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Research paper



Effect of Heat Treatment on Physicochemical Properties and Antioxidant Activity of *Quercus acutissima*

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Abstract

This study aimed to determine changes in the physicochemical characteristics and antioxidant activity in acorn (Quercus acutissima) under different thermal treatments: A (140 °C), B (150 °C), C (160 °C), D (170 °C), and E (180 °C). A hot water extract (60 °C, 4 h) of each sample was prepared for measuring the physicochemical properties. The total phenolic and flavonoid content was determined spectrophotometrically. The pH of the heat-treated acorn was significantly lower than that of native acorn, and was inversely proportional to temperature ($p < 0.05$). The sugar content of acorn was significantly higher in groups heated below 160 °C than that of the control ($p < 0.05$). Total polyphenol content was the highest (0.031.59 ± 0.21 mg GAE/mg) in the group treated at the lowest temperature (140 °C) ($p < 0.05$). Antioxidant activity depended on dose ($p < 0.05$), 31.03–95.99% and 20.49–99.51% for DPPH and ABTS radical scavenging activities, respectively, in heat-treated groups. The IC50 value of DPPH and ABTS in acorn was as low as 0.13 mg/mL and 0.15 mg/mL in A group, respectively. Thus, heat treatment of acorn significantly increased the sugar and phenolic contents and antioxidant activity at lower heat treatment temperatures.

Keywords: *Quercus acutissima; acorn; heat treatment; polyphenol; antioxidant*

1. Introduction

Acorn (*Quercus* spp.) encompass all fruits in the family Fagaceae, which are found worldwide [1,2]. In South Korea, acorn mainly refers to the fruit of *Q. acutissima* [2]. Since acorns are non-toxic and contain approximately 65–70% starch [3], they have traditionally been used as a food resource during times of shortages in spring. To completely remove the tannins that create the characteristic astringent taste of acorns, the fruits are treated with water, from which starch powder is produced to produce foods such as Muk and Jeonbyeong [4]. Moreover, in Donguibogam (東醫寶鑑) and Ben Cao Gang Mu (本草綱目), acorns are effective in treating gastroenteritis, fatigue recovery, and relief from hangovers [4]. Recent studies have reported that acorns are rich in gallic acid, digallic acid, and gallotannin components, as well as in tannic acid, all of which are known to be excellent antioxidants. These all include antioxidant, anticancer, and antiviral activities; are effective in eliminating radioactive materials; and may be effective as anti-dementia drugs [1,2,5]. Thermal processes such as cooking, frying, toasting, sterilization, and roasting of foods induce reactions such as degradation, synthesis, and condensation in raw material components, leading to elevation in solid contents and alterations in the activities of various bioactive substances [6]. During heat treatment, a non-enzymatic browning reaction (Maillard Reaction, MR) occurs, which creates a brown, melanoidin pigment from the interaction between the carbonyl compounds of sugars and nitrogenous compounds of the amino group via various intermediate steps. In addition, during the MR, various flavor components are generated in the food, which undergo color change, and subsequently, induce antioxidants that have strong antioxidant effects on lipid acidification [1,7]. Therefore, the MR is responsible for aroma and color improvement in foods with heat application [7,8]. The formation of some substances is desired for flavor and aroma development and color improvement, for example, melanoidins from some foods such as coffee, malt, cacao, and bread [7]. However, some undesirable substances are also formed during the thermal process, such as furans, under different time and temperature conditions [9]. Total phenolics significantly decreased at a higher temperature and longer roasting time. The major coffee flavor component, pyrazines, decreased rapidly at 250 °C with an increase in the roasting temperature. Moreover, the highest antioxidant properties of coffee beans were found for the medium-dark roasted coffee brews under 200 °C for 15 min roasting under various roasting conditions [9]. When mung beans were roasted from 90 °C to 120 °C for 20 min, moisture decreased with increasing roasting temperature, whereas proximate contents including crude fat, crude ash, crude protein, and carbohydrate did not change significantly. The highest total phenol and flavonoids were achieved under roasting conditions of 110 °C. Moreover, radical scavenging activities were the highest under the same roasting conditions [11]. On the other hand, when a small black bean (*Rhynchosia nulubilis*) was roasted under temperatures ranging from 90 to 120 °C for 20 min, antioxidant components and activities were the highest at 120 °C [12]. The extracts of native and thermally treated acorn kernels showed high antioxidant activity, with the extracts of thermally treated kernels being more active than those of native ones. Hydrolysable tannins and gallic acid were identified in all samples, non-tannin phenolics including gallic acid were present in significantly higher quantities in thermally treated samples, and the tannin content decreased. This means that hydrolysable tannins were degraded during the thermal treatment [6]. Therefore, optimal heat treatment conditions can be determined under various conditions to release bioactive substances and antioxidant activities. The studies conducted so far have mainly focused on producing foods such as muk, tteok, dasik, cookies, and muffins using acorn starch and improvements in serum lipid content that depend on the addition of acorn starch and

