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Characterisation of nutritional, physiochemical, and mineral compositions of aril and seed of longan fruit (*Dimocarpus longan* L.)

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Abstract

Longan (*Dimocarpus longan* L.) seeds are usually discarded without further utilisation in food production. In the present work, to determine the food resource value of longan seeds, their nutritional components, bioactive materials, and antioxidant activity were compared with those of the longan aril, the edible portion. The dried longan aril and seeds produced in Thailand were examined for their primary nutritional composition, organic acid composition, phenolic contents (gallic acid and ellagic acid), and antioxidant activity; including the DPPH radical-scavenging activity, superoxide dismutase (SOD)-like activity, and reducing power. Based on the proximate composition, the longan aril had higher moisture (9.85%) and crude ash (4.07%) contents, whereas the seed had higher crude protein (7.38%), crude lipid (4.91%), and carbohydrate (83.63%) contents. Among the minerals, calcium (122.60 mg/100 g), potassium (2053.50 mg/100 g), phosphorus (191.21 mg/100 g), and sodium (25.24 mg/100 g) contents were higher in the longan aril, whereas magnesium (69.83 mg/100 g) and iron (2.97 mg/100 g) contents were higher in the seed. The contents of most free sugars and organic acids were higher in the longan aril than in the seed. UHPLC-MS/MS analysis showed the contents of gallic acid and ellagic acid were higher in the seed (1.54 and 1249.50 µg/g, respectively) than in the aril. The total phenolic and total flavonoid contents, which indicate the antioxidant activity, were higher in the longan seed (248.42 mg GAE/g and 6.37 mg CAE/g, respectively) than in the longan aril, whereas the seed showed higher values for all the antioxidant activities than the aril. Our findings suggest that the longan seed exhibits not only high contents of nutrients but also an outstanding antioxidant activity, thereby verifying the high value of longan seeds as a potential food resource.

Introduction

Longan (*Dimocarpus longan* L.) is an arilloid fruit species belonging to the family Sapindaceae. It originated in the southern parts of China, and is currently being produced by China as well as other Southeast Asian countries (Choo, 2000; Yang *et al.,* 2011; Zhu *et al.,* 2019). As per the United States Department of Agriculture (USDA) food composition databases (USDA, 2018), the proximate composition of the dried longan fruit includes 17.60% moisture, 4.90% protein, 0.40% lipid, and 74.00% carbohydrate. Longan fruit is rich in nutrients such as carbohydrates, proteins, fats, fibres, amino acids, minerals, and vitamin C which led to its wide use as a medicinal herb or a food ingredient in China and Korea since historic times. Nevertheless, the production of longan is yet to be established in Korea, which currently imports all longan fruits (Yang *et al.,* 2011; Zhu *et al.,* 2019).

Longan fruit is composed of a thin, leather-like pericarp and an edible, juicy aril wrapped around a large dark-brown seed (Yang *et al.,* 2011). Extracts of the longan aril, pericarp, and seed have been reported to contain an abundance of phenolic compounds with highly beneficial effects such as antioxidant, anti-tyrosinase, and anticancer activities which increase the possibility of using the fruit and its components in various food products as functional food ingredients (Jiang *et al.,* 2013; Panyathep *et al.,* 2013). Notably, a combination of longan extracts has been reported to enhance the therapeutic effects of certain commercial drugs which further suggests potential benefits of longan use as a natural biological resource (Yang *et al.,* 2011).

Longan is mostly consumed as fresh; however, it has a short shelf life. Therefore, commercial products are based on various processed forms, including frozen, dried, and canned longan fruits (Choo, 2000; Chen *et al.,* 2014). Nevertheless, most of processed products only use the edible part of longan. Consequently, the proportion of waste such as seeds, which account for 17% of the total weight, is considerably high

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(Chen *et al.,* 2014). The longan seed could be useful in the food or pharmaceutical industry because it is easily separated from the aril, and exhibits excellent nutritional properties (Zhu *et al.,* 2019). In the past few years, several studies have found that the longan seed is enriched in phenolic compounds including gallic acid, ellagic acid, corilagin, acetonyl geraniin, and phenolic glycosides (Hsu *et al.,* 1994; Soong and Barlow, 2006; Chen *et al.,* 2014). Owing to the presence of these bioactive substances, the seed has been reported to show excellent antioxidant, anticancer, anti-tyrosinase, anti-glycation, anti-inflammatory, antimicrobial, and antihypertensive activities (Chen *et al.,* 2014; Zhu *et al.,* 2019). A toxicology study has reported no toxicity for acute and repeated doses of a longan seed extract (Worasuttayangkurn *et al.,* 2012). Thus, the likelihood of developing a health-beneficial functional food product using longan seeds is considered to be high (Yang *et al.,* 2011).

The present work thus aimed to investigate the main nutritional composition and bioactive compounds as well as the antioxidant activity of the longan aril, the main edible part of the fruit, and of the seed to evaluate the potential of the longan seed to be used as a food resource.

Materials and methods

Plant materials

Longan fruits (cv. *Daw*), produced in January 2016 in the northern part of Thailand (Chiang Rai), were obtained and separated into the aril and seed, which were then dried following the methods of Harahap *et al.* (2012) and Panyathep *et al.* (2013) for 48 h in a 50°C dryer (ON-22GW; Jeio Tech, Seoul, Korea). The samples were ground using a food grinder (FM-680W; Hanil, Seoul, Korea), and stored in a -55°C deep freezer (SF-53U; Nihon Freezer, Tokyo, Japan) until further analysis.

