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Physiological Functionalities and Anti-oxidant activity of heated radish extract

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Abstract

This study proceeded an experiment that can increase such physiological properties of heated radish extract. During the extraction of radish, including the byproduct, an increase in antioxidant properties of radish according the heating temperature was performed. Upon the extracts of radish bark and radish green extract(mucheong), the physiological functionalities and antioxidant activity were investigated. As a result, the color of radish ethanol extract in dependence of heating temperature, showed light brown color at low heating temperature and black color from 150℃. The total polyphenol content significantly increased as a result of heat treatment; 6.7 times and 22 times higher than the control at 110℃ and 150℃, respectively. DPPH radical scavenging ability and antioxidant property increased with increasing heating temperature; in comparison to heat-treated radish at 110℃ and 150℃, IC_{50} *decreased by 1/22 times.* IC_{50} *of the control was 23times higher than 150℃ heat treated radish (Control* IC_{50}*:130.305). According to the graph that represents ABTS activity, antioxidant activity increased in dependence of heat treatment likewise to the total polyphenol content and DDPH radical scavenging activity. Upon heat treatment at 150℃, antioxidant activity in consequence of ABTS assay increased 23 times higher than the control.*

Keywords: *Anti-Oxidant Activity, Heated Radish Extract, PolyPhenol, Functionality*

1. Introduction

With the beginning of the era of well-being, maintenance and promotion of health has become a central issue in South Korea as it entered aged society from aging society. As the consumption of functional food has been trending upwards, studies on the development of functional products for prevention of various adult diseases are on attention. In particular, functional foods manufactured using extract of radish, a representative vegetable of Korea, have high abundance of enzymes and physiologically active substances that have been reported for new physiological regulating functions, promotion of metabolism and prevention of various diseases. For that reason, demand for functional foods based on radish extract are increasing and both domestic and overseas markets are expanding. The radish displays antioxidant properties and many research for using the antioxidant features of the radish are performed. Radish skin and radish greens (*mucheong*) are an edible part of radish. But they are removed before eating radish and used as a byproduct or

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an animal feed material because of their tough and rough texture. In an attempt to investigate the processing methods for increasing the physiological activities of the radish, this study examined whether antioxidant properties of radish can be increased by a simple processing method of radish including the byproduct [1, 2, 3]. The study relates to an antioxidant composition comprising a heated radish extract that is processed to increase the antioxidant activities of radish. The radish (*Raphanus sativus L.*) is a vegetable of the family Cruciferae containing volatile sulfur-compounds that cause its unique spiciness. The spiciness peculiar to the radish is caused by the production of thiocyanate and isothiocyanate released enzymatically by glucosidase from the thioglucoside when the radish is cut into broken cells. The radish contains a larger amount of free amino acids, sugars, calcium, phosphorus, etc. than other vegetables. The root of radish contains sugar components like glucose and fructose and other ingredients, such as coumaric acid, caffeic aid, ferulic acid, phenylpyruvic acid, gentidin acid, hydroxyl benzoic acid, and a variety of amino acids. Particularly, it contains vitamin C amounting up to 20 to 25 mg and becomes an important source of vitamin C in winter. According to the ancient medicinal records, the root of radish, *nabok*, has curative effects on phlegm, coughing, dysentery, etc. and eliminates food poisoning associated with fish, shellfish, and noodles. Diastase contained in the radish is used to promote digestion, neutralize the effects of food poisoning, and ease a hangover. Rapine is known as an antibiotic component against germs, fungus, parasites, etc [4, 5, 6].

The human body has antioxidant compounds (radical scavengers) like superoxide dismutase (SOD), catalase, vitamin E, vitamin C, uviquinol, etc. But, aging, air pollution, UV radiations, and stress weaken those antioxidant systems and cause an increase in the concentration of reactive radical oxygen species with high reactivity, such as superoxide radicals, hydroxyl radicals, hydrogen peroxide, and singlet oxygen, leading to the occurrence of oxygen toxicity which is fatal to the human body. Hence, antioxidants such as compounds capable of removing reactive radical oxygen species or the substances for preventing formation of peroxide compounds are expected to be anti-aging agents or curative agents against various diseases caused by those oxide compounds. These compounds are applicable in various fields including medicinal, agricultural, or food industries as antioxidants for preventing deterioration of nutrition and quality of foods caused by acidification [6, 7, 8]. Therefore, this study proceeded an experiment that can increase such physiological properties of heated radish extract. During the extraction of radish, including the byproduct, an increase in antioxidant properties of radish according the heating temperature was performed. Upon the extracts of radish bark and radish green extract, the physiological functionalities and antioxidant activity were investigated.