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antidiabetic and anti-obesic efficacies of acorn powder. Recently, the use of acorn as tea through heat treatment such as frying or steaming has been proposed [13]. However, studies about changes in the characteristics of acorn that might depend on temperature during heat treatment are not enough. Therefore, the present study aims to provide fundamental data to identify the characteristics of acorn under different heat treatments. To achieve this, acorns heated to different temperatures and changes in their physicochemical components and antioxidants and their activities are compared with native acorn kernels.

2. Materials and Methods

2.1. Materials

Acorn (*Quercus acutissima*) was purchased from a traditional market in Chungbuk Province in 2015. An expert performed heat treatments using a roaster (Duett-M, Probat, Emmerich, Germany) under various temperature conditions--A (140 ℃), B (150 ℃), C (160 ℃), D (170 ℃), and E (180 ℃)--for 4 min and the samples were stored in the freezer until analysis. All acorn samples were ground using a food grinder (KSP-35, Koreamei Co. Ltd. Korea) for 30 s, homogenized with a 50-mesh, and extracted with 20× hot water (60 ℃, 4 h), followed by centrifugation (4,000 rpm, 4 ℃, 15 min). The supernatant was then collected and stored in a freezer until analysis. 1,1- Diphenyl-1-picrylhydrazy (DPPH), Folin-Ciocalteu phenol reagent, and ascorbic acid were obtained from Sigma-Aldrich (St. Louis, USA). All other reagents used for the analysis were purchased from Sigma-Aldrich and were of analytical grade.

2.2. Methods

2.2.1. Measurement of pH

Acorn extracts after heat treatment in a series of temperatures were diluted 50-fold in distilled water and the pH values were determined using a pH meter (Orion 3 Star, Thermo, Singapore). The pH values were triplicates of each sample and the average value was reported.

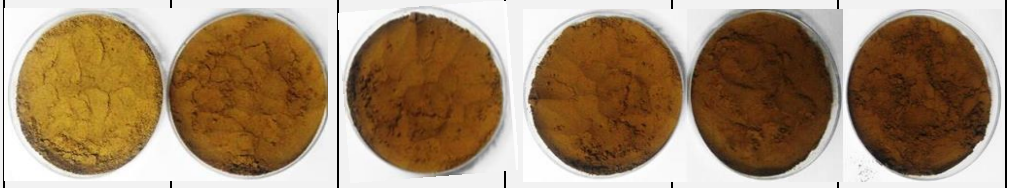
2.2.2. Measurement of sugar contents

Reducing sugar was measured using the dinitrosalicylic acid (DNS) method. The DNS reagent was prepared by mixing 1% 3,5-dinitrosalicylic acid (Sigma-Aldrich, St. Louis, MO, USA) and Rochelle salt (Sigma). One milliliter of acorn extracts was added to 2.0 mL of the DNS reagent, reacted in a boiling water bath for 10 min, and then cooled immediately for 10 min on ice. The absorbance was measured at 550 nm using a spectrophotometer (Optizen 3220UV, Mecasys, Daejeon, Korea), and the analyses were performed in triplicate. To determine the amount of reducing sugar, the values were averaged and a standard calibration curve was created using glucose (Sigma-Aldrich, St. Louis, MO, USA) as a reference.

2.2.3. Measurement of color value

A colorimeter (JC801S, Japan) was used for all color determinations. Measurements were initiated by inserting the sample into a round-bottomed light-projection tube (41 × 12.5 mm^2), as described in the previous study [14].