Extract preparation

Longan aril and seed extract preparation was carried out following a previously described method (Sim, 2017). Briefly, the longan aril and seed samples were extracted twice, at a 3-h interval, with 70% ethanol (1:20, w/v) by refluxing in a water bath at 80°C. The extracts were filtered through Whatman No. 2 filter paper (Whatman, Maidstone, UK) and then concentrated in a 60°C rotary evaporator (N-1000; EYELA, Tokyo, Japan), followed by freeze-drying (Bondiro MCFD 8508; Ilsin Co., Seoul, Korea) for 48 h. The obtained powder was sieved through a 30-mesh sieve (Chunggye Sanggong Co., Ltd., Gunpo, Korea), and stored in a deep freezer for subsequent analyses of gallic acid and ellagic acid, and antioxidant activity.

Determination of the proximate composition

The moisture content of the longan aril and seed was measured using an infrared moisture analyser (MB45; Ohaus, Zurich, Switzerland), and the rest of the composition was measured using the AOAC method (AOAC, 2000). Crude protein was measured using an automated Kjeldahl analyser (Kjeltec Auto 2400/2460; Foss Tecator AB, Höganas, Sweden) based on the Kjeldahl nitrogen determination method. The crude fat content was measured using automatic lipid extraction (Soxhlet Avanti 2050; Foss Co., Hillerod, Denmark) based on the Soxhlet extraction method. Crude ash was determined using a 550 - 600°C muffle furnace (LEF-105S; Daihan LabTech, Namyangju, Korea) based on the direct painting method. The carbohydrate content was calculated as a percentage by subtracting the moisture, crude protein, crude lipid, and crude ash contents from 100%. In addition, the levels of all proximate components, except moisture, were adjusted for the moisture content and calculated on a dry-weight basis for comparison.

Determination of mineral content

To analyse the mineral content, the longan aril and seed were pre-treated by acid digestion in a microwave based on the AOAC method (AOAC, 2000). Minerals were analysed using an inductively coupled plasma-optical emission spectrometer (Optima 8300; PerkinElmer, Waltham, MA, USA), with the device conditions set as follows: radio frequency power, 1500 W; pump flow rate, 1.5 mL/min; plasma gas flow, 12 L/min; auxiliary gas flow, 0.2 L/min; and nebulizer gas flow 0.55 L/min. The selected wavelengths for each mineral were as follows: calcium, 317.933 nm; potassium, 766.490 nm; phosphorus, 213.618 nm; iron, 238.204 nm; magnesium, 285.213 nm; and sodium, 589.592 nm. The contents of all minerals were adjusted for the moisture content, and calculated on a dry-weight basis for comparison.

Determination of free sugars and organic acids

Free sugars were analysed in the longan aril and seed following the method by Kim *et al.* (2017). To each 1-g sample, 30 mL of distilled water was added, and the mixture was filtered through a 0.45-μ m membrane filter (Millipore Corp., Bedford, MA, USA), followed by ultra-high-performance liquid chromatography (UHPLC) analysis (Dionex Ultimate 3000 series; Thermo Fisher Scientific, Waltham, MA,

USA). The device conditions were as follows: Sugar-Pak column, 300 × 6.5 mm (Waters, Milford, MA, USA); oven temperature, 70°C; flow rate, 0.5 mL/min; tertiary distilled water (Division of Millipore, Waters, Milford, MA, USA) as a mobile phase; and injection volume, 10 µL. For free sugar detection, a refraction index (RI) detector (Shodex RI-101; Showa Denko, Tokyo, Japan) was used. All standard substances were diluted with HPLC water (Samchun Pure Chemical Co., Ltd., Pyeongtaek, Korea). After generating a standard curve for different concentrations, the peak area was used to measure the free sugar content in each sample. All contents were adjusted for the moisture content, and calculated on a dry-weight basis for comparison.

Organic acids were analysed in the longan aril and seed following the method described by Sung *et al.* (2008). To each 1-g sample, 30 mL of distilled water was added, and the mixture was filtered through a 0.45-µm membrane filter for the analysis using the UHPLC Ultimate 3000 system. The device conditions were as follows: Aminex HPX-87H column, 300 × 7.8 mm (Bio-Rad Laboratories, Inc., Hercules, CA, USA); oven temperature, 40°C; 0.01 N sulfuric acid (Fluka, Neu-Ulm, Germany) as a mobile phase; flow rate, 0.5 mL/min; and injection volume, 10 µL. An RI detector (RefractoMAX; ERC, Inc., Saitama, Japan) was used for the detection at 210 nm. All standard substances were diluted with HPLC water. After generating a standard curve for different concentrations, the peak area was used to measure the organic acid content in each sample. All contents were adjusted for the moisture content, and calculated on a dry-weight basis for comparison.

UHPLC-MS/MS analysis of gallic acid and ellagic acid

The longan aril and seed extracts were analysed by UHPLC-MS/MS for gallic acid and ellagic acid, the main components of the longan fruit. Gallic acid and ellagic acid standards (Sigma-Aldrich, St. Louis, MO, USA) were diluted with methanol to 2 - 1250 µg/mL for gallic acid and 16 - 10,000 µg/mL for ellagic acid and used as standard solutions.

