Chemical composition and antioxidant capacity of black pepper pericarp

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Abstract

Piper nigrum L. is a widely used spice because of its flavour and health effects. It is prepared as black and white pepper, according to the harvest time and inclusion of the outer skin. Pepper pericarp is usually considered waste when making white pepper. In this study, bioactive and flavour compounds and minerals in the pericarp of black pepper were determined to identify its applications. The pericarp contained total phenol, total flavonoid and piperine contents of 1421.95 ± 22.35 mg GAE/100 g, 983.82 ± 8.19 mg CE/100 g and 2352.19 ± 68.88 mg/100 g, respectively. There were higher levels of total phenols and total flavonoids in the pericarp compared with black pepper and white pepper. Piperine content was lower in the pericarp than in black pepper. The principal monoterpenes in the pericarp were α-pinene (9.2%), 2-β-pinene (14.3%), δ-3-carene (21.5%) and dl-limonene (18.8%), and the primary sesquiterpenes were α-copaene (5.1%) and caryophyllene (17.2%). The higher percentages of flavour compounds found in the pericarp would impart a more potent odour, and the pericarp exhibited higher minor and tiny differences based on electronic nose analysis. It had more minerals than black pepper and peeled black pepper.

Keywords: Black pepper, Pericarp, Antioxidant, Flavour compound, Mineral, Piperine

Introduction

Piper nigrum L. is an early and widely used spice in human history and is highly valued for its characteristic pungency. It is prepared as black and white pepper, according to the harvest time and processing method [1, 2]. Black pepper is the dried immature but fully developed fruit of pepper plants, whereas white pepper consists of the mature fruit devoid of the outer skin (pericarp) [3, 4]. Black pepper has been used as a spice and in herbal medicines. It also has applications as a preservative and biocontrol agent [5]. The essential oil or extract of black pepper displays antioxidant, anti-fungal, anti-amoebic, anti-asthmatic, anti-diabetic and immunomodulatory activities [3, 6, 7]. White pepper has many different applications too, such as a preservative, insecticide, anti-bacterial, anti-fungal and anti-protozoal herb [5, 8, 9]. Especially, black and white peppers contain a bioactive compound named piperine, also found in other members of the pepper family (Piperaceae), including long pepper (Piper longum). Piperine is the most abundant and active alkaloid in pepper. It is used as a therapeutic agent because of its many health benefits associated with its antioxidant, anti-inflammatory and drug activity-enhancing activities, respiratory effects and inhibitory action on lung metastasis [10]. Spatial memory and neurodegeneration of Alzheimer can be improved by piperine in animal models [11].

Black pepper is composed of carbohydrate of 37.4%, proteins of 25.5%, fibres of 23.6%, moisture of 4.7% and fat of 5.3%, as well as minerals, including 0.66% potassium (K), 0.20% calcium (Ca), 0.16% phosphorus and 0.16% magnesium (Mg) [12, 13]. The main volatile flavour compounds in black pepper are terpenes, and black pepper oils contain nitrogen-containing compounds [4, 14]. Key odorants of black pepper are α- and β-pinene, myrcene, α-phellandrene, limonene, linalool, methyl propanal, 2- and 3-methylbutanal, butyric acid and 3-methylbutyric acid. Compounds 2,3-diethyl-5-methylpyrazine and 2-isopropyl-3-methoxypyrazine are responsible for
Bioactive compounds and antioxidant activities of pepper pericarp

Total flavonoids

The total flavonoids were determined by a colorimetric assay [19, 20]. A 1 mL of sample extract and distilled water (4 mL) were placed in a 15-mL tube, then 0.3 mL of 5% NaNO₂ was added to the tube, and the mixture was left to react at room temperature for 5 min. After adding 0.3 mL of 10% AlCl₃, the mixture was left to react further at room temperature for 6 min before 2 mL of 1 N NaOH was added. Distilled water was added to adjust the total volume to 10 mL. The absorbance was recorded by a spectrophotometer (Optizen POP, Mecasys, Daejeon, Korea) at 510 nm. Catechin was used as the standard to establish the calibration curve. Total flavonoids were expressed as milligrams of catechin equivalents (CE)/100 g fresh weight (FW).