Table 1: Heat treatment conditions of acorn kernel

	control	A	B	C	D	E
Temperature (℃)	-	140	150	160	170	180
Representative picture of acorn sample						

The color measurements were performed in triplicate for all samples. L^* is a measure of the brightness from black (0) to white (100). The parameter a^* denotes red (100) to green color (-80), with positive a^* values indicating greenness. The parameter b^* denotes yellow to blue color, with positive b^* values indicating yellowness and negative b^* values indicating blueness.

2.2.4. Proximate component analyses

Each sample was ground to pass through a 100-mesh screen, and analyzed for percentage proximate analysis and mineral element content. The moisture content (105 ℃/12 h), ash content (550 ℃/5 h), crude fat in Soxhlet apparatus (solvent ether), crude protein by nitrogen determination using Kjeldhal's method (N × 5.95), and carbohydrate content by formula difference method were determined according to their respective methods given in AOAC [15].

2.2.5. Measurement of total polyphenol content

The content of total polyphenol compounds was measured based on the method described by Folin and Denis [16]. One milliliter of the extract after adjustment of the concentration (1 mg/mL) was mixed with 2 mL of 2% sodium carbonate, and incubated at room temperature for 3 min, to which 1 mL of 1 N Folin-Ciocalteu phenol reagent was added and the mixture was incubated in the dark for 30 min, followed by measurement of optical density (Optizen 3220UV, Mecasys, Daejeon, Korea) at 750 nm. After creating a standard curve using gallic acid as the reference, the total polyphenol content of each sample was calculated.

2.2.6. Measurement of flavonoid content

The total flavonoid content was analyzed using Moreno et al.'s method [17]. The respective extracted samples (0.5 mL) were mixed with 0.1 mL of 10% aluminum nitrate and 0.1 mL of 1 M potassium acetate and 4 mL of ethyl alcohol (80%), and the reaction was allowed to complete for 40 min at room temperature. Then, the absorbance was measured using a spectrophotometer (Optizen 3220 UV, Mecasys, Daejeon, Korea) at 415 nm. Quercetin (QE) was used as a standard and the results were calculated as QE equivalents (mg/mg) of the sample.

2.2.7. Measurement of DPPH radical scavenging activity

The DPPH radical scavenging activity was measured using the Blois method [18] with partial modification. Acorn extracts after heat treatment at various temperatures were prepared at different concentrations (0.12, 0.25, 0.50, and 1.00 mg/mL), and 4 mL of 0.1 mM DPPH was added to 1 mL of each extract, followed by incubation in the dark for 30 min, after which the sample was mixed for 30 s and the optical density (Optizen 3220UV, Mecasys, Daejeon, Korea) was measured at 517 nm.

$$\text{DPPH radical scavenging activity (\%)} = [(Abs_{DPPH} - Abs_{Sample})/Abs_{DPPH}] \times 100$$

Abs_{DPPH}: absorbance of the DPPH solution without extract
Abs_{Sample}: absorbance of the sample solution

2.2.8. Measurement of ABTS radical scavenging activity

The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity was measured using the Re method [19] with partial modification. A 900 µL sample of diluted ABTS solution was mixed with 100 µL of roasted acorn extract prepared at different concentrations (0.12, 0.25, 0.50, and 1.00 mg/mL) and reacted in the dark for 10 min, followed by measurement of optical density (Optizen 3220UV, Mecasys, Daejeon, Korea) at 734 nm. The equations for DPPH and ABTS scavenging activities were presented as optical density ratio (%) between groups with and without sample solution, with distilled water used as a negative control.

$$\text{ABTS radical scavenging activity (\%)} = [(Abs_{control} - Abs_{Sample})/Abs_{control}] \times 100$$

$Abs_{control}$: absorbance of the control solution without extract
Abs_{Sample} : absorbance of the sample solution

2.2.9. Statistical analysis

All measurements were repeated three times and a statistical analysis was performed using SPSS (version 18.0, package for Social Science, Chicago, IL, USA). A statistical significance test was conducted using the ANOVA test, and when there were statistically significant differences, the post-hoc test was performed by the Duncan's multiple test at $p < 0.05$.