To quantify gallic acid, each extract (0.5 mg/mL) was mixed with 20 mL of methanol for 10 min on a vortex mixer (Vortex-Genie 2; Scientific Industries, Inc., Bohemia, NY, USA). The samples were then centrifuged for 10 min at 4°C, 4,000 rpm (COMBI-514R; Hanil Science Industrial Co., Ltd., Gimpo, Korea). The supernatant was filtered through a 0.45-µm membrane filter, then extracted with 2 mL of methanol using a solid-phase extraction cartridge (C18, 100 mg, 3 mL; Machery-Nagel, Düren, Germany), and 2 µL of the sample was used for analysis. To quantify ellagic acid, 0.1 g of each extract was stirred for 30 min with 5 mL of methanol, then diluted 1:100 with methanol, and centrifuged for 15 min at 4°C, 4000 rpm. The supernatant (2 mL) was filtered through a 0.45-µm membrane filter, and 2 µL was used for analysis.

For the analysis of gallic acid and ellagic acid in the longan aril and seed, the Dionex Ultimate 3000 UHPLC system (Thermo Fisher Scientific), coupled with a TSQ Quantiva triple-quadrupole mass spectrometer system (Thermo Fisher Scientific) was used with a selected reaction monitoring (SRM) method in negative-ion electrospray ionisation mode. The UHPLC-MS/MS system was operated under the following conditions: reversed-phase C18 Hypersil gold column, 1.9 µm, 2.1 × 100 mm (Thermo, Hemel Hempstead, UK); flow rate, 3.0 mL/min; injection volume, 2 µL; and column oven temperature, 35°C. The mobile phase was composed of 10 mM ammonium acetate in water (solvent A) and 10 mM ammonium acetate in methanol (solvent B), and the following elution gradients were used: 0 - 5 min, 20% B; 6 - 9 min, 70% B; 9.1 - 10 min, 20% B for gallic acid; and 0 - 1 min, 5% B; 2 - 5 min, 25% B; 6 - 10 min, 100% B; and 10.1 - 12 min, 5% B for ellagic acid. For MS/MS analysis, the temperatures of the vaporiser and ion transfer tube were 317 and 333°C, respectively. The ion spray voltage was 40 Arb (arbitrary units), and the capillary voltage energy was 2500 V.

Determination of total phenolic and flavonoid contents

The total phenolic contents of the longan aril and seed extracts were measured based on the Folin-Ciocalteu method. To 200 µL of each 0.1% (w/v) extract, 400 µL of 0.2 N Folin-Ciocalteu phenol reagent was added for a 3-min reaction. To the resulting solution, 800 µL of 10% sodium carbonate was added for a 1-h reaction in the dark, after which OD was measured at 765 nm using a UV/VIS spectrophotometer (V-530; Jasco, Tokyo, Japan) three times. Gallic acid (Sigma-Aldrich) was used as the standard compound, and the results were expressed as milligram gallic acid equivalents (GAE) per gram.

The total flavonoid contents of the longan aril and seed extracts were measured based on the method by Jeong *et al.* (2012). After adding 10 mL of 90% diethylene glycol and 1 mL of 1 N NaOH to 1 mL of each 0.1% (w/v) extract, the mixture was thoroughly stirred, and left to react at 37°C for 1 h. Thereafter, OD was measured at 430 nm. Catechin (Sigma-Aldrich) was used as the standard compound, and the results were expressed as milligram catechin equivalents (CAE) per gram extract.

DPPH radical-scavenging activity assay

To measure DPPH radical-scavenging activities of the longan aril and seed extracts, the method described by Sim (2017) was used. A 4-mL aliquot of each extract (10 - 3000 µg/mL) was mixed with 1 mL of a DPPH solution (4×10^{-4} M), and the mixture was left to react in the dark at ambient temperature for 30 min. OD was measured at 517 nm three times using a UV/VIS spectrophotometer, and the difference in OD with the control group was expressed as a percentage to estimate the DPPH radical-scavenging activity. The half maximal inhibitory concentration (IC_{50}) indicating the concentration at which 50% of DPPH radicals was scavenged, and was calculated for each extract.

Superoxide dismutase (SOD)-like activity assay

To measure SOD-like activities of the longan aril and seed extracts, the method described by Sim (2017) was used with some modifications. To 0.2 mL of each extract (100 - 3000 µg/mL), 1.5 mL of Tris-HCI buffer, pH 8.5, and 0.1 mL of 7.2 mM pyrogallol were added for a 10-min reaction at 25°C, followed by termination of the reaction by addition of 1 mL of 1 N HCl. OD was measured at 420 nm three times, and the difference in OD with the control group was expressed as a percentage to estimate the SOD-like activity. The IC_{50} value indicating the concentration at which 50% of pyrogallol was oxidised and calculated for each extract.

Reducing power assay

To measure the reducing power of the longan aril and seed extracts, the method described by Sim (2017) was used. A 2.5-mL aliquot of each extract (10 - 3000 µg/mL) was mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated in a 50°C water bath for 20 min, followed by the addition of 2.5 mL of 10% trichloroacetic acid. The resulting solution was centrifuged at 3,000 rpm for 10 min, and then 5 mL of the supernatant was mixed with 5 mL of distilled water. After adding 1 mL of 0.1% ferric chloride, OD was measured at 700 nm three times, and the values were used to express the reducing power.

Statistical analysis

All experimental results were analysed using the SPSS for Windows 23.0 (SPSS, Inc., Chicago, IL, USA), and expressed as the mean ± standard deviation. An independent *t*-test was used to analyse significance of the differences between samples ($p < 0.05$).

