碩士學位論文

Subcritical Water Hydrolysis of Pumpkin Leaves and their Antioxidant Activities

濟州大學校 大學院

食品工學科

高 禎 妍

2016年 8月

Subcritical Water Hydrolysis of Pumpkin Leaves and their Antioxidant Activities

指導敎授 任 尙 彬

高 禎 妍

이 論文을 工學 碩士學位 論文으로 提出함

2016年 8月

高禎妍의 工學 碩士學位 論文을 認准함

審査委員長 金 賢 貞 印

委 員 千 智 連 印

委 員 任 尙 彬 印

濟州大學校 大學院

2016年 8月

Subcritical Water Hydrolysis of Pumpkin Leaves and their Antioxidant Activities

Jeong-Yeon Ko
(Supervised by professor Sang-Bin Lim)

A thesis submitted in partial fulfillment of the requirement for the degree of Master of Engineering

2016. 08.

This thesis has been examined and approved.

Hyun Jung Kim, Thesis director, Prof. of Food Science and Engineering
Ji-Yeon Chun, Prof. of Food Science and Engineering
Sang-Bin Lim, Prof. of Food Science and Engineering

Aug. 2016

Department of Food Science and Engineering

GRADUATE SCHOOL

JEJU NATIONAL UNIVERSITY

Contents

Abstract

Enhanced production of individual phenolic compounds by subcritical water hydrolysis of pumpkin leaves was investigated at various temperatures ranging from 100°C to 220°C for 20 min and at various reaction times ranging from 10 min to 50 min at 160°C. Caffeic acid, *p*-coumaric acid, ferulic acid, and gentisic acid were major phenolic compounds in the hydrosate of pumpkin leaves. All phenolic compounds except gentisic acid showed the highest yield at 160°C, and gentisic acid showed the highest yield at 180°C. The cumulative amount of individual phenolic compound gradually increased by 48.1, 52.2, and 78.4 μg/g dry sample at 100°C, 120°C, and 140°C, respectively, and then greatly increased by 1,477.1 μg/g dry matter at 160°C. The yields of caffeic acid and ferulic acid showed peaks at 20 min while those of cinnamic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, and procatechuic acid showed peaks at 30 min. Antioxidant activities such as 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) values gradually increased with hydrolysis temperature, and were ranged from 6.77 to 12.42 mg ascorbic acid equivalents/g dry matter and from 4.25 to 8.92 mmol Fe^{2+}/100 g dry matter, respectively. *L* and *b* values were gradually decreased as hydrolysis temperature increased from 100°C to 140°C. At high temperatures (160°C to 220°C), *L* and *b* values were decreased suddenly and were leveled off. *a* value showed peak at 160°C and then decreased as temperature increased from 160°C to 220°C. These results suggest that subcritical water hydrolysis of pumpkin leaves was strongly influenced by temperature and may enhanced production of phenolic compounds and antioxidant activities.

1. Introduction

Phenolic compounds are secondary plant metabolites ubiquitous in the plant kingdom and their chemical structures vary from simple phenolics to complex polymer [Mukhopadhyay et al., 2006]. They have various functionality such as antioxidant, antimicrobial, anticancer, anti-obesity, antidiabetic, anti-hypertensive and anti-mutagenic properties [Chiou et al., 2007; Kunyanga et al., 2012]. Most naturally occurring phenolic compounds are present as conjugates with mono- and polysaccharides and linked to one or more of the phenolic groups [Balasundram et al., 2006]. Once phenolic glycosides convert into their corresponding aglycones, the biological activity may be increased. The major technologies for hydrolysis of phenolic glycosides include acid or base treatments which are not acceptable because of environmental concerns.

Subcritical water is defined as a liquid state water at temperatures between the usual boiling point 100°C and ther critical point 374°C under adequate pressure. Subcritical water may be an attractive reaction medium for hydrolyzing specially glycosides to aglycones because it is non-toxic, inexpensive, and environmentally friendly. Subcritical water has two unique properties such as a lower relative dielectric constant and a higher ion product than ambient water. At the room temperature and pressure, the dielectric constant is 80. However, heating water to 150°C, 200°C, 225°C, and 250°C, the dielectric constant is decreased to 45, 35, 31, and 27 which are similar to those of dimethyl sulfoxide, acetonitrile, methanol, and acetone at ambient temperature, respectively. Thus, the ability of water to elute non-polar compounds is increased [Amashukeli et al., 2007; Shitu et al., 2015]. In addition, the ion product of water substantially increases with temperature. The dissociation constant of water at room temperature is 1.0×10^{-14}. However, heating water to 100°C, 250°C, and 350°C, the dissociation constant of water is increased to 5.6

× 10^{-13}, 4.9 × 10^{-12}, and 1.2 × 10^{-12}, respectively [Plaza and Turner, 2015]. This indicates that pH of water is decreased from about 7.0 to 5.5 and it provides ability of acidic hydrolysis to water [Shitu et al., 2015].