3. Results

3.1 Effect of heat treatment on pH of acorn kernel

Table 2 presents the pH values of the acorn extracts, ranging from 5.43 to 5.70, at different heat treatment temperatures. The pH significantly decreased as the temperature was increased ($p < 0.05$). The pH value (6.18) of raw acorn extract in the present study was higher than that of the acorn crude starch powder, 5.26 [20]. Nam and Kang reported that the pH value of acorn was 5.99 after roasting at 250 °C for 10 min, which was comparable to our roasted sample's pH value. They also showed that the pretreatment of acorn with steaming or hot-air drying reduced the pH of acorn extracts, although they did not present the pH value of raw acorn [10].

3.2. Effect of heat treatment on sugar content of acorn kernel

The sugar content was 0.50–1.11 °Bx and increased in the A–C groups and then decreased at higher temperatures, as shown in Table 2. The heat treatment at low temperatures would have destroyed the acorn tissue, leading to changes in water-soluble substances and facilitating the extraction of water-soluble substances. However, at high temperatures over 170 °C, it can be thought that the sucrose derived from acorn kernels constantly decomposed into organic compounds such as hydroxymethylfurfural, furfural, and 5-methylfurfural and organic acids through the MR [6,7].

3.3. Effect of heat treatment on color value of acorn kernel

The L^* value was significantly decreased with heat treatment, and was lower in higher-temperature groups such as 170–180 °C, while the a^* values fluctuated by the heat treatment, that is, significantly decreased in the lower-temperature groups such as 140–160 °C and increased with higher heat treatment, e.g., that at 170–180 °C, compared to untreated acorn. The b^* values significantly decreased after heat treatment compared to the untreated acorn (Table 2). Different heating conditions can affect the color, phytochemical concentration, and antioxidant capacity of foods including acorns [10]. Since the non-enzymatic browning reaction typically proceeds rapidly from around

140 °C to 165 °C and caramelization, and subsequently, pyrolysis become more pronounced at higher temperatures [7], the darker color in the acorn extract with the higher heat treatment is natural in the present study.

Table 2: Physicochemical characteristics of acorn extract under various heat treatment conditions

Sample	pH	Sugar content (°Bx)	Color value					
			L^*		a^*		b^*	
control	†6.18 (0.02)[a]	0.50 (0.01)[d]	61.24	(0.08) [a]	8.82	(0.18) [c]	55.15	(0.35) [a]
A	5.70 (0.01)[b]	1.11 (0.01)[a]	50.46	(0.12) [b]	6.01	(0.08) [d]	33.12	(0.10) [d]
B	5.70 (0.02)[b]	1.00 (0.02)[b]	47.17	(0.11) [bc]	7.79	(0.05) [cd]	34.01	(0.05) [d]
C	5.63 (0.03)[c]	1.10 (0.02)[a]	43.66	(0.25) [c]	8.43	(0.01) [c]	36.02	(0.07) [c]
D	5.56 (0.01)[d]	0.90 (0.03)[c]	38.35	(0.22) [d]	10.24	(0.11)[b]	39.11	(0.14) [b]
E	5.43 (0.03)[e]	0.93 (0.06) [c]	37.15	(0.12) [d]	11.10	(0.21) [a]	42.20	(0.20)[b]

Control (native), heat treatment condition: A (140 °C), B (150 °C), C (160 °C), D (170 °C), and E (180 °C)
†mean (SD)
Values with different letters in superscripts within a column are significantly different at $p < 0.05$ according to Duncan's multiple range test.

3.4. Effect of heat treatment on proximate components of acorn kernel

The proximate compositions of acorn are shown in Table 3. The moisture content was naturally higher in native acorn than that in thermally processed acorn. After the heat treatment, the moisture content of acorn was reduced by 12.78–15.88% with an increase in temperature. The carbohydrate content was increased significantly by the heat treatment, but there was no significant difference in the increase in temperature among the experimental groups. There were no significant differences in other proximate compositions in acorn according to the thermal treatment. Acorns have been studied for their nutritional profile and phytochemical contents, presenting great variability among species and sometimes even within the same species. On the other hand, acorns are constantly considered as nutritionally rich products, justifying their use as secondary human foods, especially as the sources of carbohydrates, proteins, and fat or food ingredients for thousands of years wherever oak trees have been found. Rakić et al. reported that acorns were used as flour, generally for bread production, or as a coffee substitute beverage after the roasting process [1,6,21]. Before the last decade, acorns were mainly used for hog feeding due to their high contents of nutrients such as carbohydrates, proteins, and lipids, or as raw material for acorn oil production. However, recent scientific evidences have proposed several advantageous properties of acorns, such as rheological effects in bread, since the incorporation of limited amounts of acorn flour increased the bread volume and improved the crumb characteristics [22]. Above all, the main interest in acorns is derived from their plentiful phytochemical profile. Therefore, we investigated the phenolic contents and antioxidant activities after heat treatment in
acorns.