Results and discussion

Nutritional composition of the longan aril and seed

The results of the proximate composition analysis of the longan aril and seed are presented in Table 1. The longan aril contained 9.85% moisture, 7.38% crude protein, 4.91% crude lipid, 4.07% crude ash, and 83.63% total carbohydrate. The longan seed contained 1.18% moisture, 7.97% crude protein, 6.17% crude lipid, 1.78% crude ash, and 84.08% total carbohydrate. Comparison of the proximate compositions of the longan aril and seed indicated that the moisture and crude ash contents were higher in the aril (both $p < 0.001$), while those of crude protein ($p < 0.01$), crude lipid ($p < 0.001$), and total carbohydrate ($p < 0.05$) were higher in the seed. These results could not be compared with previous findings due to the scarcity of available data regarding the proximate composition of the longan aril and seed. Only a few studies were found that reported the proximate composition analysis of fresh longan aril (Choo, 2000; Li *et al.,* 2004; Wall, 2006; Yahia, 2011) and dried longan seed (Harahap *et al.,* 2012). The studies on the nutritional composition of fresh longan reported that despite slight differences according to the longan cultivar, the range of contents were 77.83 - 81.02% for moisture, 1.0 - 1.2% for protein, 0.1 - 0.5% for lipid, 0.7% for ash, and 12.38 - 25.2% for carbohydrate (Choo, 2000; Li *et al.,* 2004; Wall, 2006; Yahia, 2011); these results showed higher moisture content than that observed in the present work, but lower contents of protein, lipid, ash, and carbohydrate. Meanwhile, Harahap *et al.* (2012) analysed the proximate composition of the seed of rambutan, a species belonging to the same family (Sapindaceae) as the longan, and their results were similar to the data obtained in the present work for the longan seed. The rambutan seed also showed a higher carbohydrate content (48.10%) than that of oil (38.90%), crude protein (12.40%), or moisture (3.31%). Our findings confirmed that the main constituents of the longan seed were carbohydrates, similar to the longan aril.

The results of the mineral content analysis of the longan aril and seed are presented in Table 1. The calcium content was 1.9-fold higher ($p < 0.001$) in the aril (122.60 mg/100 g) than in the seed (66.17 mg/100 g). The potassium content was 2.1-fold higher ($p < 0.001$) in the aril (2053.50 mg/100 g) than in the seed (964.40 mg/100 g). Furthermore, the phosphorus content was 1.1-fold higher ($p < 0.01$) in the aril (191.21 mg/100 g) than in the seed (173.21 mg/100 g); and that of sodium was 1.8-fold higher ($p < 0.001$) in the aril (25.24 mg/100 g) than in the seed

Table 1. Nutritional composition of the longan aril and seed.

Nutritional composition	Longan aril	Longan seed
Proximate composition (%)		
Moisture (ww)	9.85 ± 0.25[a]	1.18 ± 0.32[b]
Crude protein (dw)	7.38 ± 0.03[b]	7.97 ± 0.11[a]
Crude lipid (dw)	4.91 ± 0.03[b]	6.17 ± 0.07[a]
Crude ash (dw)	4.07 ± 0.02[a]	1.78 ± 0.01[b]
Carbohydrate (dw)	83.63 ± 0.04[b]	84.08 ± 0.18[a]
Minerals (mg/100 g dw)		
Calcium	122.60 ± 0.86[a]	66.17 ± 1.28[b]
Potassium	2053.50 ± 22.38[a]	964.40 ± 18.20[b]
Magnesium	69.83 ± 0.43[b]	81.90 ± 1.47[a]
Phosphorus	191.21 ± 1.22[a]	173.21 ± 3.15[b]
Sodium	25.24 ± 0.31[a]	14.44 ± 0.58[b]
Iron	1.96 ± 0.04[b]	2.97 ± 0.06[a]
Free sugars (g/100 g dw)		
Glucose	13.89 ± 0.16[a]	5.08 ± 0.17[b]
Fructose	13.83 ± 0.04[a]	4.85 ± 0.07[b]
Sucrose	41.34 ± 0.17[a]	0.18 ± 0.00[b]
Organic acids (mg/g dw)		
Citric acid	1.47 ± 0.02	ND
Malic acid	4.23 ± 0.07	ND
Shikimic acid	0.58 ± 0.00[a]	0.35 ± 0.01[b]
Fumaric acid	ND	0.62 ± 0.00

ND: not detected, ww: wet weight, and dw: dry weight. Values are mean ± SD (n = 3). Different letters in the same row indicate significant difference ($p < 0.05$).

(14.44 mg/100 g). In contrast, the magnesium content was 1.2-fold higher ($p < 0.001$) in the seed (81.90 mg/100 g) than in the aril (69.83 mg/100 g); and that of iron was 1.5-fold higher ($p < 0.001$) in the seed (2.97 mg/100 g) than in the aril (1.96 mg/100 g). Thus, when compared with the longan aril, the longan seed was found to contain higher levels of magnesium and iron, while the contents of calcium, potassium, phosphorus, and sodium were lower; although potassium was the highest among minerals in both aril and seed. According to the USDA databases (USDA, 2018), 100 g of dry longan fruits contains 45 mg of calcium, 658 mg of potassium, 46 mg of magnesium, 196 mg of phosphorus, 48 mg of sodium, and 5.40 mg of iron. Despite minor differences, our data also indicate a higher potassium content than that of other minerals. Likewise, the study by Wall (2006) showed that 100 g of fresh longan fruits cultivated in Hawaii contained 6.7 - 10.7 mg of calcium, 306.9 - 338.2 mg of potassium, 10.4 - 14.3 mg of magnesium, 30.1 - 35.7 mg of phosphorus, 5.8 - 18.7 mg of sodium, and 0.06 - 0.10 mg of iron, confirming a higher potassium content. Notably, Wall (2006) concluded that the longan was a good source of potassium as 100 g of fresh longan fruits would satisfy the criteria of the Dietary Reference Intake of 7% potassium. The longan seed is thus anticipated to be a good source of potassium as well.

