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Physicochemical Characteristics and Antioxidant Activities of Deoduck (*Codonopsis lanceolata*) with Different Aging Temperatures and Periods

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Abstract

To assess a potential possibility of Deoduck as functional food resources, this study was performed to determine the changes in chemical components and antioxidant activities on Deoduck with various aging conditions; aging temperatures were 60, 70, and 80°C, and aging periods were 5, 10, 15, 30, and 50 days. We determined pH, total acidity, browning index, 5-hydroxymethyl-furfural, total phenolic contents, DPPH and ABTS radical scavenging activities of aged Deoduck. Total acidity of aged samples increased during aging treatment, at higher temperature and longer time. The pH value of aged Deoduck ranged from 4.97 to 3.76. Aged Deoduck at 60℃ decreased slowly than 70 and 80℃, and these results were similar in total acidity. 5-HMF and total phenolic contents increased when increased aging temperature and periods. The DPPH and ABTS radical scavenging activities of Deoduck were ranged from 0.374 to 1.560 mg TEAC eq/g and from 0.302 to 1.745 mg trolox eq/g, respectively.

Key words: Deoduck, chemical components, antioxidant activities

Introduction

Recently the use of herbal preparations as functional foods and remedies for various health problems and medical conditions has been rapidly increasing in Korea. *Codonopsis lanceolata* Trautv. (Common name: Bounet bell flower, Deoduck in Korea) is a perennial herb belonging to the Campanulaceae family (Cho et al. 2008; Yongxu et al. 2008). Deoduck is distributed in Asian countries (Korea, Japan, and China), which mostly grows in moist places, under half shade of low mountain and hills (Byeon et al. 2009).

It has also been used in Korean cuisines, called Deoduck. Over the past few decades, Deoduck has attracted a growing amount of attention because of its pharmacological effects including anti-oxidant, anti-fatigue, anti-inflammatory, anti-microbial, anti-mutagenic, and immunomodulatory effects (Byeon et al. 2009; Li et al. 2009).

The Deoduck contains various active compounds such as saponins, tannins, polyphenolics, steroids, alkaloids, and triterpenes, and has used to treat bronchitis, cough, spasm, psychoneurosis, cancer and inflammation (Li et al. 2009).

According to the changing needs of consumers, they have demanded more healthful and various food. Especially, aging process is known to improve the biological activities of various agro-product such as aged black garlic, red ginseng, and black Doraji in Korea. After aging, agro-product increased phenolic compounds and antioxidant activity (Hwang et al. 2011).

Therefore, in this study, we tried to investigate the changes of chemical components and anti-oxidant activities of Deoduck with aging treatment and periods.

Materials and Methods

1. Aging of samples

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Fresh Deoduck was obtained from Hongcheon in Gangwon-do, South Korea. The Deoduck was rinsed with tap-water, dried at room temperature, and stored at −20℃.

Deoduck was placed into aluminum foil laminated film bag (Nowpack, Seoul, South Korea) and vacuum packaging (Chamber type vacuum package, DP-901, Dew Pack Korea Machinery Co, Seoul, Korea.) to keep moisture content of raw material. The packed samples were aged at 5, 10, 15, 30, and 50 days for 60, 70, and 80℃ by the temperature-controlled incubator. After aging, these samples were extracted three times with 80% (v/v) ethanol solution at room temperature for 1 hr using an ultrasonicator (SD-350H; Seong Dong, Seoul, Korea). Their extracts were concentrated using a rotary evaporator (N-1000; Eyela, Tokyo, Japan) under vacuum at 40℃ for remove the solvent, and then were dried using a freeze dryer (Ilshin Biobase FD5508, Kyungggi-do, Korea). These extracts were used for measuring total phenolic content and antioxidant activities.

2. Change of chemical components with aging conditions

The samples with various aging conditions were extracted with 20 mL of distilled water using an ultrasonicator for 1 hr, then pH, total acidity, browning index, and HMF of the extracts were measured, and all samples were analyzed in triplicate.