Pumpkin such as potiron, squash, sweet pumpkin has been widely used in Korea as health and functional vegetables, because it is rich in phenolics, flavonoids, vitamin A, vitamin C, β-carotene, α-tocopherol, amino acids, carbohydrates, minerals, and fibers. Pumpkin has been known to have physiological activities such as anticancer, antioxidant, and anti-obesity [Kim et al., 2005; Que et al., 2008; Lee et al., 2010; Valenzuela et al., 2014]. Pumpkin leaves have distinct taste and texture, and are used as "ssam" to a piece of meat such as pork, beef. Cha [2009] reported that DPPH radical scavenging activity of the water extract from pumpkin leaves was 44.6% at the concentration of 20 mg/mL. Kim et al. [2011] reported that the ethanol extract of pumpkin leaves had the highest total phenolic contents (29.62 mg GAE/g dry matter (DM)), followed by skin (12.08), flesh (7.08), and seed (1.52), and had stronger DPPH-, ABTS-radical scavenging activities, and ferric-reducing antioxidant power value than any other parts.

To date, there have been no reports on the study of subcritical water hydrolysis of pumpkin leaves. The objective of this study were to investigate the production of phenolic compounds from pumpkin leaves by subcritical water and the effect of temperature and reaction time on the individual phenolic compounds, antioxidant activities and color.

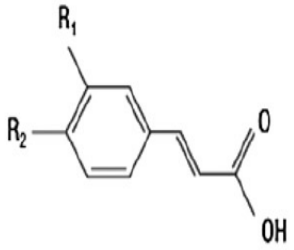
2. Materials and methods

2.1. Materials

Pumpkin (*Cucurbita moschata* Duch.) leaves were purchased from a local market, and washed, dried, grinded (-30 mesh), and stored in a refrigerator at 4°C until needed. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and 2, 4, 6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), respectively. Potassium persulfate, ferric chloride hexahydrate, and ferrous sulfate heptahydrate were purchased from Daejung Chemicals, Ltd. (Siheung-si, Korea), Junsei Chemical Co., Ltd. (Tokyo, Japan), and Wako Pure Chemicals Industries, Ltd. (Osaka, Japan), respectively. N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) was purchased from Supelco (Bellefonte, PA, USA). All phenolic standards were purchased from Fluka (Steinheim, Switzerland).

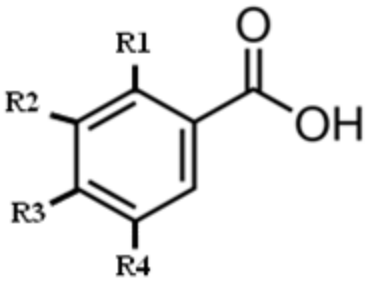
Table 1. Chemical structure of phenolic compounds in pumpkin leaves.

Hydroxycinnamic acids



	R1	R2
Cinnamic acid	H	H
p-coumaric acid	H	OH
Ferulic acid	OCH_3	OH
Caffeic acid	OH	OH

Benzoic acids



	R1	R2	R3	R4
p-hydroxybenzoic acid	H	H	OH	H
Gentisic acid	OH	H	H	OH
Protocatechuic acid	H	OH	OH	H

2.2. Subcritical water hydrolysis

The lab-scale subcritical water hydrolysis system was self-built as shown in Fig. 1. The system consists of reaction vessel, heating mantle, nitrogen gas tank, auxiliary valve and piping systems. subcritical water hydrolysis of pumpkin leaves was carried out in a 500 mL high-temperature and high-pressure stainless steel batch type reactor (Autoclave Engineers, Erie, PA, USA) designed for maximum operating temperature of 238°C at 413 bar. Powdered pumpkin leaves (about 2.0 g) suspended in 100 mL distilled water were loaded into the reactor with heating mantle. After being tightly capped, the reactor was purged with inert nitrogen gas to remove dissolved atmospheric oxygen. The reactor was heated electrically at the desired temperature. After treatment for the desired reaction time, the reactor was quickly cooled by soaking in a water bath at room temperature. The hydrolyzed sample was collected and filtered through a filter paper (Toyo No. 5A, Advantec Toyo Kaisha, Ltd., Tokyo, Japan). The filtered solution was brought to a total volume of 100 mL with distilled water (hereafter referred to as the "hydrolysate"). The hydrolysis of pumpkin leaves was performed at different temperatures at 100, 120, 140, 160, 180, and 220°C for 20 min. The effect of hydrolysis time was also investigated at 160°C for 10, 20, 30, 40, and 50 min.

