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Effect of Heat Treatment on the Antioxidant Properties of Yacon (*Smallanthus sonchifolius*)

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열처리에 따른 야콘의 항산화 활성 변화

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국문요약

본 연구에서는 열처리가 야콘의 항산화 활성에 미치는 영향을 살펴보기 위하여 열처리 온도(100 및 121℃)와 시간(15, 30 및 60분)에 따른 항산화 성분 및 항산화 활성 변화를 조사하였다. 열처리 후 야콘의 갈변도, 유리형 및 결합형 폴리페놀 함량, 유리형 및 결합형 플라보노이드 함량, DPPH radical 소거 활성 및 ABTS radical 소거 활성을 측정하였다. 열처리 후 야콘의 갈변도, 유리형 폴리페놀 및 플라보노이드 함량과 항산화 활성은 열처리 온도와 시간에 따라 유의적(p<0.05)으로 증가하였고, 결합형 폴리페놀 및 플라보노이드 함량은 감소하였다. 야콘의 유리형 폴리페놀 및 플라보노이드 함량은 121℃, 60분 열처리 시 생야콘에 비해 각각 1.2배 및 1.1배로 유의적(p<0.05)으로 증가하였다. 또한, 야콘의 DPPH radical 소거 활성 및 ABTS Radical 소거 활성도 121℃, 60분 열처리 시 생야콘에 비해 각각 1.7배 및 2.0배로 유의적(p<0.05)으로 증가였다. 야콘의 갈변도, 폴리페놀 및 플라보노이드 함량과 DPPH radical 및 ABTS radical 소거 활성 간의 상관관계를 분석한 결과, 높은 상관관계(p<0.01)가 있는 것으로 나타났다. 본 연구결과, 열처리 방법을 통해 야콘의 항산화 성분과 항산화 활성을 강화시킬 수 있으며, 이를 활용한 기능성 식품 소재 개발이 가능할 것으로 생각된다.

Key words: yacon, heat treatment, polyphenolics, antioxidant activity

Introduction

Yacon (*Smallanthus sonchifolius*), which belongs to the genus *Smallanthus* in the Asteraceae family, originates from the Andean region, and has spread worldwide, including to South Korea (Ojansivu et al. 2011). Yacon tubers are used for the production of natural sweeteners and syrups suitable for persons suffering from diabetes or various digestive problems. In South Korea, yacon tubers have gradually received more attention as a functional food supplement, because they contain abundant amounts of oligofructans and phenolic compounds (Habib et al. 2011; Kim et al. 2010b). Recent studies have shown its medicinal attributes, including hypoglycemic, anti-obesity, cholesterol-lowering, and antioxidant activities (Habib et al. 2011; Kim et al. 2010a; Kim et al. 2010b; Park et al. 2009). Yan et al. (1999) identified chlorogenic acid and l-tryptophan from yacon tubers as major antioxidants. Further, five different caffeic acid derivatives have been isolated from the roots of yacon (Takenaka et al. 2003).

Reactive oxygen species (ROS) are generated by irradiation, chemical reactions, and redox reactions of various compounds. It has been reported that ROS may contribute to oxidative damage of the lipids, proteins, and nucleic acids in living tissues

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and cells (Ratnam et al. 2006; Wootton-Beard & Ryan 2011). If ROS are not effectively scavenged by cellular constituents, the oxidative stress from ROS or free radicals may lead to many chronic diseases such as aging, cancer, inflammation, rheumatoid arthritis, hypertension, and atherosclerosis. Humans have antioxidant defenses, including enzymatic and non-enzymatic scavengers, to minimize ROS accumulation (Gill & Tuteja 2010; Niki E 2010; Wang et al. 2011). Recent studies have indicated that consumption of fruits, vegetables, and cereals correlates with reduced chronic disease risk. These effects are due to the presence of antioxidant substances like vitamins and phenolic compounds (Du et al. 2009; Wang et al. 2011; Wootton-Beard & Ryan 2011).