The pH value was measured by a pH meter (Orion4 STAR, Thermo Scientific, Beverly, MA, USA), and total acidity was determined as the amount of standardized 0.01N NaOH solution required to neutralize using phenolphthalein as indicator and expressed as percent lactic acid (Chun et al. 1995).

The browning index was measured according to the method of Ajandouz et al. (2001). Appropriate dilution (10-fold) was made using distilled water and the absorbance was measured using an UV-vis spectrophotometer (DU650; Beckman, Fullerton, CA, USA) at 420 nm.

5-HMF content was determined according to a modification of the method of Kwon et al. (2006). The extracts were filtered through a 0.45 μm-membrane filter (Millipore, Billerica, MA, USA) and analyzed using a HPLC-UVD (Younglin, Anyang, Korea). The analytical column was a C18 column (Mightysil RP-18 GP, 4.6×250 mm, 5 μm, Kanto Chemical, Tokyo, Japan). Water-acetonitrile (80:20, v/v) was used as mobile phase. The injection volume, flow rate, and wavelength were 20 μL, 0.6 mL/min, and 280 nm, respectively. 5-HMF was used as standard, and all samples were analyzed in triplicate.

3. Measurement of antioxidant activities with aging conditions

The total phenolic content of ethanolic extracts from aged Deoduck was determined using the method of Hwang et al. (2011). The sample (0.1 mL), 2 mL of 2% Na_2CO_3, and 0.1 mL of 50% Folin-Ciocalteu phenol reagent (Sigma-Aldrich, St. Louis, MO, USA) were into a test tube and mixed. After exactly 30 min, the absorbance was measured at 750 nm, and the phenolic content was calculated from a calibration curve (R^2=0.998) that was obtained using gallic acid as a standard. All extracts were measured in triplicate.

The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging activity of the aged Deoduck was measured according to the method of Yang et al. (2006) with some modifications. 0.2 mM DPPH (Sigma-Aldrich) methanolic solution (1 mL) were mixed with 0.5 mL of the samples. The mixture was shaken and then kept at room temperature for 30 min under the dark condition. The absorbance was measured at 520 nm using a UV-vis spectrophotometer. The inhibitory activity was calculated as $(A_0 - A_1)/A_0 \times 100$, where A_0 is the absorbance of control and A_1 is the absorbance with the sample. DPPH radical scavenging activity was expressed as TEAC (μmol Trolox equivalent/g, dry basis). All samples were measured in triplicate.

The ABTS (2,2'-azino-3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activity was measured by the method of Lee et al. (2007). The ABTS radical solution was adjusted with distilled water to obtain an absorbance of 1.4-1.5 at 735 nm. A 1 mL of diluted ABTS radical solution was mixed with 50 μL of the samples. The absorbance at 735 nm was determined using a UV-vis spectrophotometer after 30 min. The radical scavenging activity was calculated as $(A_0 - A_1)/A_0 \times 100$, where A_0 is the absorbance of control and A_1 is the absorbance with the sample. ABTS radical scavenging activity was expressed as TEAC (μmol Trolox equivalent/g, dry basis). All samples were measured in triplicate.

4. Statistical analysis

The results were expressed as mean±standard deviation. The significant differences among samples treated with different conditions was determined by one-way analysis of variance (ANOVA), using SPSS version 12 (SPSS Institute, Chicago, IL, USA) in $p<0.05$.

Results and Discussion

1. Changes of chemical components with aging conditions

The pH value of aged Deoduck with aging conditions are shown in Fig. 1. The pH value decreased with increasing temperature from 60 to 80℃ and aging periods from 5 to 50 days.

Aged Deoduck also became more acidic than control. pH values of aged Deoduck extracts slowly decreased from 4.96 to 3.76 during aging treatment, and those of Deoduck treated at 60℃ decreased slowly than 70℃ and 80℃. Especially, 30 and 50 days aged Deoduck were lower pH value other aging periods. Similar results were reported that the pH of aged garlic was acidified as increased temperature and periods (Shin et al. 2008).