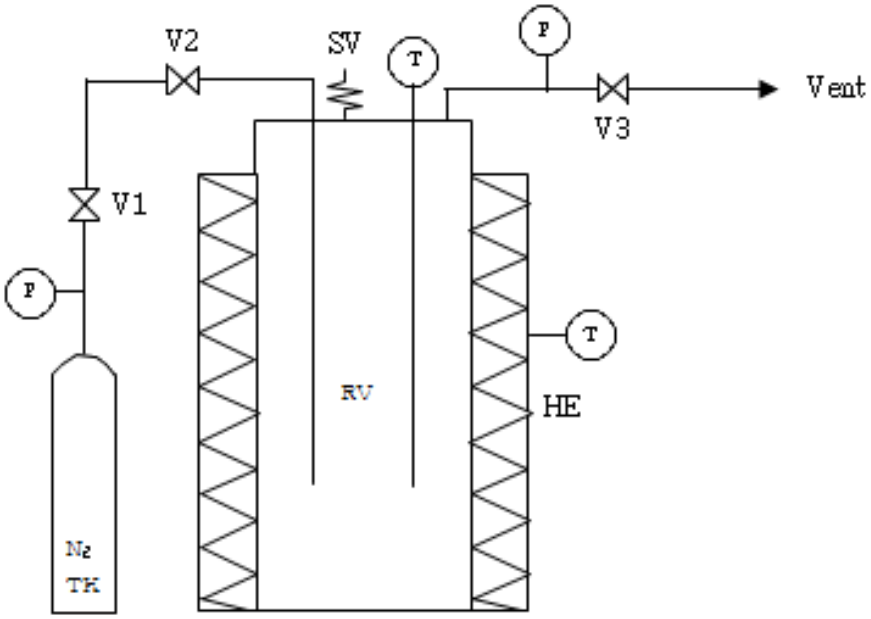


Fig. 1 Schematic diagram of subritical water hydrolysis system (HE : heat mantel, N_2 TK : nitrogen gas tank, P : pressure gauge, RV : reaction vessel, T : temperature gauge, SV : safety valve, V : on/off valve).

2.3. Determination of phenolic compounds by GC/MS

The hydrolysate (10 mL) was mixed with 15 mL ethyl acetate in a conical tube and vortexed. After centrifugation (10,000 × g) for 10 min, the supernatant was recovered, and the residue was further re-extracted twice. Anhydrous sodium sulfate was added to the mixed supernatant to remove moisture. The ethyl acetate layer was evaporated to remove the solvent using a rotary vacuum evaporator (Rotavapro R-124, Büchi Labortechnik AG, Flawil, Switzerland) at 40°C. The dried residue was dissolved in 0.25 mL BSTFA and incubated for 40 min at 75°C for derivatization.

Individual phenolic compounds were qualified and quantified using an Agilent series GC 6890N coupled with an HP 5973 MS detector and an HP 7683 autosampler (Agilent Technologies, Santa Clara, CA, USA) [Kim et al., 2010]. An HP-5 MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness) was used. The injector and transfer line temperatures were 280 and 300°C, respectively. The oven temperature was held at 120°C for 1 min, increased to 220°C at 5°C/min, then to 300°C at 10°C/min, and held for 10 min. Helium was used as a carrier gas at a flow rate of 0.6 mL/min. Injection volume was 1 μL at a split ratio of 1:5. Chromatographic peaks were identified by comparing the retention times and three fragment ions of each phenolic compound with those of reference compounds. Target and qualifier ions for seven phenolic compounds were set as follows: cinnamic acid, 205, 220, 161; *p*-hydroxybenzoic acid, 267, 223, 193; gentisic acid, 355, 356, 357; protocatechuic acid, 193, 355, 370; *p*-coumaric acid; 293, 308, 219; ferulic acid, 338, 323, 308; and caffeic acid, 396, 381, 219.

2.4. Determination of antioxidant activity

Antioxidant activities of the hydrolysates were measured in terms of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and ferric reducing antioxidant power (FRAP). DPPH value was measured according to the method of Kamiloglu et al. [2015]. Briefly a 0.1 mL of the hydrolysate was added to 1.5 mL of 0.1 mM DPPH in methanol. After vortexing, the mixed solution was incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm using a Spectronic Genesys 2 spectrophotometer (Spectronic Instruments, Rochester, NY, USA). Ascorbic acid was used as the standard, and DPPH value was expressed as mg ascorbic acid equivalents/g dry matter.

FRAP of the hydrolysate was measured according to the method of Thaipong et al. [2006] with minor modifications. The FRAP solution consists of 300 mM acetate buffer, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution at the ratio 10:1:1 (v/v), respectively. The hydrolysate (50 μL) was mixed with 150 μL distilled water and 1.5 mL FRAP solution, and incubated at 37℃ in a water bath for 10 min. The absorbance was measured at 595 nm using a Multiskan EX microplate reader (Thermo Electron Corp.). Ferrous sulfate ($FeSO_4 \cdot 7H_2O$) was used as the standard, and FRAP value was expressed as mmol Fe^{2+}/100 g dry matter.