3.5. Effect of heat treatment on total polyphenol contents of acorn kernel

Phenolic compounds are classified under the group of bioactive compounds possessing strong antioxidant activity [23]. The contents of phenolic compounds in the extract of native and thermally treated kernels of acorns are presented in Table 4. The total polyphenol content of acorn extracts under different temperatures was significantly higher after heat treatment than in the control (0.193 ± 0.01 mg gallic acid equivalent [GAE]/mg). The A group, which had the lowest temperature (140 °C), contained the highest total polyphenol content (0.315 ± 0.02 mg GAE/mg), which was 163% greater than that in the control. However, the total phenolic content of the acorn extract significantly decreased as the temperature increased in the heat treatment groups ($p < 0.05$). Although the thermal effect on phenolic compounds of acorn kernels was determined in different cultivars (*Q. robur* and *Q. cerris.*) treated under much higher temperature conditions, such as 200 °C for 10 min, Rakić et al. [6] observed that the extracts of thermally treated acorn kernels had lower total phenolic content compared to those of native ones. Interestingly, the content of non-tannin phenolics, including gallic acid, was higher in thermally treated acorn kernels compared to the native ones; their content was about either 2.1 or 2.6 times higher according to the different cultivars (*Q. robur* and *Q. cerris.*). On the other hand, the tannin content was lower in thermally treated samples. This may describe the presence of thermally degradable hydrolysable tannins in native Oak kernels. It is reasonable to consider that the hydrolysable tannins present in the extracts of native kernels were degraded under high temperature, causing an increase in non-tannin phenolics and gallic acid content, and consequently, an increase in antioxidant activity of heat treatment. The potential effects of thermal treatment on the physical and nutritional characteristics of acorns were also evaluated in *Q. rober*. Recently, Toori et al. [24] evaluated the antioxidant activity and hepatoprotective effects of acorn extracts on carbon tetrachloride-induced liver damage in rats. According to the study results, chloroform and methanolic and aqueous extracts of the internal layer of oak fruit (cotyledons) exhibited high antioxidant power. Aqueous extracts at 250 and 500 mg/kg exhibited the highest antioxidant activity and superior hepatoprotective potential, suggesting that this solvent is a better alternative with no toxic effects. This is also the reason why water was chosen as the solvent in the present study. The results of the previous and current studies indicate that a higher thermal condition can expect different results on the degradation of total polyphenol for *Q. acutissima* in the further study.

Table 3: Proximate components of acorn kernel under various heat treatment conditions

Sample	Moisture (%)		Crude ash (%)		Crude fat (%)		Crude protein (%)		Carbohydrate (%)	
control	†35.24	(0.02) [b††]	1.38	(0.01) [ns]	2.42	(0.21) [ns]	1.45	(0.21) [ns]	58.69	(0.01) [ns]
A	15.88	(0.01)[c]	1.37	(0.02)	2.60	(0.12)	1.20	(0.01)	76.95	(0.41)
B	15.01.	(0.01)[c]	1.30	(0.04)	2.54	(0.44)	1.15	(0.24)	77.45	(0.04)
C	14.45	(0.05)[c]	1.30	(0.01)	2.33	(0.31)	1.04	(0.14)	78.56	(0.15)
D	13.12	(0.02)[c]	1.35	(0.05)	2.45	(0.45)	0.98	(0.05)	78.42	(0.01)
E	12.78	(0.01)[c]	1.33	(0.01)	2.56	(0.20)	1.05	(0.10)	78.91	(0.10)

control(native), heat treatment condition: A(140°C), B(150°C), C(160°C), D(170°C), E(180°C)
†mean (SD)
Values with different letters in superscripts within a column are significantly different at $p < 0.05$ according to Duncan's multiple range test.