The total acidity of aged Deoduck with aging conditions increased significantly with increasing aging period (Fig. 2). The total acidity of Deoduck was 0.541 lactic acid eq % at control, whereas it was increased to 0.95 lactic acid eq % when aged at 80℃ for 15 days. Aged Deoduck became more acidic during aging treatment, with total acidity ranging from 0.54 to 1.04. This result showed the opposite trends to those of pH value, and increasing of total acidity is considered due to a combination of sugars and amino acids during the non-enzymatic browning reaction. Woo et al. (2011) reported that the organic acid content significantly increased when increased heating temperature and time in sugar-amino browning reaction. Aida et al. (2007) reported that organic acid content is strongly related to heating condition. In this study, the pH in aged samples generally decreased as aging temperature and periods increase, because of the production of acidic substances.

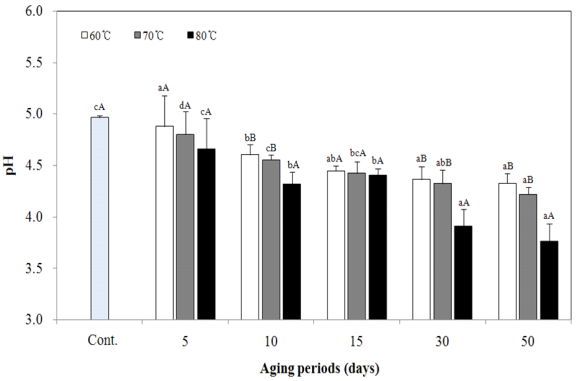

Fig. 1. Change of pH of Deoduck with different aging temperature and periods. [1] Different small letters in the same items indicate a significant difference (p<0.05) among different aging periods. [2] Different capital letters in the same items indicate a significant difference (p<0.05) among different aging temperatures.

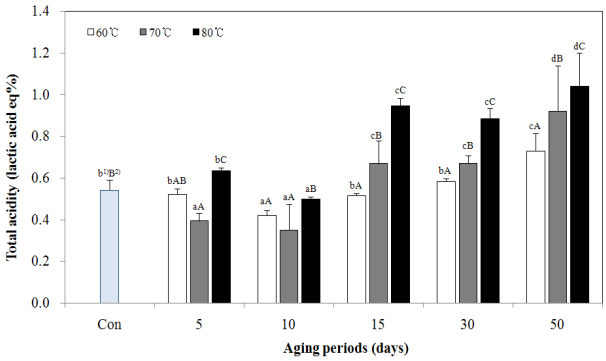

Fig. 2. Change of total acidity of Deoduck with different aging temperature and periods. [1] Different small letters in the same items indicate a significant difference (p<0.05) among different aging periods. [2] Different capital letters in the same items indicate a significant difference (p<0.05) among different aging temperatures.

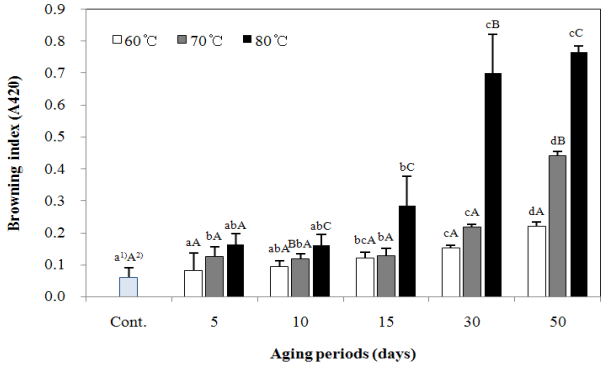

Fig. 3. Change of browning index of Deoduck with different aging temperature and periods. [1] Different small letters in the same items indicate a significant difference (p<0.05) among different aging periods. [2] Different capital letters in the same items indicate a significant difference (p<0.05) among different aging temperatures.

The browning index of aged Deoduck is shown in Fig. 3, and those of aged Deoduck were the highest value of 0.77 and 1.00 at 80℃ for 50 days, whereas control was 0.06. This results indicated that the browning intensity of Deoduck is related with aging temperature and periods. Hwang et al. (2011) reported that final compound from browning reaction of hydroponics ginseng roots was the highest value of 1.33 at 150℃, whereas raw hydroponic ginseng roots was 0.16.