2.5. Color measurement

Color of the hydrolysate was determined using a UltraScanVIS color spectrophotometer (Hunter Associates Lab., Inc., Reston, VA, USA) in terms of *L* (lightness), *a* (redness to greenness), and *b* (yellowness to blueness) values.

2.6. Statistical analysis

All measurements were performed in triplicate. Results were presented as mean ± standard deviation. Statistical analysis was made by SPSS version 18.0 software (SPSS Inc., Chicago, IL, USA). The difference was considered to be significant when the p value was less than 0.05 among the treatment means determined by Duncan's multiple range tests.

3. Results and discussion

3.1. Yields of phenolic compounds

In order to apply subcritical water for production of phenolic compounds from pumpkin leaves, experiments were performed over a temperature range of 100-220°C at reaction time of 20 min and over a reaction time range of 10-50 min at hydrolysis temperature of 160°C.

Table 2 shows the effect of hydrolysis temperature on the yield of individual phenolic compounds from pumpkin leaves at reaction time of 20 min. Seven phenolic compounds were detected in the hydrolysate of pumpkin leaves: cinnamic acid, *p*-hydroxybenzoic acid, gentisic acid, protocatechuic acid, *p*-coumaric acid, ferulic acid, and caffeic acid. Caffeic acid, *p*-coumaric acid, ferulic acid, and gentisic acid were greatly high in quantities among the others and were considered as major phenolics in pumpkin leaves. All phenolic compounds except gentisic acid showed the highest yields at 160°C, but gentisic acid showed the highest yield at 180°C. The cumulative amount of individual phenolic compound gradually increased by 48.1, 52.2, and 78.4 μg/g dry matter at 100°C, 120°C, and 140°C, respectively, and then greatly increased by 1,477.1 μg/g dry matter at 160°C. It was drastically decreased at higher hydrolysis temperatures than 160°C due to the decomposition reactions. Singh and Saldana [2011] also found that higher concentrations of phenolic compounds were recovered at temperatures from 140°C to 180°C and further increase in the reaction temperature from 180°C to 240°C lowered the yield of recovered phenolic compounds in subcritical water extraction of potato peel. This could be attributed to sample pyrolysis above 180°C which resulted in degradation of phenolic compounds.

Table 3 shows the effect of reaction time on the yield of individual phenolic compounds in subcritical water hydrolysates of pumpkin leaves at hy-

drolysis temperature of 160°C. The hydrolysis temperature was set at 160°C due to the highest yield of phenolic compounds as shown in Table 2. The yields of caffeic acid and ferulic acid showed peaks at 20 min, while those of cinnamic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, and procatechuic acid showed peaks at 30 min. Specially the yield of gentisic acid consistently increased with reaction time. The cumulative amount of individual phenolic compound gradually increased with reaction time up to 30 min and then decreased gradually at longer reaction times which may happen due to the decomposition of phenolic compounds. Longer reaction times as well as higher temperatures had destructive effects on the yields of phenolic compounds due to decomposition reactions which may occur under subcritical water condition [Singh and Saldana, 2011].

Cheigh et al. [2012] reported that the yields of hesperidin and narirutin increased for 10 min and then decreased with increasing extraction time at 160°C and 170°C in subcritical water extraction of *Citrus unshiu* peel. Ko et al. [2012] also reported that total amount of quercetin increased for 5-15 min, and then decreased for 20-30 min at 165°C in subcritical water extraction of onion skin.

Table 2. Yield of phenolic compounds in pumpkin leaves hydrolyzed by subcritical water at various temperature for 20 min.

Phenolic compounds	Yields (μg/g dry sample)						
	100°C	120°C	140°C	160°C	180°C	200°C	220°C
Cinnamic acid	ND	4.0±0.8^{d}	4.0±0.8^{d}	16.2±0.8^{b}	8.7±0.8^{c}	19.8±1.3^{a}	18.6±0.9^{a}
p-Hydroxybenzoic acid	2.5±0.6^{e}	4.4±1.1^{e}	8.1±1.7^{d}	24.0±1.2^{b}	8.7±1.4^{d}	30.1±1.7^{a}	14.8±0.5^{c}
Gentisic acid	7.9±2.3^{d}	6.2±0.1^{d}	17.8±1.7^{d}	237.1±16.3^{b}	263.9±18.5^{a}	61.7±3.8^{c}	23.6±1.0^{d}
Protocathecuic acid	1.1±0.0^{cd}	1.0±0.1^{d}	1.7±0.4^{c}	11.4±0.3^{a}	3.9±0.6^{b}	11.6±0.5^{a}	4.4±0.1^{b}
p-Coumaric acid	7.9±2.3^{d}	5.9±0.0^{d}	6.2±0.2^{d}	363.2±13.9^{a}	100.3±11.0^{b}	36.4±1.3^{c}	26.2±1.3^{c}
Ferulic acid	11.1±1.1^{d}	13.7±1.2^{d}	19.1±2.9^{c}	237.5±5.0^{a}	24.9±2.6^{b}	ND	ND
Caffeic acid	17.5±2.3^{b}	16.9±0.4^{b}	21.3±2.9^{b}	587.8±24.2^{a}	20.1±1.1^{b}	19.9±0.6^{b}	16.5±0.3^{b}
Total	48.1±9.5^{d}	52.2±3.5^{d}	78.4±7.7^{d}	1,477.2±57.6^{a}	430.6±31.5^{b}	179.5±8.5^{c}	104.1±2.9^{c}

Data are given as means ± SD (n=3)

"ND": not detected

The means in each row followed by a common letter are not significantly different by Duncan's multiple range tests at $p < 0.05$.