ns: not significant

3.6. Effect of heat treatment on flavonoid contents of acorn kernel

Flavonoids are a common class of phenolic compounds that is attributed to antioxidant and lipid-reducing properties [23]. Table 4 shows the content of flavonoids in the extracts of native and thermally treated kernels of acorn. Flavonoid content of acorn extracts under different temperatures was significantly higher after heat treatment than in the control (0.047 ± 0.02 mg quercetin equivalent [QE]/mg). The E group, which had the highest temperature (180 °C), contained the highest flavonoid content (0.088 ± 0.02 mg QE/mg). The flavonoid content of acorn extract increased significantly only in the highest thermal treatment in the current study ($p < 0.05$). In a previous study, the flavonoid content in acorn kernels was very small in all investigated samples, *Q. robur* and *Q. cerris* regardless of the thermal treatment, although the heat treatment induced a little higher content of flavonoids in the kernel extract. These thermal processing effects on acorns agree with the present results, although *Q. acutissima* have slightly more flavonoids than *Q. robur* and *Q. cerris*. In a study conducted on the leaves of five *Quercus* species (*Q. acuta, Q. glauca, Q. mysinacfolia, Q. phylliracoides, and Q. salicina*), high levels (especially in *Q. salicina*) of gentisic, chlorogenic acids, as well as of the flavonoids naringin and rutin, were reported but none was detected in any other *Quercus* species [25]. On the other hand, flavanols and flavonols are the major constituents in holm oak (*Q. ilex*) leaves, some of them being exclusively found in this species.

3.7. Effect of heat treatment on antioxidant activity of acorn kernel

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [23]. The dark color of the DPPH radical solution becomes lighter when mixed with an antioxidant. It is widely used to evaluate the antioxidant activity of various compounds. Figs. 2 and 3 show the antioxidant activity of the acorn kernel extracts. Both DPPH and ABTS radical scavenging activities of acorn extracts exhibited strong and concentration-dependent manner. When the native kernel exhibited 20.78–87.18% DPPH radical scavenging activity at doses of 0.12–1.0 mg/mL, the heat treatment group exhibited 31.03–95.99% DPPH radical scavenging activity in dose-dependent manner, which was significantly different. Among the various thermal conditions, the IC_{50} value of acorn extracts was the lowest, i.e., 0.13 mg/mL, with the lowest thermal processing, such as 140 °C, in Table 5.