2. Experiment Materials

2.1 Heat treatment of radish

Radishes were purchased from the wholesale market. Radish skin and radish greens (*mucheong*) were washed and then used. The heat treatment device used was heat treatment furnace (Jisco, Seoul, Korea) designed to tolerate a pressure of at least 10 kg/cm^2. The whole radish sample was put in a container, which was put in an outer container filled with a defined amount of water and then heated to a predetermined temperature for a predetermined period of time to prevent carbonization of the sample by direct heat transfer. During the heat treatment, the water vapor was eliminated. The temperature for the heat treatment was set to 110 °C, 120 °C, 130 °C, 140 °C, or 150 °C. The time for the heat treatment was 2 hours [9].

2.2 Preparation of extract of heated radish

After cooled down, the heated radish sample was pulverized with a grinder and fluxed in 1/10 (v/v) diluted 70% ethanol to perform extraction for 3 hours. The extraction liquid obtained was filtered and then

concentrated for use. For a comparison, an extract was prepared from a sample without heat treatment in the same manner as described above and used as a control. All the following tests were repeatedly performed three times[10].

3. Experiments Method

3.1 Chromaticity and browning degree

The color change of the extract heated at each temperature in Example was measured with a color difference meter (Minolta, CR-200, Tokyo, Japan) to determine the "L" value (lightness), the "a" value (redness), and the "b" value (yellowness). To determine the browning degree, the heated extract sample was subjected to a 15-fold dilution and then measured in regards to absorbance at 420 nm using a spectrophotometer (Shimadzu, UV-1650, PC, Tokyo, Japan) [11].

3.2 Determination of total polyphenol content

The total polyphenol content was determined according to the Folin-Ciocalteu method using the phenomenon that the polyphenol material reacts with a phosphomolybdic acid to take a blue color. For this procedure, 2 ml of a 2% Na_2CO_3 solution was added to 100 µl of the extract. The mixture was stood for 3 minutes, and 100 µl of a 50% Folin-Ciocalteu reagent was added to the mixture. After a 30-minute reaction in darkness, the absorbance was measured at 750 nm to determine the phenol content. Tannic acid was used as a reference material [12].

3.3 Determination of DPPH radical scavenging capacity

0.8 ml of a 0.02mM DPPH (1,1-diphenyl-2-picryl hydrazyl, Sigma Aldrich) solution was added to the extract. The mixture thus obtained was shaken for 10 seconds with a vortex, kept at room temperature for 30 minutes, and then measured in regards to the absorbance at 525 nm using a spectrophotometer (Beckman Coulter, DU-650, Anaheim, CA, USA). The radical scavenging capacity (%) as a function of the concentration of the extract was calculated according to the following equation, and the concentration IC_{50} representing the 50% radical scavenging capacity was determined [13].

DPPH Radical Scavenging Capacity (%) = = [1-{A(sample)- B (sample blank)}/ C(control)] x 100

3.4 ABTS cation decolorization assay

The total antioxidant capacity of each extract was determined according to the $ABTS^+$ cation decolorization assay method. 7.4 mM 2,2-azino-bis-(3-ethylbenozothiazoline-6-sulfonic acid) (ABTS) and 2.6 mM potassium persulphate were left in darkness for one day to form $ABTS^+$ cations. The solution thus obtained was diluted with distilled water using a water absorption coefficient ($\varepsilon = 3.6 \times 10^4\ M^{-1}cm^{-1}$) so that the absorbance at 735 nm was 1.4 to 1.5. 50 µl of the extraction liquid was added to 1 ml of the diluted $ABTS^+$ solution, and the change of the absorbance thus obtained was measured exactly in 60 minutes after the addition. The reference material was L-ascorbic acid (Sigma Aldrich) to indicate the ascorbic acid equivalent antioxidant capacity (AEAC) [14].

3.5 Estimation of physiological function

The SOD-like activity was homogenized after adding 20mL of sample solution, following the method of Markland et al [15]. The pH of the supernatant, obtained from centrifuging, was adjusted to 8.2 and used as a sample solution after being made up to 50mL using Tris-cacodylic acid buffer (TCB). To 950µL of the sample

solution, 50μL of 24mM pyrogallol was added to measure the increasing rate of absorbance at 420nm during the first two minutes. The sample solution was compared with the no-adding solution [15].

SOD-like activity (%) = [{A(sample)-B(control)}/ A(sample)] x 100

Xanthine oxidase inhibitory activity was measured via the following. To 600μL of 0.1M potassium phosphate buffer (pH 7.5), 100μL of the sample dissolved in 1 mg/mL was added. The solution was left for five minutes at 37°C. 200μL of 1NHCl was added to cease the reaction. Then, the solution was centrifuged at 12,000 rpm for ten minutes. After the removal of proteins, uric acid was measured at 292nm.