Table 3. Yield of phenolic compounds in pumpkin leaves hydrolyzed by subcritical water at various residence time at 160°C.

Phenolic compounds	Yields (μg/g dry sample)				
	10 min	20 min	30 min	40 min	50 min
Cinnmic acid	13.2±0.2^{b}	16.2±0.8^{a}	16.7±0.7^{a}	15.5±0.8^{a}	16.2±0.3^{a}
p-Hydroxybenzoic acid	17.4±0.7^{c}	24.0±1.2^{b}	30.9±1.5^{a}	28.8±2.4^{a}	30.6±1.3^{a}
Gentisic acid	76.5±1.2^{d}	237.1±16.3^{c}	342.5±22.9^{b}	354.8±21.1^{ab}	373.5±7.2^{a}
Protocathecuic acid	8.1±0.5^{d}	11.4±0.3^{c}	15.6±0.8^{ab}	14.0±1.5^{b}	15.9±0.8^{a}
p-Coumaric acid	258.4±11.4^{d}	363.2±13.9^{b}	399.3±18.1^{a}	348.1±25.3^{bc}	330.4±10.3^{c}
Ferulic acid	184.3±12.2^{c}	237.5±5.0^{a}	217.8±8.8^{b}	161.8±15.5^{d}	130.1±6.8^{e}
Caffeic acid	592.7±41.7^{a}	587.8±24.2^{a}	503.0±21.0^{b}	308.8±29.2^{c}	203.8±10.0^{d}
Total	1,150.6±67.4^{bc}	1,477.2±57.6^{a}	1,525.7±67.1^{a}	1,231.9±94.8^{b}	1,100.4±35.0^{c}

Data are given as means ± SD (n=3)

The means in each row followed by a common letter are not significantly different by Duncan's multiple range tests at $p < 0.05$

3.2. Antioxidant activities

Table 4 shows the effect of hydrolysis temperature on antioxidant activities, such as DPPH free radical scavenging activity and FRAP value, of pumpkin leaves hydrolyzed by subcritical water hydrolysis for 20 min. Antioxidant activities such as DPPH and FRAP values gradually increased with hydrolysis temperature, and were ranged from 6.77 to 12.42 mg ascorbic acid equivalents/g dry matter and from 4.25 to 8.92 mmol Fe^{2+}/100 g dry matter, respectively. Even though the cumulative yields of individual phenolic compounds were low at higher hydrolysis temperatures (> 160°C), DPPH and FRAP values were high. This suggested the possibility that degradation compounds from phenolic compounds or other highly antioxidative nonphenolic compounds with high antioxidant activity could be formed or extracted at such higher hydrolysis temperatures.

Jose et al. [2013] reported that increasing hydrolysis temperature above 160°C decreased polyphenol contents but increased antioxidant activity because high temperatures favor the formation of derived antioxidant compounds from phenolic compounds in grape pomace. Rangsriwong et al. [2009] also reported that despite the lowest total phenolic contents in the subcritical water extract from *Terminalia chebula* obtained at 220°C, the antioxidant activity was comparable to that of those obtained at lower temperatures because at high temperature, other highly antioxidative nonphenolic compounds including triterpenes, coumarins, steroids and benzenoids could be extracted.

Table 5 shows the effect of reaction time on antioxidant activities, such as DPPH free radical scavenging activity and FRAP value, of of pumpkin leaves hydrolyzed by subcritical water hydrolysis at 160°C. DPPH and FRAP values increased up to 20 min of reaction time and then stayed almost the same value. Khuwijitjaru et al. [2012] reported that DPPH free radical scavenging activity of subcritical water extract from cinnamon bark didn't increased even though the extraction time was longer than 30 min.

Table 4. DPPH free radical scavenging activities and ferric reduction antioxidant power (FRAP) values of pumpkin leaves hydrolyzed by subcritical water at various temperatures for 20 min.

Hydrolysis temperature (°C)	DPPH scavenging activity (mg ascorbic acid equivalents /g dry matter)	FRAP value (mmol Fe^{2+}/100 g dry matter)
100	6.77±0.24^{f}	4.25±0.08^{e}
120	7.05±0.26^{f}	4.44±0.15^{e}
140	8.03±0.19^{e}	6.02±0.27^{d}
160	9.71±0.21^{c}	7.36±0.46^{b}
180	9.16±0.03^{d}	6.55±0.05^{c}
200	12.42±0.08^{a}	8.67±0.30^{a}
220	12.03±0.14^{b}	8.92±0.12^{a}

Data are given as means ± SD (n=3)

The means in each column followed by a common letter are not significantly different by Duncan's multiple range tests at $p < 0.05$.