The results of free sugar analysis (Table 1) showed that both longan aril and seed contained free sugars, including glucose, fructose, and sucrose. The glucose content was 2.7-fold higher ($p < 0.001$) in the aril (13.89 g/100 g) than in the seed (5.08 g/100 g); while the fructose content was 2.9-fold higher ($p < 0.001$) in the aril (13.83 g/100 g) than in the seed (4.85 g/100). The content of sucrose, which confers strong sweetness, was found to be 229.8-fold higher ($p < 0.001$) in the aril (41.34 g/100 g) than in the seed (0.18 g/100 g). Similar to our finding, Son *et al.* (2007) and Shi *et al.* (2016) reported that sucrose, glucose, and fructose were among the main sugar components in the longan fruit with sucrose exhibiting the highest content. The relative

proportion of free sugars, especially those that confer sweetness to fruits is closely associated with fruit maturity (Florkowski *et al.,* 2009). Whereas fruits such as apples, pears, grapes, and strawberries contain an abundance of glucose and fructose, the longan aril was found to contain the highest level of sucrose, which were found in abundance in ripe fruit (Florkowski *et al.,* 2009). The finding that sucrose is the main free sugar in the longan fruit is consistent with its far higher sweetness than that in other fruits, and is likely to lead to a strong consumer preference.

The results of organic acid analysis showed some differences in the composition between the longan aril and seed (Table 1). Citric acid (1.47 mg/g), malic acid (4.23 mg/g), and shikimic acid (0.58 mg/g) were detected in the aril, while shikimic acid (0.35 mg/g) and fumaric acid (0.62 mg/g) were detected in the seed. Among the organic acids found in both the longan aril and seed, although in small amounts, the content of shikimic acid was 1.7-fold higher in the aril ($p < 0.001$). Shikimic acid is a precursor for the biosynthesis of most phenolic compounds in plants. Most secondary metabolites with antioxidant properties, including the diverse group of phenolic compounds, are synthesised through the shikimic acid pathway (Ghasemzadeh and Ghasemzadeh, 2011). As shikimic acid influences the synthesis of phenolic compounds, the shikimic acid content in the longan aril and seed was predicted to have a significant impact on the phenolic content and antioxidant activity. However, based on the observed, there is lower phenolic content and antioxidant activity with higher shikimic acid content in the longan aril than that in the longan seed. It was determined that a small amount of shikimic acid results in a negligible increase in antioxidant activity. While the longan aril contained both citric and malic acids, the organic acids was abundant in fruits, the seed contained fumaric acid. In a previous study on longan aril, Son *et al.* (2007) reported formic acid and malic acid as the main organic acids. Meanwhile, Yang *et al.* (2009) reported malic acid and citric acid as the main organic acids, and Li *et al.* (2004) reported malic acid and tartaric acid as the main organic acids. Thus, different studies have reported slight differences in the organic acid composition in the longan aril. The organic acid composition of fruits may vary, and the contents may decrease during fruit maturation (Florkowski *et al.,* 2009). Therefore, the organic acid content in the longan fruit may also vary, depending on the fruit maturation. Notably, the contents of malic acid and citric acid, and the main organic acids in the longan aril were shown to reach the highest levels at the intermediate maturity stage, and gradually decrease afterward (Yang *et al.,* 2011). It has been reported that bagging treatment, used to prevent damage by harmful insects, may decrease the malic acid content in the longan aril, which suggests that not only the process of fruit maturation, but also agricultural techniques used for pest control may result in fluctuations in the organic acid content in the longan fruit, thereby affecting its taste and quality.

Contents of gallic acid and ellagic acid in the longan aril and seed

Quantification of gallic acid and ellagic acid, the major phenolic compounds in the longan aril and seed, showed that both produced molecular ion peaks in the negative-ion mode in the form of $[M\text{-}H]^-$, detected at *m/z* 169 and 301, respectively, leading to the determination of the precursor ion and the product ion (Table 2). The product ion with the highest intensity was selected as the quantitation ion, and the ion with the second highest intensity was selected as the confirmation ion for qualitative confirmation. For gallic acid (exact mass: 170.02), the form without H^- at *m/z* 169 (precursor ion) and the form without CO_2 at *m/z* 125 (quantitation ion) were selected. For ellagic acid (exact mass: 302.01), the form without H^- at *m/z* 301 (precursor ion) and the $[M\text{-}H\text{-}CO_2\text{-}CO]^-$ form at *m/z* 229 (quantitation ion) were selected. The calibration curves obtained with the standard solutions of gallic acid and ellagic acid at different concentrations showed a good linearity, with R^2 values of 0.9999 (regression curve:

Table 2. SRM mass detection conditions and average contents of gallic acid and ellagic acid in longan aril and seed extracts

Phenolic compound	Longan fruit component	Retention time (min)	Exact mass (g/mol)	Precursor ion (*m/z*)	Confirmation ion (*m/z*)	Quantitation ion (*m/z*)	Collision energy (eV)	Average content (µg/g)
Gallic acid	Aril	0.76	170.02	169	125, 81, 79	125	15	0.08 ± 0.01^b
(MW 170.12)	Seed	0.75						1.54 ± 0.02^a
Ellagic acid	Aril	6.14	302.01	300	257, 229, 185	229	28	1249.50 ± 304.06^b
(MW 302.19)	Seed	6.15						8262.85 ± 203.93^a

Values are mean ± SD ($n = 3$). Different letters in the same column indicate significant difference ($p < 0.05$).

y = -916.782 + 553.867x) and 0.9995 (regression curve: y = -15,250 + 189.675x), respectively. Based on these results, the longan aril and seed extracts were confirmed to be suitable for gallic acid and ellagic acid quantification.

For gallic acid, the retention time of the longan aril and seed when the deprotonated molecule ion showed m/z 125 was 0.76 and 0.75 min, respectively (Figure 1). For ellagic acid, the retention time of the longan aril and seed when the deprotonated molecule ion showed m/z 229 was 6.14 and 6.15 min, respectively (Figure 2). The gallic acid contents in the aril and seed were 0.08 and 1.54 µg/g, respectively (p < 0.001), while the ellagic acid contents were 1249.50 and 8262.85 µg/g, respectively (p < 0.01). These results indicated that the contents of gallic acid and ellagic acid were higher in the longan seed than in the aril.

Previous studies in which the contents of gallic acid and ellagic acid were analysed in the longan aril and seed showed that the contents of both acids were higher in the longan seed than in the longan aril. Rerk-am *et al.* (2016) compared the contents of gallic acid and ellagic acid in longan seed with those of the longan peel, and found the contents to be higher in the 20% ethanolic extract of the longan seed (each 8.24 and 8.21 mg/g, respectively) than in that of the longan peel (4.16 and 5.20 mg/g, respectively). Moreover, Tang *et al.* (2019) compared the contents of gallic acid and ellagic acid in longan seed to those of the longan pericarp, and found the contents to be greater in the 50 - 70% ethanolic extract of the longan seed (each 0.47 - 0.49 and 0.21 - 0.83 mg/g, respectively) than in that of the longan pericarp (0.07 - 0.08 and 0.15 - 0.18 mg/g, respectively). Evidently, the higher gallic acid and ellagic acid contents in the longan seed than in the longan aril is attributed to the variation in the types of phytochemicals present in the different parts of the longan fruit (Yang *et al.,* 2011). The longan aril has been reported to contain lysophosphatidylcholine, phosphatidyl choline, phosphatidyl inositol, phosphatidyl serine, phosphatidyl ethanolamine, phosphatidate, and phosphatidic acid glycerol (Sheng and Wang, 2010). Conversely, the longan seed has been reported to contain ethyl gallate 1-β-O-galloyl-D-glucopyranose, methyl brevifolin carboxylate, grevifolinand 4-O-α-L-rhamnopyranosyl-ellagic acid, gallic acid, corilagin, and ellagic acid (Zheng *et al.,* 2009; Zhu *et al.,* 2019). Moreover,