Thermal processing is the most widely used method for preserving and extending the shelf life of food products. Application of heat to food is commonly done by roasting, blanching, boiling, and autoclaving. Heat treatments reduce the sensory and nutritional qualities of these products. Moreover, it is well known that natural nutrients can be depleted during heat treatment because most bioactive compounds are relatively unstable when subjected to heat (Hwang et al. 2010). However, many recent studies have showed that the increase in biological activities due to thermal processing can disrupt the cell wall, liberate antioxidant compounds from insoluble portion of foods, and cause various chemical changes (Dewanto et al. 2002; Hwang et al. 2010; Jeong et al. 2004; Manzocco et al. 2001; Woo et al. 2006). Therefore, the objective of this study was to evaluate the effects of heat treatment on the changes in antioxidant compounds and antioxidant activities of yacon with different temperatures and heating time.

Material and Methods

1. Chemicals and Reagents

Folin-Ciocalteu reagent, gallic acid, (+)-catechin, 1,1-diphenyl-2-picrylhydrazyl(DPPH), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)(ABTS), and potassium persulfate were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and all other reagents were of analytical grade.

2. Heat Treatment

Fresh yacon was purchased from a local market in Suwon, South Korea, in August 2011. Sliced yacon (100 g) was put into a sample bottle, tightly capped, and heated in an autoclaving apparatus at 100℃ and 121℃ for 15, 30, or 60 min.

3. Extraction of Free and Bound Phenolic Compounds

Free and bound phenolic compounds from yacon were extracted as described by Choi et al. (2006), with some modifications. Samples were transferred into a 1 ℓ flask. After adding 500 mℓ of 80% ethanol-water solution (v/v), the contents were sonicated at room temperature for 30 min in an ultrasonic bath (frequency, 40 Hz, power, 300 W, SD-350H; Seong Dong, Seoul, South Korea), and then filtered through Whatman No. 4 filter paper. The residue was re-extracted as described above. The combined extracts were concentrated to approximately 20 mℓ with a rotary vacuum evaporator at 40℃. The free extracts were diluted to a final volume of 100 mℓ with distilled water and stored at −70℃ until analysis.

The residue from free phenolic compound extraction was hydrolyzed with 20 mℓ of 4 N NaOH for 1 hr under nitrogen and adjusted to pH 2 with 6 N HCl. The bound polyphenolic compounds were extracted 3 times with 40 mℓ of ethyl acetate. The organic extracts were then evaporated at 40℃ to dryness, redissolved in 5 mℓ of methanol, and stored at −70℃ until analysis.

4. Measurement of Browning Index

The browning index of the yacon extract was measured according to the method of Hwang et al. (2011). Appropriate dilution (10-fold) was prepared using distilled water, and the absorbance was measured at 420 nm by using a spectrophotometer (UV-1650PC; Shimadzu, Kyoto, Japan).

5. Determination of Free and Bound Phenolic Compound Levels

The levels of free and bound phenolic compounds in the extract were determined using the Folin-Ciocalteu method (Tepe et al. 2006). Standard solution or extract (0.2 mℓ) was mixed with 2 mℓ of 2% Na_2CO_3 solution and 0.1 mℓ of 50% Folin-Ciocalteu reagent. After 30 min, the absorbance was read at 750 nm, and the phenolic levels were calculated from a calibration curve that was obtained using gallic acid as a standard. The results were expressed as mg of gallic acid equivalents per 100 g of yacon. All extracts were analyzed in triplicate.

6. Determination of Free and Bound Flavonoid Content

The levels of free and bound flavonoid in the extracts were

determined using a colorimetric method described by Jia et al. (1999). Standard solution or extract (0.25 ㎖) was mixed with 1.25 ㎖ of distilled water and 0.75 ㎖ of 5% $NaNO_2$ solution. After 5 min, 0.15 ㎖ of 10% $AlCl_3 \cdot H_2O$ solution was added. After 6 min, 0.5 ㎖ of 1 M NaOH solution and 0.275 ㎖ of distilled water were added to the mixture. The solution was mixed well, and the absorbance was measured with a spectrophotometer at 510 nm. The results were expressed as ㎎ of (+)-catechin equivalents per 100 g of yacon. All extracts were analyzed in triplicate.