Table 5. DPPH free radical activities and ferric reduction antioxidant power (FRAP) values of pumpkin leaves hydrolyzed by subcritical water at various residence times at 160°C.

Residence time (min)	DPPH scavenging activity (mg ascorbic acid equivalents /g dry matter)	FRAP value (mmol Fe^{2+}/100 g dry matter)
10	7.39±0.02^{d}	5.74±0.01^{c}
20	9.71±0.21^{b}	7.36±0.46^{a}
30	9.35±0.07^{c}	6.71±0.04^{b}
40	9.40±0.16^{c}	7.12±0.16^{ab}
50	10.16±0.06^{a}	7.16±0.14^{a}

Data are given as means ± SD (n=3)

The means in each column followed by a common letter are not significantly different by Duncan's multiple range tests at $p < 0.05$.

3.3. Color values

Color values of subcritical water hydrolysate from pumpkin leaves are shown in Table 6 at various temperatures for 20 min and in Table 7 at various reaction times at 160°C. As hydrolysis temperature increased from 100°C to 140°C, color values L and b of the hydrolysate gradually decreased. At high temperatures (160°C to 220°C), color values L and b decreased suddenly and were leveled off. Color value a showed peak at 160°C and then decreased as temperature increased from 160°C to 220°C. All color values L, a, and b decreased gradually with reaction time.

Singh and Saldana [2011] reported that at higher temperatures (140°C-180°C) color of potato peel extract was darker than at lower temperatures (100°C-120°C). But, at temperatures of 180°C to 240°C, the color of the extract became very dark due to sample pyrolysis. Watchararuji et al. [2008] also reported that L values of subcritical water hydrolysates from rice bran and soybean meal decreased when hydrolysis time increased from 10 to 30 min at 200°C, 210°C, and 220°C because of maillard reaction.

Table 6. Color values of pumpkin leaves hydrolyzed by subcritical water at various temperatures for 20 min.

Hydrolysis temperature (°C)	*L*	*a*	*b*
100	48.42±0.19^{a}	3.47±0.07^{d}	11.57±0.31^{a}
120	48.35±0.09^{a}	3.76±0.02^{c}	11.67±0.10^{a}
140	47.62±0.37^{b}	4.26±0.03^{b}	11.04±0.52^{b}
160	43.97±0.09^{c}	5.49±0.07^{a}	6.14±0.14^{c}
180	41.34±0.02^{d}	3.14±0.02^{e}	1.85±0.03^{d}
200	40.19±0.01^{e}	1.98±0.03^{f}	0.07±0.02^{e}
220	40.00±0.04^{e}	1.62±0.02^{g}	-0.29±0.03^{e}

Data are given as means ± SD (n=3)

The means in each column followed by a common letter are not significantly different by Duncan's multiple range tests at $p < 0.05$.

Table 7. Color values of pumpkin leaves hydrolyzed by subcritical water at various residence time at 160°C.

Residence time (min)	*L*	*a*	*b*
10	46.36±0.13[a]	5.49±0.08[a]	9.72±0.23[a]
20	43.97±0.09[b]	5.49±0.07[a]	6.14±0.14[b]
30	43.30±0.05[c]	5.22±0.02[b]	4.90±0.02[c]
40	43.34±0.04[c]	5.17±0.05[b]	4.92±0.06[c]
50	42.65±0.01[d]	4.60±0.02[c]	3.75±0.04[d]

Data are given as means ± SD (n=3)

The means in each column followed by a common letter are not significantly different by Duncan's multiple range tests at $p < 0.05$.

4. Conclusions

When phenolic compounds analysis, subcritical water hydrolysis compared to the well-known solvent extraction has many advantages such as non-toxic, shorter extraction time, low costs of the extracting agents. It can be seen through the study, production of phenolic compounds from pumpkin leaves was strongly influenced by hydrolysis temperature instead of reaction time. The experimental results of many the best condition was 160℃, 20 min.

The results of this study suggest that at 160℃ for 20 min, the highest yield of phenolic compounds and favorable color of the hydrolysate from pumpkin leaves was obtained. But higher temperature and longer time were showed that decreased yield of pehnolic compounds, and were unfavorable color. Antioxidant activity of the hydrolysate increased as the increase of hydrolysis temperature, but was not proportional to the content of phenolic compounds in the hydrolysates at higher temperatures.

We analysed the individual phenolic compounds and antioxidant activities by subcritical water hydrolysis of pumpkin leaves. However, further studies with different sample are needed because it is still lacking by the individual phenolic compounds analysis study by subcritical water hydrolysis, also for clarifying degradation products from phenolic compounds with high antioxidant activity.