The ABTS radical scavenging activity of acorn had a similar pattern to the DPPH radical scavenging activity. When the native kernel exhibited 12.14–83.58% of ABTS radical scavenging activity at a dose of 0.12–1.0 mg/mL, those of thermally treated group exhibited 20.49–99.51% with concentration dependency. When comparing the temperature in the heat treatments, A group with the lowest heat treatment temperature (140 °C) had the highest antioxidant activity according to the ABTS radical scavenging activity test ($p < 0.05$). Among the various thermal conditions, the IC_{50} value of acorn extracts was the lowest, i.e., 0.15 mg/mL, with the lowest thermal processing, such as 140 °C, in Table 5. The DPPH activity significantly decreased in the D group (170 °C) and E group (180 °C) ($p < 0.05$), whereas at lower temperatures, the ABTS radical scavenging activity also decreased with increasing heat treatment temperature ($p < 0.05$). The difference in antioxidant activity was particularly prominent at lower thermal processing. The concentration-dependent DPPH radical scavenging activities of ascorbic acid exhibited 27.44–98.09% as reference. As for the concentration-dependent ABTS radical scavenging activities of ascorbic acid exhibited 32.57–100% as reference. This is in accordance with the results of some previous investigations concerning the type of *Quercus* acorns polyphenols and their antioxidant activity. In the ethyl-acetate fraction of *Q. acutissima* acorns, gallic acid, digallic acid, and gallotannin were identified as its high antioxidant activity [1,27]. Methanol extracts of three different *Quercus* acorns (*Q. ilex, Q. rotundifolia, and Q. suber*) presented hydrolysable tannins. These are all gallic acid derivatives, and exhibited antioxidant activity similar to those of BHA and Troloxs [27]. The acorns of *Q. rober* were also proposed to be convenient nutritional contents with significant antioxidant effects. The antioxidant activity of the aqueous extracts of thermally treated *Q. rober* kernels (0.04%) in the Schall oven test was comparable to the activity of 0.02% BHA [26]. Two of the most common extraction solvents are acidified methanol and ethanol [1]. Nevertheless, several other solvents have been described in the literature, in terms of their phytochemical properties and a further evaluation of their biological activity [1]. Shim [3] observed the acorn's antioxidant components and activity using extracts of *Q. acutissima* acorn with various solvent systems including water, methanol, ethanol, 75% ethanol, and $CHCl_3$. The water extract was the best antioxidant activity among various solvent systems through the DPPH radical scavenging activity. In a similar study, the antioxidant and inhibitory activities of acorn leaves and fruits of *Q. suber* were examined using three solvents including hexane, methanol, and water. The aqueous extracts showed the highest antioxidant activity as measured by the DPPH and ABTS assays [28]. Water proved to be a better extraction solvent for tannins, which explains the better results obtained via aqueous extracts in a study performed to assess the antioxidant activity of twigs, leaves, and acorns of *Q. robur* and *Q. petraea* [1,30]. The phenolic compounds presented as the phytochemicals have the highest number of reported studies on acorns. Phenolic compounds are classified as one of the five categories of phytochemicals found in foods including carotenoids, alkaloids, nitrogen-containing compounds, and organosulfur compounds, exhibiting a wide range of physiological activities such as activating or limiting the gene expression associated with some diseases or with the production of natural antioxidant enzymes [1,30]. Phenolic compounds are responsible for physiological, biological, and biochemical functions, mainly because of their strong antioxidant activity, but also due to their properties as membrane stabilizers [1,32,35]. Furthermore, these compounds are important in the human diet to maintain an adequate level of antioxidant and to cointertact the production of reactive oxygen species, reactive nitrogen species, and reactive sulfur species, as well as their subsequent neutralization [33]. Although there exist phylogenetic differences, phenolic acids (particularly gallic acid and ellagic acids and their derivative compounds), flavonoids (especially flavan-3-ols), and tannins are somewhat ubiquitous in all *Quercus* species. Most reports describing the biological activity of acorns are focused on their strong antioxidant activity, which might be related to other biological functions including antimutagenicity, anticarcinogenicity, and antiaging effects, as well as reducing risks or symptoms of cardiovascular diseases, diabetes, microbial infection, and other inflammatory diseases [1,31,34,36]. Therefore, it is suggested that acorns are valuable sources of phytochemicals with potential use in food and pharmaceutical industries.

Table 4: Total polyphenol content and flavonoid content of acorn samples with various heat treatments

Sample	†Total polyphenol content		††Flavonoids content	
control	0.193	(0.01)^{d}	0.047	(0.02)^{b}
A	0.315	(0.02)^{a}	0.050	(0.01)^{b}
B	0.309	(0.10)^{b}	0.057	(0.00)^{b}
C	0.308	(0.02)^{b}	0.061	(0.02)^{b}
D	0.296	(0.05)^{c}	0.078	(0.12)^{b}
E	0.287	(0.03)^{c}	0.088	(0.02)^{a}

control(native), heat treatment condition: A (140 °C), B (150 °C), C (160 °C), D (170 °C), E (180 °C)
†total polyphenolic content is expressed as mg gallic acid equivalent (GAE)/mg extract
††total flavonoid content is expressed as mg quercetin equivalent (QE)/mg extract
Each value is mean (SD) of triplicate determinations.
Values with different small letters in superscripts within a column are significantly different at $p < 0.05$ according to Duncan's multiple range test.

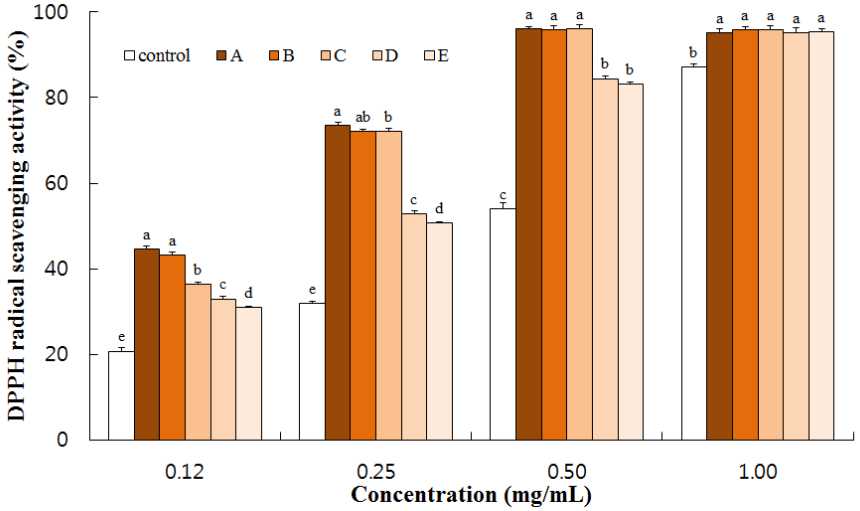


Fig. 1. DPPH radical scavenging activities of acorn extracts under various heat treatment conditions.