Xanthine oxidase inhibitory activity (%) = [1 – {A(sample) – B(sample blank)}/ C(control)] x 100

Angiotensin I-converting Enzyme (ACE) inhibitory activity was measured via Cushman et al method [14] 50μL of the extract was obtained from treating the sample solution with ethylacetate of its equal amount. The extract was mixed with 150μL of ACE solution extracted from rabbit lung powder and 50μL of substrate solution (2.5 mL solution of 100mM sodium borate buffer solution at pH 8.3, 300mM NaCl and 25mg Hip-His-Leu). The latter solution was left for 30 minutes at 37°C. 200μL of 1NHCl was added to cease the reaction. The amount of hippuric acid, a product of this reaction, was calculated by measuring the absorbance of the solution at 228nm. The inhibition rate was calculated by using no-added sample as a control.

ACE inhibitory activity (%) =
[{C(control) – T(sample treatment)}/ {C(control) – B(substrate treatment)}] x 100

HMG-COA reductase inhibitory activity was measured via the following [15] 50mM phosphate buffer solution (pH 7.0) 100μL, 2mM DTT 100μL, 0.5mM β-NADPH 100μL, HMG-CoA reductase crude enzyme solution 50μL (produced from E. coli BL21 (DE3) carrying pKFT 7-21 recombinant DNA), 0.3mM HMG-CoA 100μL and 10μL of extract dissolved in distilled water at 10μg/μl were mixed. The latter solution was left for three minutes and its absorbance at 340nm was measured using spectrometer. Simultaneously, the absorbance of a control, which was a distilled water, at 340nm was measured and compared with the sample to calculate inhibitory activity.

HMG-CoA Inhibitory Activity (%) = [1-(sample/control)] x 100

Acetylcholinesterase (AChE) inhibitory activity was measured via Ellman method [16] After the addition of 0.1M sodium phosphate buffer (pH 7.3) 100μL, 20μL of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) was added and mixed. Then, 30μL of acethylthiocholine chloride was added. At 37°C, the production of reactive substance, 5-thio-2-nitrobenzoiate, was measured at 415nm for 60 minutes.

AChE inhibitory activity (%) = [1-(sample/control)] x 100

Fibrinolytic activity was solidified via the addition of 0.6% fibrinogen dissolved in phosphate buffer at pH 7.0 to a plate medium containing 0.1 unit thrombin per μL. A paper disc containing 25μL of the sample was incubated at 37°C for six hours, and the size of the transparent ring was measured to indicate the thrombolytic activity in mm [17].

4. Result and Discussion

4.1 Yied of extract of heated radish

The yield of the ethanol extract, which was 4.883 wt.% for the control, was increased to 5.709 wt.% at 130 °C with an increase in the temperature of the heat treatment and decreased to 5.584 wt.% and 5.186 wt.% at 140 °C and 150 °C, respectively.

4.2 Chromaticity and browning degree

The color change of the extract heated at each temperature in Example was measured with a color to determine the "L" value (lightness), the "a" value (redness), and the "b" value (yellowness).According to the measurement results in Table 1, the "L" value representing the lightness, which was 98.65 for the control (without heat treatment), decreased with an increase in the temperature of the heat treatment and amounted to 57.48 at 150 °C. The "a" value representing the redness had no significant change (0.34 to 1.31) at relatively low temperature of the heat treatment and abruptly increased to 9.22 and 14.83 at 140 °C and 150 °C, respectively. The "b" value representing the yellowness had no significant change (1.54 for the control and 3.96 at 110 °C). But it was abruptly increased to 26.37 and 25.99 at 120 °C and 150 °C, respectively. Overall, the ethanol extract of radish according to the temperature of the heat treatment took light brown at relatively low temperatures and black at 140 °C or above.

Table 1. The color change of the extract heated at each temperature in radish

Samples	Redness (at 420 nm)	Color		
		L-value	a-value	b-value
Control	0.118±0.005	98.65±0.41	2.39±0.03	1.54±0.02
110 °C	0.150±0.002	97.61±0.16	1.25±0.02	3.96±0.02
120 °C	0.468±0.004	90.55±0.10	0.34±0.03	26.37±0.09
130 °C	0.705±0.014	81.73±0.32	1.31±0.02	28.55±0.08
140 °C	1.022±0.046	64.58±0.15	9.22±0.04	29.67±0.05
150 °C	1.436±0.009	57.48±0.11	14.83±0.10	25.99±0.02

4.3 Determination of total polyphenol content

The total polyphenol content was determined according to the Folin-Ciocalteu method using the phenomenon that the polyphenol material reacts with a phosphomolybdic acid to take a blue color. As shown in FIG. 1, there was an abrupt increase in the polyphenol content upon the heat treatment, so the polyphenol content of the extract heated at 150 °C was 22 times as high as that of the control and about 6.7 times as high as that of the extract heated at 110 °C.