국문요약

페놀 화합물의 생산을 증진시키기 위해 호박잎을 아임계수 처리 온도((100-220°C, 20 min)와 처리 시간(10-50 min, 160°C)을 달리하여 가수분해하였다. 호박잎 가수분해물의 주요 페놀 성분은 caffeic acid, *p*-coumaric acid, ferulic acid와 gentisic acid이었다. 대부분의 페놀 화합물은 160°C에서 최고 함량을 보였으나 gentisic acid의 경우는 180°C에서 최고 함량을 나타내었다. 총페놀 화합물의 양은 100°C, 120°C, 140°C에서 각각 48.1, 52.2, 78.4 μg/g dry matter로 가수분해 온도의 증가에 따라 점차 증가하는 경향을 보였으며, 160°C에서 1,447.1 μg/g dry matter로 최고 함량을 나타내었다. 그러나 160°C 이상의 온도에서는 총페놀 화합물의 수율은 감소하는 경향을 보였다. Caffeic acid와 ferulic acid는 20 min에서, cinnamic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, 그리고 protocatechuic acid는 30 min에서 가장 높은 함량을 보였다. 항산화 활성은 온도 증가에 따라 DPPH는 6.77-12.42 mg ascorbic acid equivalents/g dry matter, FRAP은 4.25-8.92 mmol Fe^{2+}/100 g dry matter 값을 보였다. 색차 분석에서는 *L*과 *b* 값이 가수분해 온도가 100-140°C로 증가했을 때는 서서히 감소하는 경향을 보였으나, 높은 온도 (160-220°C)에서는 급격하게 감소하는 경향을 보였다. *a* 값의 경우 100-160°C에 이르기 까지 서서히 올라가고 160°C에서 최고점을 나타냈지만, 온도가 증가할수록 감소하는 경향을 보였다. 이상의 결과로부터 호박잎의 아임계수 가수분해물은 가수분해 시간보다는 온도의 영향이 더 큰 것으로 나타났으며, 페놀 화합물 함량과 항산화 활성을 증가시키는데 도움을 줄 것으로 추정되었다.

References

1. Mukhopadhyay S, Luthria DL, Robbins RJ. Optimization of extraction process for phenolic acids from black cohosh (*Cimicfuga racemosa*) by pressurized liquid extraction. J. Sci. Food Agric. 86: 156-162 (2006)

2. Chiou A, Karathanos VT, Mylona A, Salta FN, Preventi F, Andrikopoulos NK. Currants (*Vitis vinifera* L.) content of simple phenolics and antioxidant activity. Food Chem. 102: 516-522 (2007)

3. Kunyanga CN, Imungi JK, Okoth MW, Biesalski HK, Vadivel V. Total phenolic content, antioxidant and antidiabetic properties of methanolic extract of raw and traditionally processed Kenyan indigenous food ingredients. *LWT*-Food Sci. Technol. 45: 269-276 (2012)

4. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chem. 99: 191-203 (2006)

5. Amashukeli X, Pelletier CC, Kirby JP, Grunthaner FJ. Subcritical water extraction of amino acids from Atacama desert soils. J. Geophys. Res. 112: 1-10 (2007)

6. Shitu A, Izhar A, Tahir TM. Sub-critical water as a green solvent for production of valuable materials from agricultural waste biomass: A review of recent work. Global J. Environ. Sci. Manage 1: 255-264 (2015)

7. Plaza M, Turner C. Pressurized hot water extraction of bioactives. Trends

Anal. Chem. 71: 39-54 (2015)

8. Kim SR, Ha TY, Song HN, Kim YS, Park YK. Comparison of nutritional composition and antioxidative activity for Kabocha squash and pumpkin. Korean J. Food Sci. Technol. 37: 171-177 (2005)

9. Que F, Mao L, Fang X, Wu T. Comparison of hot air-drying and freeze-drying on the physicochemical properties and antioxidant activities of pumpkin (*Cucurbita moschata* Duch.) flours. Int. J. Food Sci. Technol. 43: 1195-1201 (2008)

10. Lee HJ, Do JR, Kwon JH, Kim HK. Physiological activities of *Cucurbita moschata* Duch. Extracts with different extraction conditions. J. Korean Soc. Food Sci. Nutr. 39: 165-171 (2010)

11. Valenzuela GM, Soro AS, Tauguinas AL, Gruszycki MR, Cravzov AL, Gimenez MC, Wirth A. Evaluation polyphenol content and antioxidant activity in extracts of *Cucurbita* spp. Open Access Library J. 1: 1-6 (2014)

12. Cha YY. Experimental study on effects of *Cucurbita moschata* Duch. on antioxidation. J. Soc. Korean Med. Obesity Res. 9: 57-63 (2009)