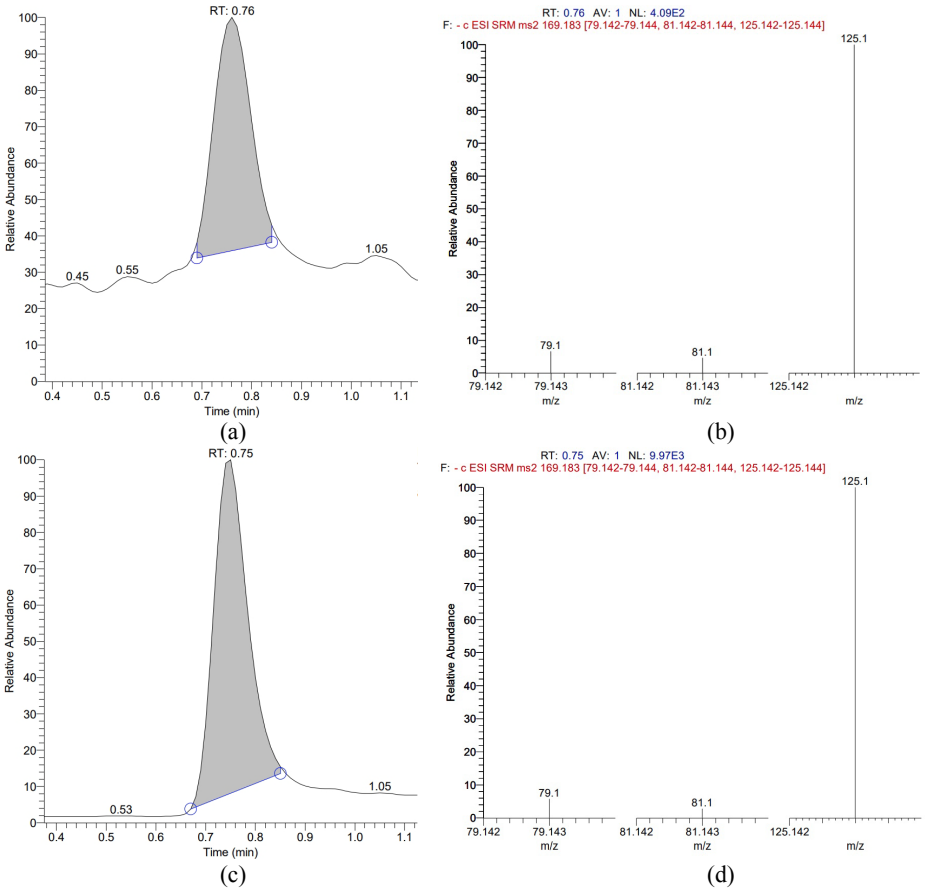

Figure 1. UHPLC-MS/MS chromatograms and electrospray product ion mass spectra of gallic acid in longan aril and seed extracts. (a, c) UHPLC-MS/MS chromatogram of gallic acid in longan (a) aril extract and (c) seed extract. (b, d) Electrospray product ion mass spectra of gallic acid in longan (b) aril extract and (d) seed extract.

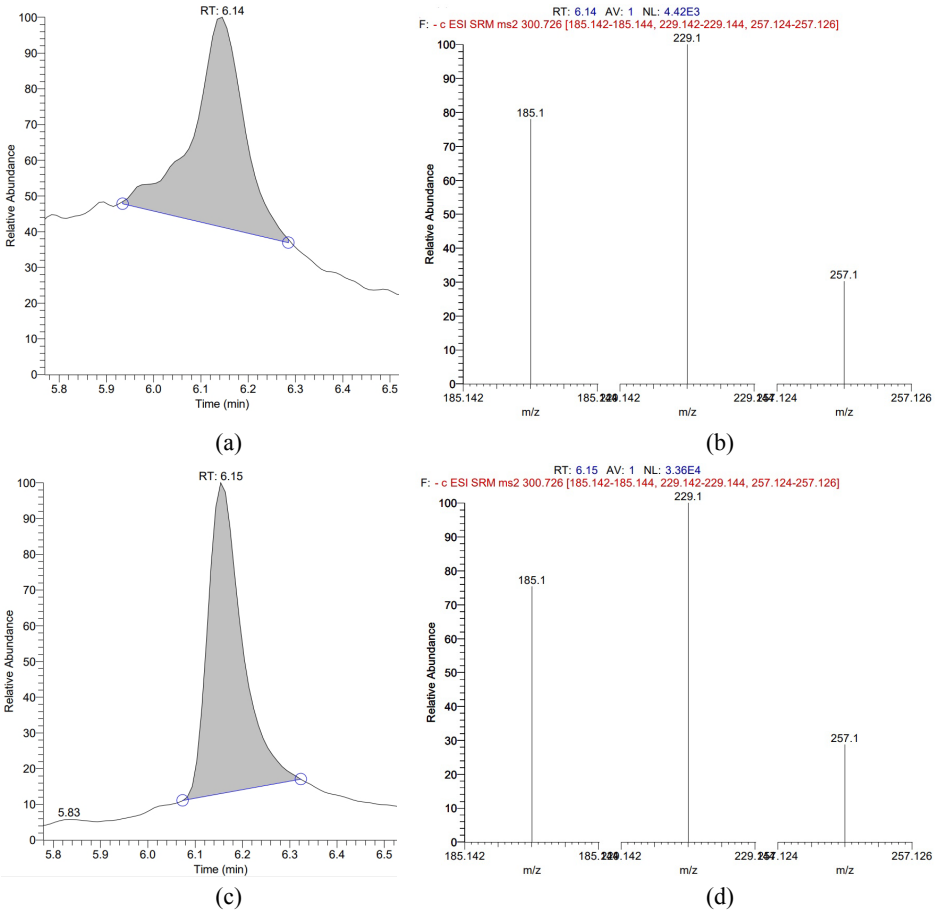

Figure 2. UHPLC-MS/MS chromatograms and electrospray product ion mass spectra of ellagic acid in longan aril and seed extracts. (a, c) UHPLC-MS/MS chromatogram of ellagic acid in longan (a) aril extract and (c) seed extract. (b, d) Electrospray product ion mass spectra of ellagic acid in longan (b) aril extract and (d) seed extract.

as compared to the other parts of the longan fruit, the longan seed mainly comprises phenolic compounds including gallic acid and ellagic acid, which could explain the high antioxidant activity in the longan seed observed in the present work.

Antioxidant properties of the longan aril and seed

The antioxidant properties were determined based on the total phenolic contents, total flavonoid contents, DPPH scavenging activity, SOD-like activity and reducing power of each longan extract. The total phenolic content and total flavonoid content in the longan seed were higher than those in the longan aril (Table 3). The total phenolic content was approximately 50-fold higher ($p < 0.001$) in the seed than in the aril (248.42 95 and 4.95 mg GAE/g,

Table 3. Antioxidant properties of extracts from the longan aril and seed.