The change in 5-HMF content of the aged Deoduck are shown in Fig. 4. Deoduck slowly increased from 0.3 to 6.4 below 70℃ according to aging periods. Before aging, 5-HMF content not de-

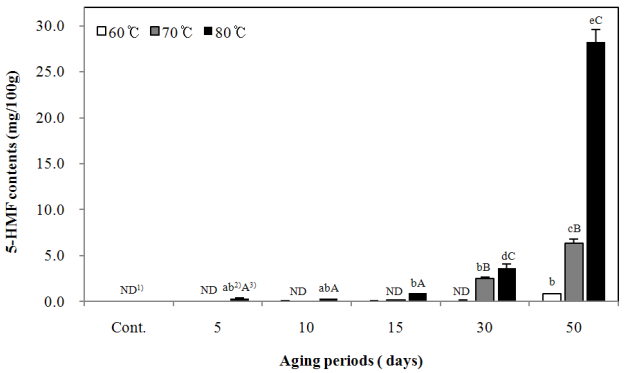

Fig. 4. Changes in 5-HMF contents of Deoduck with different aging temperature and periods. [1] ND: Not detected. [2] Different small letters in the same items indicate a significant difference (p<0.05) among different aging periods. [3] Different capital letters in the same items indicate a significant difference (p<0.05) among different aging temperatures.

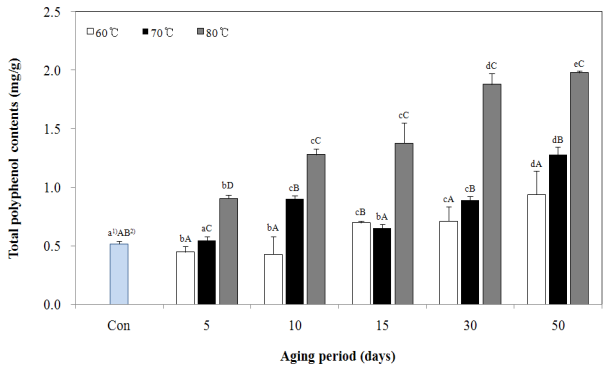

Fig. 5. Changes in total polyphenol contents of Deoduck with different aging temperature and periods. [1] Different small letters in the same items indicate a significant difference (p<0.05) among different aging periods. [2] Different capital letters in the same items indicate a significant difference (p<0.05) among different aging temperatures.

tected in raw Deoduck, but increased 28.227 and 50.400 mg/100g at 80℃ for 50 days.

5-HMF, an important intermediate, is widely used as an indicator of maillard reaction (Cohen et al. 1998). 5-HMF is one of the major products of carbohydrate degradation in food, known as non-enzymatic browning, and derived from dehydration of sugars. The physiological effects of 5-HMF have revealed as anti-sickling agent and tyrosinase inhibitor (Woo et al. 2011). Antal et al. (1990) and Woo et al. (2011) reported that HMF was generated from sucrose degradation and that the low pH promoted the formation and successive fragmentation to levulinic acid and formic acid (Fig. 4).

2. Changes of antioxidant activities with aging conditions

The major contribution on the antioxidant activities of foods from plants was the amount of phenolics. Thus, it's important to quantify of phenolics and to assess its contribution to antioxidant activities (Cohen et al. 1998).

The changes in total phenolic content of the aged Deoduck are shown in Fig. 5. The phenolic contents of aged Deoduck was significantly increased relative to that of raw material, and it was measured the highest in Deoduck aged at 80℃ for 50 days. Lee et al. (1998) reported that the total phenolic content and antioxidant activity were the highest in 15 days aged black garlic when garlic was treated with different aging periods (10, 15, 20 days) at 70℃. Jang et al. (2008) was reported effect of aging treatment. The extract from aged garlic had a 2.5-fold higher total phenolic content than that of the fresh garlic extract, showing levels of 10.0 mg/g and 3.7 mg/g, respectively.