13. Kim MJ, Hong CO, Nam MH, Lee KW. Antioxidant effects and physiological activities of pumpkin (*Cucurbita moschata* Duch.) extract from different aerial parts. Korean J. Food Sci. Technol. 43: 195-199 (2011)

14. Kim MB, Park JS, Lim SB. Antioxidant activity and cell toxicity of pressurized liquid extracts from 20 selected plant species in Jeju, Korea. Food Chem. 122: 546-552 (2010)

15. Kamiloglu S, Pasli AA, Ozcelik B, Van Camp J, Capanoglu E. Colour retention, anthocyanin stability and antioxidant capacity in black carrot (*Daucus carota*) jams and marmalades: Effect of processing, storage conditions and *in vitro* gastrointestinal digestion. J. Funct. Food 13: 1-10 (2015)

16. Thaipong K, Bonnprakob U, Crosby K, Cisneros-Zevallos L, Hawkins DB. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extract. J. Food Composit. Anal. 19: 669-675 (2006)

17. Singh PP, Saldana MDA. Subcritical water extraction of phenolic compounds from potato peel. Food Res. Int. 44: 2452-2458 (2011)

18. Cheigh CI, Chung EY, Chung MY. Enhanced extraction of flavanones hesperidin and narirutin from *Citrus unshiu* peel using subcritical water. J. Food Eng. 110: 472-477 (2012)

19. Ko MJ, Cheigh CI, Cho SW, Chung MS. Subcritical water extraction of flavonol quercetin from onion skin. J. Food. Eng. 102: 327-333 (2012)

20. Jose RVS, Bulnes P, Zuniga MC, Jimenez JP, Torres JL, Mateos-martin ML, Agosin E, Jose RPC. Effect of pressurized hot water extraction on antioxidants from grape pomace before and after enological fermentation. J. Agric. Food Chem. 61: 6929-6936 (2013)

21. Rangsriwong P, Rangkadilok N, Satayavivad J, Goto M, Shotipruk A. Subcritical water extraction of polyphenolic compounds from *Terminalia*

chebula Retz. fruits. Sep. Purif. Technol. 66: 51-56 (2009)

22. Khuwijitjaru P, Sayputicasikorn N, Samuhasaneetoo S, Penroj P, Siriwongwilaichat P, Adachi S. Subcritical water extraction of flavoring and phenolic compounds from cinnamon bark (*Cinnamomun zeylanicum*). J. Oleo Sci. 61: 349-355 (2012)

23. Watchararuji K, Goto M, Sasaki M, Shotipruk A. Value-added subcritical water hydrolysate from rice bran and soybean meal. Bioresour. Technol. 99: 6207-6213 (2008)

논문 발표 및 투고 실적

Kim MB, Ko JY, Ko MO, Lim SB, Formulation optimization of antioxidant-rich juice powders based on experimental mixture design, 2015 KoSFoST International Symposium and Annual Meeting, Korean Society of Food Science and Technology, June 3-5, 2014, Bexco, Busan, Korea, P11-016, p. 344

Ko MO, Ko JY, Kim MB, Lim SB, Antimicrobial activity of benzyl isothiocyanate against oral and fish microorganisms. 2015 KoSFoST International Symposium and Annual Meeting, Korean Society of Food Science and Technology, June 3-5, 2014, Bexco, Busan, Korea, P12-078, p. 432

Kim MB, Ko JY, Lim SB. Optimization of high hydrostatic pressure extraction of phenolic compounds from *Brassica juncea* using response surface methodology, 2015 KFN International Symposium and Annual Meeting, The Korean Society of Food Science and Nutrition, Aug. 24-26, 2015, Alpensia Convention Center, Pyeongchang, Korea, P03-40, p. 288

Ko MO, Ko JY, Kim MB, Lim SB. Antimicrobial activity of isothiocyanates against oral microorganisms, 2015 KFN International Symposium and Annual Meeting, The Korean Society of Food Science and Nutrition, Aug. 24-26, 2015, Alpensia Convention Center, Pyeongchang, Korea, P05-28, p. 317

Ko MO, Ko JY, Kim MB, Lim SB. Antimicrobial activity of radish root extract against oral microorganisms, 2015 KFN International Symposium and Annual Meeting, The Korean Society of Food Science and Nutrition, Aug. 24-26, 2015, Alpensia Convention Center, Pyeongchang, Korea, P05-29, p. 317

고미옥, 고정연, 김미보, 임상빈, 십자화과 채소 유래 isothiocyanates의 넙치 어병세균에 대한 항균활성, 한국식품저장유통학회지 22(6), 886-892(2015)

Kim MB, Ko JY, Lim SB, Formulation optimization of antiodidant-rich juice powders based on experimental mixture design, International Journal of Food Processing and Preservation (2016), Accepted

Ko JY, Ko MO Kim DS, Lim SB, Enhanced production of phenolic compounds from pumpkin leaves by subcritical water hydrolysis, Preventive Nutrition and Food Science (2016), Submitted