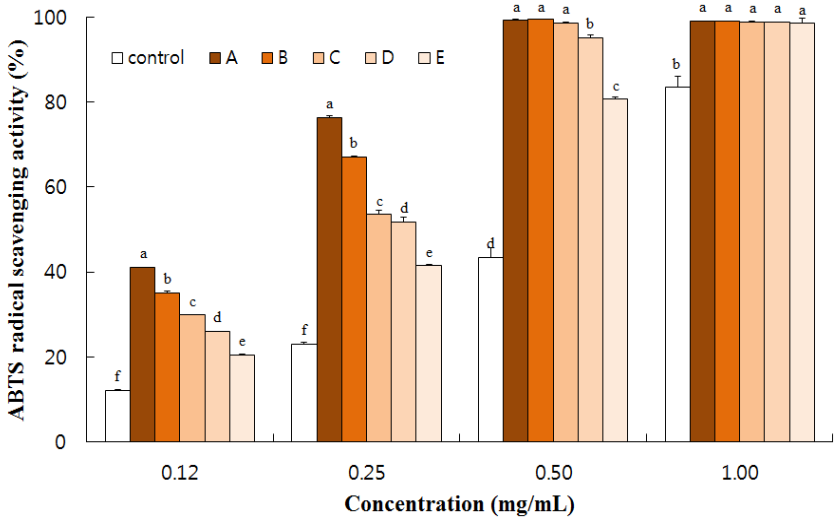


Fig. 2. ABTS radical scavenging activities of acorn extracts under various heat treatment conditions.

Table 5: Radical scavenging IC_{50} value of acorn samples according to heat treatment

Sample	DPPH IC_{50} (mg/mL)		ABTS IC_{50} (mg/mL)	
control	0.46	(0.01)^{a}	0.57	(0.02)^{a}
A	0.13	(0.00)^{e}	0.15	(0.00)^{f}
B	0.15	(0.02)^{d}	0.19	(0.00)^{e}
C	0.18	(0.02)^{c}	0.25	(0.01)^{d}
D	0.27	(0.05)^{bc}	0.28	(0.00)^{c}
E	0.29	(0.00)^{b}	0.34	(0.00)^{b}

control(native), heat treatment condition: A (140 °C), B (150 °C), C (160 °C), and D (170 °C) for 10 min
Each value is mean (SD) of triplicate determinations.
Values with different small letters in superscripts within a column are significantly different at $p < 0.05$ according to Duncan's multiple range test.

4. Conclusion

The present study investigated the changes in physicochemical characteristics, proximate contents, and antioxidant components and their activities in acorn extracts after heat treatment under various temperature conditions from 140 °C to 180 °C. When acorns were subjected

to a thermal process under various temperature conditions, the pH of acorn extracts decreased depending on the heat treatment temperature, whereas their sugar content increased. As the color value, the L^* value was significantly decreased with heat treatment and was lower in higher-temperature groups such as 170–180 ℃, while a^* values fluctuated by heat treatment, that is, significantly decreased in lower-temperature groups such as 140–160 ℃ and increased at higher temperatures such as 170–180 ℃ compared to native acorn. The b^* values significantly decreased after heat treatment compared to native acorn. The moisture content of acorn was significantly decreased with an increase in temperature, but carbohydrate was significantly increased by the heat treatment. In addition, total polyphenol contents and antioxidant activities strongly increased by thermal processing, where the group with the lowest treatment temperature (140 ℃) had the highest polyphenol content and antioxidant activity. Besides, acorn is a natural low-cost edible food source, and can provide essential constituents for a healthy diet but may also be used for biomedical potential. The current study investigated the effect of different temperatures on the various physiologically active components of acorn, whose findings indicate that the use of acorn as a potential macrobiotic, other than a starch source, will be emphasized on.

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