7. DPPH Radical-Scavenging Activity

The DPPH radical-scavenging activity of the free extracts was based on the scavenging activity of the stable DPPH free radical, which was measured according to the method of Tepe et al. (2006), with some modifications. Aliquots of 0.8 ㎖ of 0.2 mM DPPH methanolic solution were mixed with 0.2 ㎖ of the samples. The mixture was shaken vigorously, and then kept at room temperature for 30 min in the dark. The absorbance was measured at 520 nm by using a spectrophotometer (UV-1650PC; Shimadzu, Kyoto, Japan). The DPPH radical-scavenging activity was expressed as ㎎ of ascorbic acid equivalents per 100 g of yacon (㎎ AA eq/100 g). The AA eq antioxidant activity was calculated as (△A/△AAA) × CAA, where △A is the change in absorbance after the addition of the extract, △AAA is the change in absorbance after the addition of ascorbic acid standard solution, and CAA is the concentration of the ascorbic acid standard solution. All samples were analyzed in triplicate.

8. ABTS Radical-Scavenging Activity

The ABTS radical cation-scavenging activity of the free extracts was measured according to the method described by Hwang et al. (2010) and Re et al. (1999), with some modifications. The ABTS radical cation was generated by adding 7 mM ABTS to 2.45 mM potassium persulfate solution, and leaving the mixture to stand overnight in the dark at room temperature. The ABTS radical cation solution was diluted with distilled water to obtain an absorbance of 1.4-1.5 at 735 nm. A 1 ㎖ aliquot of diluted ABTS radical cation solution was added to 50 ㎕ of the sample or distilled water. The absorbance at 735 nm was determined using a spectrophotometer (UV-1650PC; Shimadzu, Kyoto, Japan) after 30 min. The ABTS radical cation scavenging activity was expressed as ㎎ AA eq/100 g yacon. The ascorbic acid equivalent antioxidant activity was calculated as (△A/△AAA) × CAA, where △A is the change in absorbance after the addition of the extract, △AAA is the change in absorbance after the addition of ascorbic acid standard solution, and CAA is the concentration of the ascorbic acid standard solution. All samples were analyzed in triplicate.

9. Statistical Analysis

The results were reported as the mean ± standard deviation (SD) values. The significance of differences among the means was determined using one-way analysis of variance (ANOVA), using SPSS version 12 (SPSS Institute, Chicago, IL, USA), at a significance level of 0.05. Pearson's correlation test was used to assess correlations between means.

Result and Discussion

1. Browning Intensity

The browning reaction is one of the most common and complex reactions that takes place in foods during thermal processing and home cooking. Absorbance at 420 nm has commonly been used as an indicator of the browning pigment level developed during the final stage of the browning reaction (Ajandouz et al. 2001). The effects of heat treatment on the browning intensity of yacon extracts are shown in Fig. 1. An increase in the browning intensity of heated yacon was observed as the heating temperature and time increased (p<0.05). The browning intensity of raw yacon was 0.76. After treatment at 100℃ for 15, 30, or 60 min, the browning intensities of heated yacon were 0.73, 0.74, and 0.99, respectively. After treatment at 121℃ for 15, 30, or 60 min, the browning intensities of

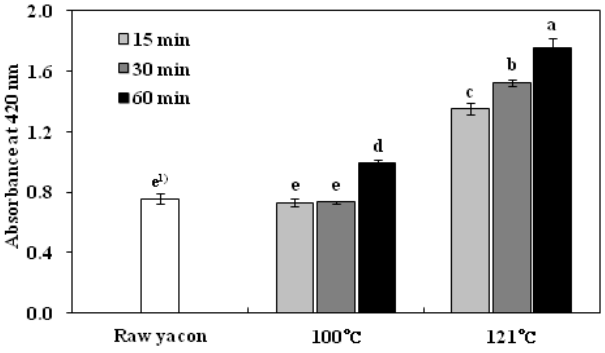

Fig. 1. Browning intensity of yacon produced by heat treatment at 100 and 121℃ for 15, 30, or 60 min. [1)] The different letters in the same bar are significantly different (p<0.05).