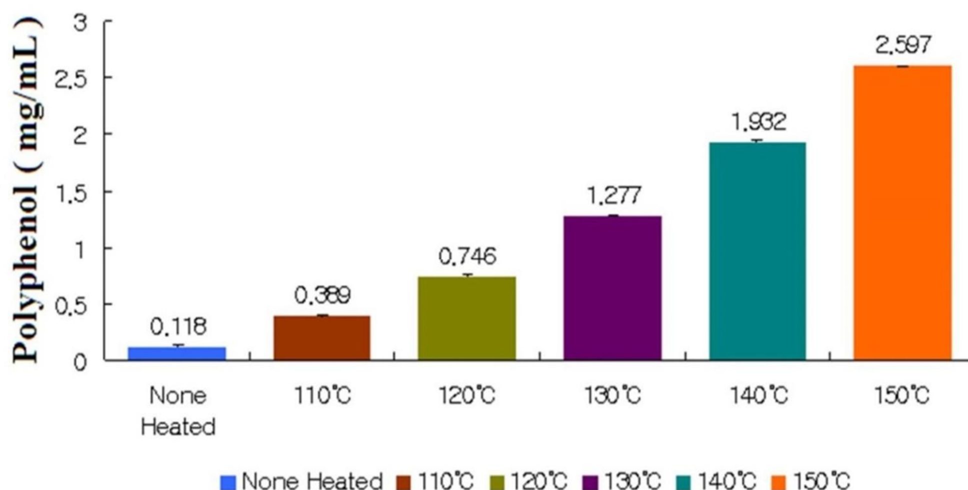

Figure 1. The change of the polyphenol content in the extract by the heat treatment of radish.

4.4 Determination of DPPH radical scavenging capacity

The radical scavenging capacity as a function of the concentration of the extract was calculated according to the equation, and the concentration IC_{50} representing the 50% radical scavenging capacity was determined. The measurement results were presented in FIG. 2. The DPPH radical scavenging capacity increased dependently on the heat treatment temperature of the radish. For example, the IC_{50} value of the radish heated at 150 °C was decreased to the 22th of that of the radish heated at 110 °C, which explicitly showed a great increase in the antioxidant effect. If not shown in FIG. 2, the control had an IC_{50} value of 130.305, 234 times as high as that of the radish heated at 150 °C[18].

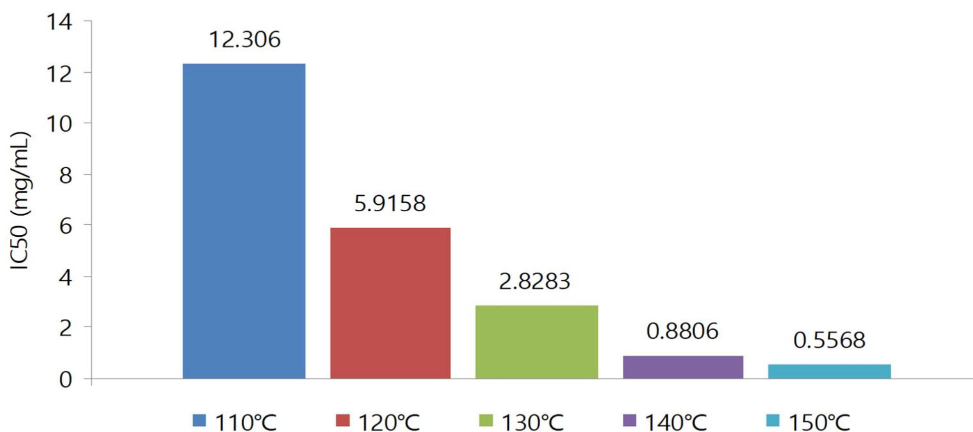

Figure 2. The change of the DPPH radical scavenging capacity of the extract by the heat treatment of radish.

4.5 ABTS cation decolorization assay

The total antioxidant capacity of each extract was determined according to the $ABTS^{+}$ cation decolorization assay method. FIG. 3 is a graph showing the evaluation results. Like the polyphenol content and the DPPH radical scavenging capacity, the antioxidant capacity had a temperature-dependent increase with an increase in

the heat treatment temperature. For example, the extract of the radish heated at 150 °C had an ABTS-assayed antioxidant capacity about 23 times as high as that of the control[19].

