Comparison of biological activities before and after roasting

	Untreated	+Roasting
DPPH[1)]	45.3±0.2	83.2±0.8
PEP[2)]	12.6±0.1	15.2±1.2
BACE1[3)]	33.2±0.0	30.6±1.0
ACE[4)]	25.8±0.6	25.6±0.8
APTT[5)]	53.6±2.0	50.6±0.6
NIH-3T3[6)]	65.3±1.1	66.8±0.9
HUVECs[7)]	75.6±0.6	72.5±2.4

[1)]Antioxidative effect (scavenging % at 100 ppm).
[2),3)]Anti-dementia (inhibition % at 40 ppm, PEP for prolyl endopeptidase and BACE1 for β-secretase).
[4)]Anti-hypertensive effect (inhibition % at 20 ppm, ACE for angiotensin converting enzyme).
[5)]Anti-coagulation effect (inhibition % at 167 ppm, APTT for activated partial thromboplastin times).
[6)]Cytotoxicity to human cell line (survival % at 40 ppm).
[7)]Cytotoxicity for normal cells (survival % at 200 ppm, HUVECs for human umbilical vein endothelial cell). All data are presented as the mean value±standard deviation of triplicate experiments.

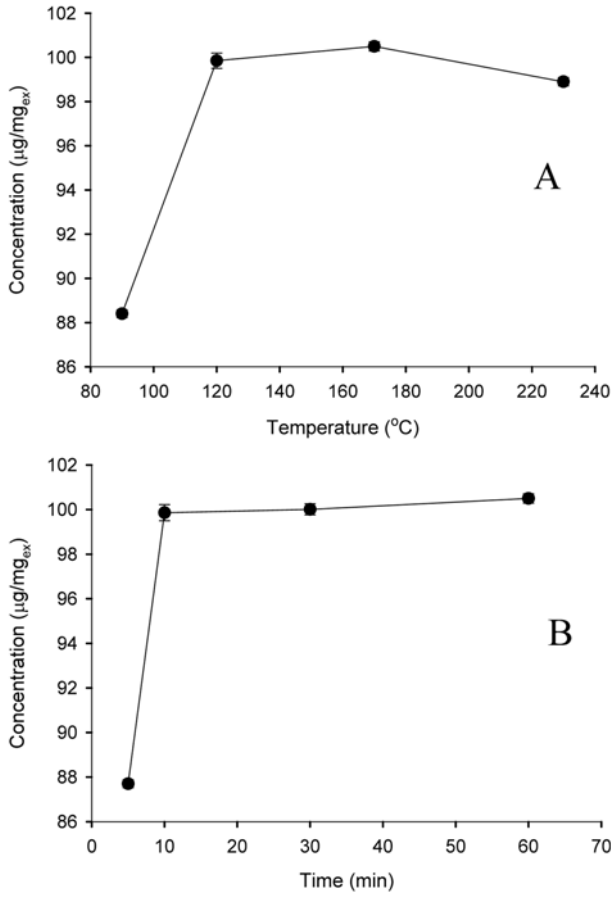

Fig. 2. Changes in cinnamaldehyde content according to the roasting temperature (A) and time (B).

the distinctive odor and flavor. This compound has been used in foods, beverages, medical products, perfumes, cosmetics and is also used as a filtering agent, a rubber reinforcing agent, a brightener in electroplating processes, an animal repellent effective against dogs and cats, an insect attractant, and as a fungicide applied to the root system of many crops. Moreover, cinnamaldehyde showed various biological properties such as an antioxidative, antimicrobial, and antidiabetic effects [Gowder and Davaraj, 2006; Ooi *et al*., 2006; Huang *et al*., 2007; Susash, 2007]. Therefore, the enhanced antioxidative effect of roasted cinnamon might be theorized to originate from the increase in the cinnamaldehyde content.

Influences of roasting time and temperature on cinnamaldehyde production in C. Cortex were investigated by means of HPLC analysis. Each 10 g of C. Cortex was roasted for 30 min at 90±2, 120±2, 170±2, and 225±2°C. The cinnamaldehyde content reached its maximum level at 120°C and was slightly decreased at 225°C (Fig. 2A). For optimizing roasting time, the temperature was fixed at 120°C and the roasting time was varied from 5 to 60 min. As shown in Fig. 2B, the cinnamaldehyde content was the highest after 10 min, and no significant change was observed thereafter. The content of cinnamaldehyde in roasted C. Cortex (108.42±0.26 mg/mg extract) was increased by 137 times that of an unroasted control (0.78 ±0.23 mg/mg extract) under optimal conditions.

The results are noteworthy in that simple stir-roasting processing might improve the values of C. cortex since cinnamaldehyde are widely used in many fields including foods and drugs as well as an antioxidant. Further study should be carried out for clarifying the heat-induction mechanism of cinnamaldehyde.

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