heated yacon were 1.35, 1.53, and 1.76, respectively. Among the heat-treated samples, the browning intensities of the heated yacon at 100℃ for 60 min and at 121℃ for 15, 30, or 60 min were significantly increased (p<0.05) relative to that of raw yacon. The velocity of the browning reaction depends upon many factors such as temperature, time, reactant types and concentration, and ratio of reducing sugars and amino acids. Moreover, studies of model systems showed that increases in temperature and/or time of heating resulted in an increase in color development, as observed in numerous other studies (Ajandouz et al. 2001; Ajandouz et al. 2008; Lan et al. 2010; Matins & Van Boekel 2005). Recently, many studies have also reported a positive correlation between the levels of Maillard reaction products (MRPs) and antioxidant properties following browning reactions in model systems and in studied food products (Manzocco et al. 2001; Yu et al. 2012).

2. Determination of Antioxidant Compounds

The phenolic compounds in herbs can act as antioxidants because of their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers, and metal chelators. Many studies have determined that fruits and vegetables are a major source of dietary antioxidative phenolics (Wootton-Beard & Ryan 2011). Therefore, it is important to consider the effect of heat treatment on the total polyphenolic compound levels in yacon extract. The free and bound phenolic contents of yacon extracts, expressed as ㎎ of gallic acid equivalent per 100 g sample, are shown in Fig. 2. Heat treatment had a significant effect (p<0.05) on the free and bound phenolic levels of yacon. The levels of free and bound phenolic compounds in raw yacon were 78.58 and 3.84 ㎎/100 g, respectively. Heat treatment at 100℃ did not significantly change the levels of free and bound phenolic compounds relative to raw yacon. However, the free phenolic content levels of yacon heated at 121℃ for 15, 30, or 60 min were significantly increased (p<0.05) from 81.92 to 91.53 ㎎/100 g (Fig. 2A), whereas the bound phenolic levels were significantly decreased (p<0.05) from 3.40 to 2.75 ㎎/100 g (Fig. 2B).

The effects of heat treatment on the flavonoid content of yacon extracts are shown in Fig. 3. The free and bound flavonoid contents of raw yacon, expressed as ㎎ of (+)-catechin equivalents per 100 g sample, were 50.97 and 1.19 ㎎/100 g, respectively. After heat treatment at 100℃, the free flavonoids were slightly increased (Fig. 3A), and the bound flavonoids were slightly

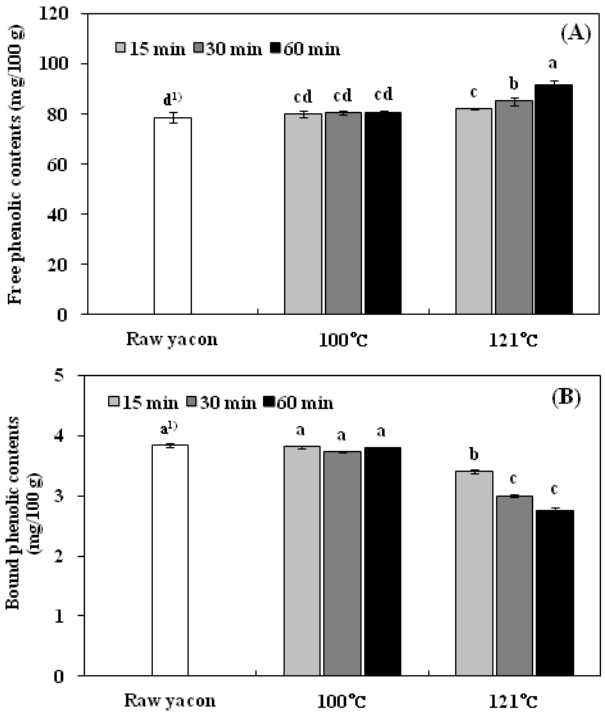

Fig. 2. Effect of heat treatment at 100 and 121℃ for 15, 30, or 60 min on free (A) and bound (B) phenolics content in yacon extract. [1)] The different letters in the same bar are significantly different (p<0.05).