Antioxidant property	Longan aril	Longan seed
Total phenolic content (mg GAE/g)	4.95 ± 1.06^b	248.42 ± 9.93^a
Total flavonoid content (mg CAE/g)	1.34 ± 1.19^b	6.37 ± 0.97^a
DPPH radical-scavenging activity, IC_{50} values (μg/mL)	1292.64 ± 153.40^a	61.88 ± 0.85^b
SOD-like activity, IC_{50} values (μg/mL)	8287.00 ± 142.74^a	1867.39 ± 224.79^b
Reducing power of different concentrations (μg/mL)		
100	0.12 ± 0.00^b	0.49 ± 0.01^a
250	0.19 ± 0.00^b	1.07 ± 0.02^a
500	0.31 ± 0.01^b	2.09 ± .01^a
1000	0.54 ± 0.03^b	3.03 ± 0.06^a

Values are mean ± SD (n = 3). Different letters in the same row indicate significant difference ($p < 0.05$).

respectively). The total flavonoid content was approximately 4.8-fold higher ($p < 0.01$) in the seed than in the aril (6.37 95 and 1.34 mg CAE/g, respectively).

All antioxidant activity assay results showed that the highest results were obtained with longan seed extracts (Table 3). The IC50 values for the DPPH radical-scavenging activity were 61.88 and 1292.64 μg/mL for the longan seed and aril, respectively, which implied an approximately 20.0-fold higher DPPH radical-scavenging activity in the longan seed extract ($p < 0.01$). The IC_{50} values for the SOD-like activity were 1867.39 and 8287.00 μ g/mL for the longan seed and aril, respectively, which implied an approximately 4.4-fold higher superoxide anion radical-scavenging activity via pyrogallol autoxidation in the longan seed extract ($p < 0.001$). The results of measuring the reducing power of each extract at different concentrations (100 - 1000 g/mL) showed that the OD values for the longan seed and aril extracts were 0.49 - 3.03 and 0.12 - 0.54, respectively, indicating an approximately 4.1- to 6.7-fold higher reducing power of the longan seed ($p < 0.001$).

The antioxidant activities of longan aril and seed extracts were correlated with their phenolic compound contents (Prasad *et al.,* 2009; Chindaluang and Sriwattana, 2014). Furthermore, longan seed extract contains substantial amounts of phenolic compounds as compared to the longan aril, and it is the extent of phenolic compounds present in this extract that is responsible for its marked antioxidant activity as proposed through various *in vitro* models (Prasad *et al.,* 2009). Several reports of longan fruit have conclusively shown a close relationship between total phenolic content and antioxidant activity (Guo *et al.,* 2003; Soong and Barlow, 2004; Rangkadilok *et al.,* 2007; Prasad *et al.,* 2009; Yang e*t al.,* 2011; Chindaluang and Sriwattana, 2014; Rerk-am *et al.,* 2016; Tang *et al.,* 2019). The phenolic compounds exhibit extensive free radical scavenging activities through their reactivity as hydrogen or electron-donating agents and metal ion chelating properties (Rice-Evans *et al.,* 1996; Prasad *et al.,* 2009). Therefore, there should be a close correlation between the content of phenolic compounds and antioxidant activities (Prasad *et al.,* 2009). These results confirmed that when compared with the longan aril, the longan seed exhibited a higher phenolic content, including total phenolic and flavonoid contents, as well as a substantially more outstanding antioxidant activity. Numerous previous studies have provided direct evidence of the antioxidant effects of the longan seed (Guo *et al.,* 2003; Soong and Barlow, 2004; Rangkadilok *et al.,* 2007; Yang *et al.,* 2011; Chindaluang and Sriwattana, 2014; Rerk-am *et al.,* 2016; Tang *et al.,* 2019). Soong and Barlow (2004) reported a higher total phenolic content and antioxidant activity for the longan seed. Panyathep *et al.* (2013) reported that dried longan seeds contained high levels of phenolic compounds such as gallic acid and ellagic acid with powerful antioxidant functions; and Zheng *et al.* (2009) reported that the longan seed contained phenolic compounds such as gallic acid, ellagic acid, and corilagin, accounting for its high antioxidant activity. Hence, the excellent antioxidant activity of the longan seed was determined to be due to phenolic compounds. Our results are in agreement with other studies that have reported parallel results. In the present work, the longan seed was found to have much higher total phenolic and total flavonoid contents than did the longan aril, with higher DPPH and superoxide anion radical-scavenging activities, as well as a stronger reducing power of the seed. In addition, gallic acid and ellagic acid were 19.2- and 6.6-fold more abundant in the longan seed than in the aril, respectively.

A comparison of these results with those of the assays performed to determine the extract antioxidant and radical-scavenging activities indicates that the separated longan seed phenolic extracts may exert powerful antioxidant effects.

Conclusion

In the present work, the nutritional components, bioactive materials, and antioxidant activity of the edible longan aril were compared with those of the inedible longan seed to determine if the longan seed could be valuable as a food resource. Results showed differences in the nutritional components and antioxidant activity of longan aril and seed. Longan seed had excellent antioxidant activity, and can be used in the development of various food products. In terms of the proximate composition, the moisture and crude ash contents were higher in the longan aril; while the crude protein, crude lipid, and carbohydrate contents were higher in the longan seed. Regarding the mineral contents, calcium, potassium, phosphorus, and sodium were higher in the longan aril; whereas magnesium and iron were higher in the longan seed. In addition, free sugars such as glucose, fructose, and sucrose whose levels are high in fruits, and organic acids such as citric acid and malic acid, were higher in the longan aril than in the seed. Analysis of gallic

acid and ellagic acid showed their higher contents in the longan seed. Furthermore, the seed exhibited not only higher total phenolic and flavonoid contents but also higher total antioxidant activity than did the aril. The present work demonstrated that the longan seed can also be a good source of nutritional value and phenolic compounds with antioxidant properties.

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