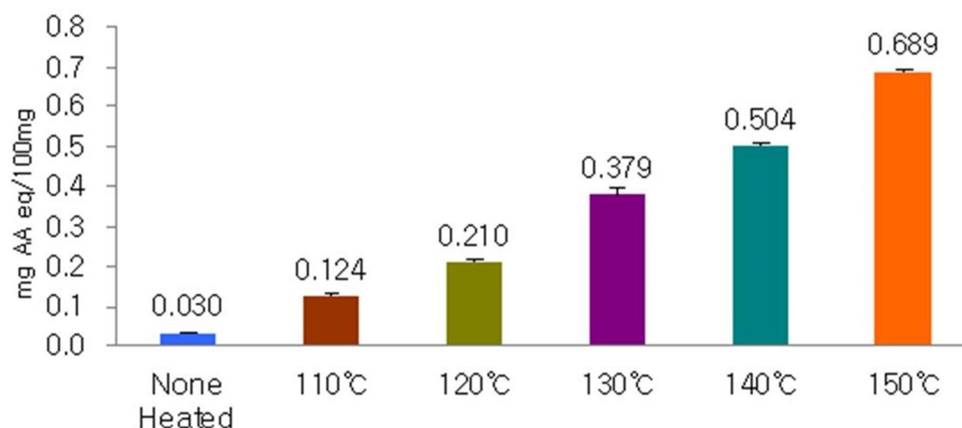

Figure 3. The results of the ABTS cation decolorization assay on the extract by the heat treatment of radish.

4.6 Physiological function of Heated Radish Extract

As high as 91.6% of physiological functions of radish extract were aging related anti-oxidative activity, while SODl-like activity and Xanthine oxidase inhibitory activity can only be as high as 21.6% and 9.3% respectively (Table 2). The higher antioxidant activity is due to the fact that the sample was a radish extract and that it has a high phenol content, an indicator of antioxidant activity of plants. Also, Kang et al [20] reported that electron donating ability increases with the increase in reducing power. Radish extract showed cholesterol synthesis inhibitory functions, 10.0% of anti-arteriosclerotic HMG-CoA reductase inhibitory activity and 5.1% of anti-dementic acethylcholinesterase inhibitory activity (Table 2). The following result was low in reference to a study by Kim et al [21]. In this study, ethanol extract of 865 species of medicinal plants showed 77% of HMG-CoA reductase inhibitory activity and 25 kinds of plant extracts showed inhibitory activity of more than 15%. Anti-hypertensive activity or thrombolytic activity was absent, which is presumed to be due to a small number of proteins or peptides in plants since most of the functional substances are peptides.

Table 2. Physiological functions of Heated Radish Extract

Antioxidant activity (%)	SOD-like activity (%)	Xanthine Oxidase Inhibitory activity (%)	HMG-CoA reductase inhibitory activity (%)	ACHE inhibitory activity (%)	Fibrinolytic activity (mm)
91.6	21.6	n.d*	10.0	5.1	n.d*

*n.d ; not detected

5. Conclusion

The study relates to an antioxidant composition comprising a heated radish extract that is processed to increase the antioxidant activities of radish. It is an object of the present invention to provide a composition with good antioxidant properties that is available in the fields of medicinal, cosmetic and food products as well as a process for increasing the antioxidant properties of the radish. To achieve the object of the present invention, there is provided an antioxidant pharmaceutical composition comprising an extract of radish heated at 100 to 200 °C. The heat treatment as used herein refers to heating the radish. Preferably, the radish is heated at higher temperature, because the antioxidant capacity increases with an increase in the temperature of the heat treatment. More preferably, the heat treatment is performed at 110 to 150 °C due to the risk of carbonization at extremely high temperature. Heating the radish contributes to the increase in the yield of the radish extract, the content of polyphenol, the DPPH radical scavenging performance, and the antioxidant capacity as evaluated by the ABTS cation decolorization assay. The ethanol extract of radish according to the temperature of the heat treatment took light brown at relatively low temperatures and black at 140 °C or above. The polyphenol content of the extract heated at 150 °C was 22 times as high as that of the control and about 6.7 times as high as that of the extract heated at 110 °C. The DPPH radical scavenging capacity increased dependently on the heat treatment temperature of the radish. Like the polyphenol content and the DPPH radical scavenging capacity, the antioxidant capacity had a temperature-dependent increase with an increase in the heat treatment temperature.

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