decreased (Fig. 3B), but there was no significant difference compared to untreated yacon. The levels of free flavonoids in yacon heated at 121℃ for 15, 30, or 60 min were significantly increased (p<0.05), with a range of 51.06-55.75 ㎎/100 g (Fig. 3A), whereas the levels of bound flavonoids were significantly decreased (p<0.05), with a range of 0.62-0.64 ㎎/100 g (Fig. 3B).

The overall levels of free phenolic and flavonoid compounds increased with both increased heating time and temperature, while the bound phenolic and flavonoid levels declined. These results suggest that phenolic compounds in yacon can be liberated by heat treatment. This result agrees with the findings of earlier studies by Adefegha & Oboh (2011), Choi et al. (2006), Dewanto et al. (2002), Jeong et al. (2004), and Ju et al. (2010), who reported a significant increase in the levels of phenolic compounds in heated foods due to the disruption of cell walls and the liberation of soluble phenolic compounds from insoluble ester bonds.

3. Determination of Antioxidant Activities

To determine antioxidant activities, we used chemical assays based on the ability of compounds to scavenge model free

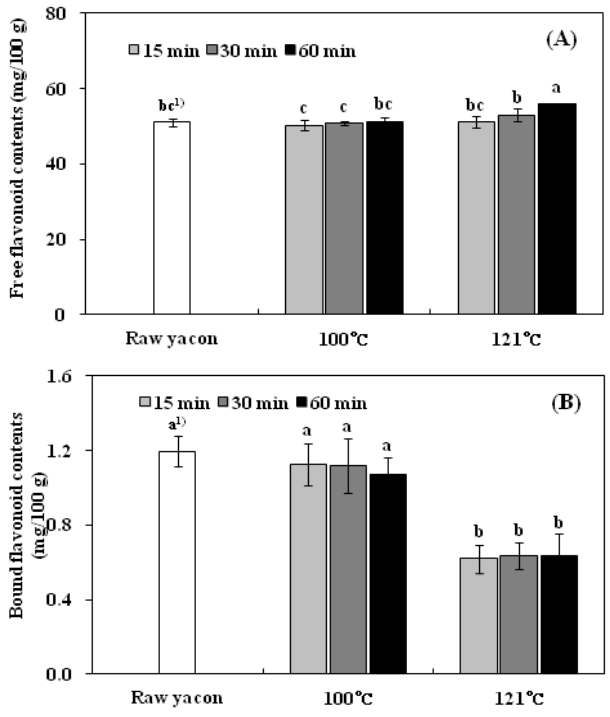

Fig. 3. Effect of heat treatment at 100 and 121℃ for 15, 30, or 60 min on free (A) and bound (B) flavonoid content in yacon extract. [1)] The different letters in the same bar are significantly different (p<0.05).

radicals, *i.e.*, DPPH and ABTS radicals, because of their simplicity and common use for comparative purposes (Cotelle et al. 1996). The DPPH radical-scavenging activities of raw and heated yacon are shown in Fig 4. The DPPH radical-scavenging activity of yacon extract was significantly increased (p<0.05) by heat treatment. The DPPH radical-scavenging activity of raw yacon, expressed as ㎎ of AA equivalents per 100 g, was 24.66 ㎎ AA eq/100 g. The DPPH radical-scavenging activity increased from 27.98 to 31.73 ㎎ AA eq/100 g by heating yacon at 100℃. After heat treatment at 121℃, DPPH radical-scavenging activity increased from 34.60 to 43.72 ㎎ AA eq/100 g. The DPPH radical-scavenging activity of the heat-treated yacon was significantly increased (p<0.05) relative to that of raw yacon. Notably, DPPH radical-scavenging activity of the yacon treated at 121℃ for 60 min was ~1.7-fold higher than that of raw yacon.