The DPPH radical scavenging activity of aged Deoduck are shown in Fig. 6 expressed as the trolox equivalent antioxidant capacity. After aging at 60, 70, and 80℃, the DPPH radical scavenging activity of samples were increased relative to that of raw material. Aged Deoduck increased from 0.36 to 0.87 mg trolox eq/g, from 0.46 to 1.56 mg trolox eq/g, and from 0.458 to 1.345 mg trolox eq/g at 60, 70, and 80℃, respectively. Several researchers also have reported that thermal treatment including aging processing causes improving the antioxidant activity in foods

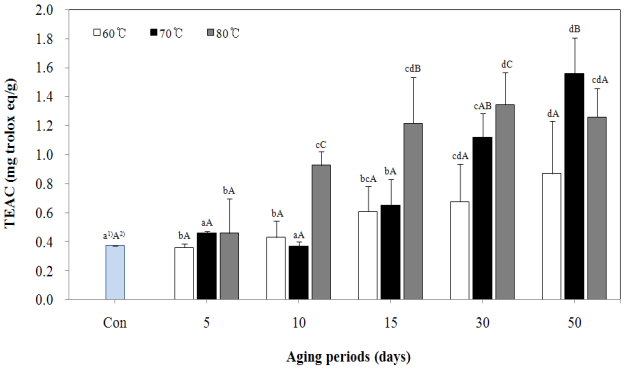

Fig. 6. Changes in DPPH radical scavenging of Deoduck with different aging temperature and periods. [1] Different small letters in the same items indicate a significant difference (p<0.05) among different aging periods. [2] Different capital letters in the same items indicate a significant difference (p<0.05) among different aging temperatures.

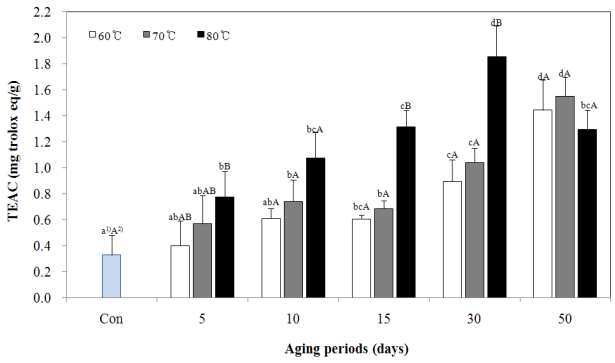

Fig. 7. Changes in ABTS of Deoduck with different aging temperature and periods. [1)] Different small letters in the same items indicate a significant difference (p<0.05) among different aging periods. [2)] Different capital letters in the same items indicate a significant difference (p<0.05) among different aging temperatures.

from plants, because of increasing the antioxidant properties of naturally occuring the formation of bio-active compounds, such as Maillard reaction products (Yen et al. 1995).

The ABTS radical scavenging activity of raw Deoduck was 0.35 mg trolox eq/g. The ABTS radical scavenging activity of aged Deoduck was ranged from 0.302 to 1.745 mg trolox eq/g (Fig. 7).

These results are showed that the overall antioxidant activities of Deoduck were improved by increasing aging temperature and periods significantly. Lee et al. (2009) determined the antioxidant effect of garlic and aged black garlic, and TEAC values of garlic and aged garlic were determined as 13.3 and 59.2 μmol/g wet weight, respectively. TEAC value of aged black garlic was 4.5-fold higher than fresh garlic, and these results demonstrated that ageing of whole garlic can enhance antioxidant activity. Woo et al. (2008) and Kim et al. (2008) reported that ABTS radical scavensing activity of ginseng extracts were increased by heat treatment. Recently, another study carried out on tomato and coffee found that prolonged thermal treatment improved the antioxidant activities of these foods (Dewanto et al. 2002); browning and antioxidant activities of the tomato and coffee sample increased with heating and roasting time.

Many researches indicated that heated products exhibit chain breaking and oxygen-scavenging activities, and the heated products enhanced activities of these components by the low-molecularization effects of the heat treatment (Manzocco et al. 2000). The aging treatments also consider that the more effective antioxidant activities by low-molecularization.

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