The ABTS radical-scavenging activities of raw and heated yacon are shown in Fig 5. The ABTS radical-scavenging activity of yacon extract was significantly increased (p<0.05) by heat treatment. The ABTS radical-scavenging activity of raw yacon, expressed as ㎎ AA eq/100 g, was 31.98 ㎎ AA eq/100 g. This activity increased from 35.70 to 40.83 ㎎ AA eq/100 g by heating yacon at 100℃. After heat treatment at 121℃, the ABTS radical-scavenging activity increased from 49.68 to 62.93 ㎎ AA eq/100 g. The ABTS radical-scavenging activity of treated yacon was significantly increased (p<0.05) relative to that of raw yacon. Specifically, the ABTS radical-scavenging activity of the yacon treated at 121℃ for 60 min was about 2.0-fold higher than that of raw yacon. The antioxidant activities of yacon were significantly increased (p<0.05) with increasing temperature and heating time.

Previously, many studies have reported that the increase in antioxidant activity due to thermal processing might disrupt the cell wall and liberate antioxidant compounds from insoluble portions of food samples such as ginseng (Hwang et al. 2010), tomato (Dewanto et al. 2002), citrus peel (Jeong et al. 2004), and Chaga mushroom (Ju et al. 2011). In addition, the increase in antioxidant activity with thermal processing has been attributed to the formation of MRPs such as melanoidins, which are known to act as effective antioxidants (Jeong et al. 2004; Hwang et al. 2011).

In the present study, the browning intensity, levels of free and bound phenolic and flavonoid contents, and DPPH and ABTS radical-scavenging activities of the extracts obtained through heat treatment of yacon were measured. The browning intensity, levels of free phenolic and flavonoid compounds, and antioxidant activities were significantly increased with heating temperature and time, while the levels of bound phenolic and flavonoid compounds were decreased. A significant correlation was observed among the browning intensity, levels of free phenolic and flavonoid compounds as well as bound phenolic and flavonoid compounds, and DPPH and ABTS radical-scavenging activities of heated yacon extract (Table 1). Strong correlations between the browning intensity and antioxidant activities of heated yacon extract against DPPH (R=0.971) and ABTS (R=0.984) radicals were observed at a significant level (p<0.01). Moreover, a positive correlation was observed between the levels of free phenolic or flavonoid compounds and DPPH radical-scavenging activity (R=0.954, p<0.01 for free phenolic compounds vs. DPPH radical; R=0.887, p<0.01 for free flavonoid vs. DPPH radical) of heated yacon extracts. This was also true for ABTS radical-scavenging activity (R=0.941, p<0.01 for free phenolic vs. ABTS radical; R=0.881, p<0.01 for free flavonoid vs. ABTS radical). However, a negative correlation was observed between the levels of bound phenolic or flavonoid compounds and DPPH (R=−0.952, p<0.01 for bound phenolic vs. DPPH radical; R=−0.896,

Table 1. Correlation coefficients among the browning intensity, free and bound phenolic compounds, free and bound flavonoid compounds, and DPPH and ABTS radical-scavenging activities of heated yacon extract

Factor	Browning intensity	Free phenolic	Bound phenolic	Free flavonoid	Bound flavonoid	DPPH radical	ABTS radical
Browning intensity	1	0.899**	−0.960**	0.866*	−0.941**	0.971**	0.984**
Free phenolic	-	1	−0.935**	0.972**	−0.744	0.954**	0.941**
Bound phenolic	-	-	1	−0.914**	0.877**	−0.952**	−0.957**
Free flavonoid	-	-	-	1	−0.669	0.887**	0.881**
Bound flavonoid	-	-	-	-	1	−0.896**	−0.918**
DPPH radical	-	-	-	-	-	1	0.997**
ABTS radical	-	-	-	-	-	-	1

*p<0.05, **p<0.01

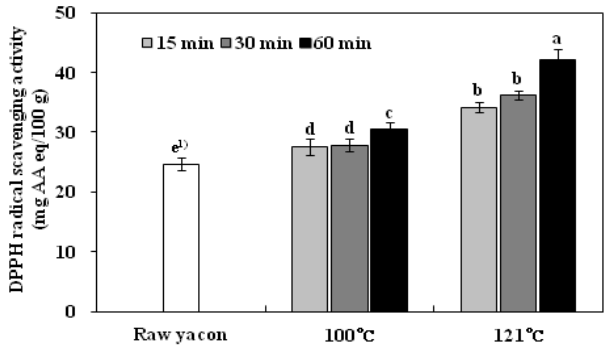

Fig. 4. Effect of heat treatment at 100 and 121℃ for 15, 30, or 60 min on DPPH radical scavenging activity in yacon extract. [1)] The different letters in the same bar are significantly different (p<0.05).

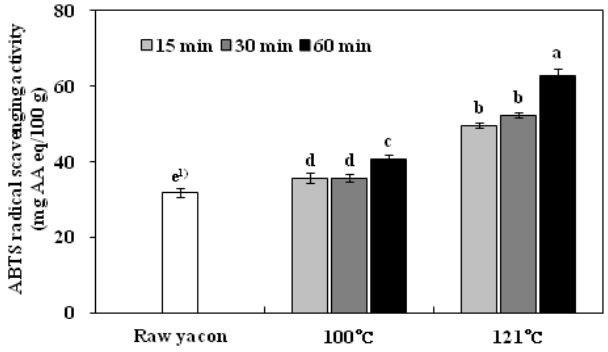

Fig. 5. Effect of heat treatment at 100 and 121℃ for 15, 30, or 60 min on ABTS radical scavenging activity in yacon extract. [1)] The different letters in the same bar are significantly different (p<0.05).

p<0.01 for bound flavonoid vs. DPPH radical) and ABTS (R=−0.957, p<0.01 for bound phenolic vs. ABTS radical; R=−0.918, p<0.01 for bound flavonoid vs. ABTS radical) radical-scavenging activity of heated yacon extracts. Therefore, the results reported in our study suggested that heat treatment might disrupt the cell and liberate the antioxidant compounds present in a bound form with insoluble polymers or liberate low-molecular-weight antioxidant compounds from the repeating subunits of high-molecular-weight polymers in yacon. In addition, the enhanced antioxidant activity could be attributed to the formation of MRPs during heat treatment. Based on the results of this study, heat treatment may be proposed as a method to enhance the antioxidant compound content in and the antioxidant activity of yacon. Future research on isolation and identification of the antioxidant substances in yacon is imperative.

Summary

This study was investigated the effects of heat treatment on polyphenolic compounds and antioxidant activities of yacon. Raw yacon was heated at 100℃ and 121℃ for 15, 30, or 60 min by using an autoclave. The browning intensity, levels of free and bound phenolic and flavonoid compounds, and 1,1-diphenyl-2-picrylhydrazyl(DPPH) and 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)(ABTS) radical-scavenging activity of yacon extracts following heat treatment were measured. The browning index, free polyphenolic content, and antioxidant activities in the extracts were significantly increased (p<0.05) with both increased heating temperature and time, while bound polyphenolic levels were decreased. The levels of free phenolic and flavonoid compounds in the yacon extract heated to 121℃ for 60 min were increased by 1.2 and 1.1 folds compared to raw yacon,

respectively. Moreover, DPPH and ABTS radical-scavenging activities of the yacon heated to 121℃ for 60 min were 1.7 fold and 2.0 fold higher, respectively, than those of raw yacon. A significant (p<0.01) correlation was observed among browning intensity, free and bound phenolic and flavonoid levels, and DPPH and ABTS radical-scavenging activities in heated yacon extract. Thus, heat treatment can be used as a method to enhance the antioxidant compound content in and the antioxidant activity of yacon.

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