Total phenols

The total phenols were analysed by the Folin–Ciocalteu colorimetric method [19, 20]. A 1 mL of sample extract and distilled water (4 mL) were placed in a 15-mL tube, then 0.2 mL of 7% Na₂CO₃ was added, followed by incubation at room temperature for 90 min in the dark. Spectrophotometric absorbance was determined at 750 nm. The total phenol contents were shown as milligrams of gallic acid equivalents (GAE)/100 g FW by evaluating a gallic acid calibration curve.

Piperine

Piperine was determined, as described by Santosh et al. with some modifications [21]. A 50 mL conical tube containing 0.1 g of the sample extract and 50 mL methanol was extracted by ultrasonication at 50 °C for 20 min. The extracted sample was cooled down at room temperature and filtered through a Minisart syringe filter (0.45 μm, regenerated cellulose). Piperine was detected using a high-performance liquid chromatography (HPLC) apparatus (Agilent, Santa Clara, CA, USA) equipped with a diode array detector (340 nm) and Eclipse C18 Plus
column (4.6 × 150 mm, 5 µm; Agilent) maintained at 25 °C. Acetonitrile–1% citric acid (45:55, v/v) was used as the mobile phase in isocratic mode at the flow rate of 1 mL/min for 20 min. The injection volume was 10 µL. Peak of piperine and its spectra in the HPLC chromatogram was identified (Fig. 1).

**DPPH radical scavenging activity**

DPPH radical scavenging activity was analyzed by the method reported by Brand-Williams et al. with some modifications [22]. DPPH solution (100 µM) was diluted with 80% methanol to have an absorbance of 0.65 ± 0.02 at a wavelength of 517 nm. The extracted sample (50 µL) was mixed with 2950 µL of DPPH diluted from 0.1 mM of DPPH solution and reacted for 30 min. The absorbance at 517 nm (Optizen POP spectrophotometer, Mecasys) was shown as milligrams of vitamin C equivalents (VCE)/100 g FW.

**ABTS radical scavenging activity**

The ABTS radical scavenging activity was measured with an analytical method described by Floegel et al. [23]. ABTS (137.175 mg) and AAPH (27.117 mg) were prepared in 100 mL of phosphate-buffer, followed by reaction in a water bath at 70 °C for 40 min to produce ABTS radicals, and then cooled down at room temperature. The working solutions were diluted with the ABTS radical solution by phosphate-buffered saline to obtain an optical density at 734 nm of between 0.63 and 0.67. Finally, the sample (20 µL) and working solution (980 µL) were mixed and reacted at 37 °C for 10 min. The absorbance of the solution at 734 nm was determined by spectrophotometer (Optizen POP, Mecasys). The antioxidant activity of the solution was expressed as milligrams of VCE/100 g FW.

**Flavour compounds by gas chromatography/mass spectrometry (GC/MS)**

Two grams of sample was added into an amber vial of 20 mL with PTFE/silicone septa, and it was equilibrated at 80 °C for 30 min with the headspace sampler under agitation at 300 rpm. GC/MS analysis was performed using a 6890 GC/MS system (Agilent Co., Santa Clara, CA, USA) installed with an HP-INNOWax column (60 m × 0.25 mm × 0.25 µm; Agilent Co.) [24]. The sample (1 mL) was injected by a syringe in split injection mode (20:1) at 230 °C, with helium (>99.9% purity) as the carrier gas at a flow rate of 1 mL/min. The oven was initially maintained at 40 °C for 2 min, then heated at a rate of 5 °C/min to 230 °C and maintained at this temperature for 10 min. The flavours were identified by comparison of the obtained spectra to library (NIST 2005, John Wiley & Sons, New York, NY, USA). Figure 2 is the GC/MS chromatogram of the peaks of flavours in the black pepper pericarp.

**Flavour compounds by electronic nose (e-nose)**

A GC-type e-nose (Heracles II, Alpha MOS, Toulouse, France) with dual column (non-polar MXT-5 and slightly polar MXT-1701, 10 m length × 180 µm diameter; Restek, Lisses, France) and dual flame ionisation detector (FID) was used for flavour component profile analysis. The oven temperature was increased from 50 °C (maintained for 2 s) to 260 °C at 1 °C/s. The sample (0.8 g) was transferred to a 20-mL headspace vial and incubated at 50 °C for 5 min, with agitation at 500 rpm. Then, 1000 µL was injected at a flow rate of 1 mL/min and measured five times. For peak identification and principal component analysis (PCA), AroChemBase (NIST retention index database) and AlphaSoft (version 17.0; Alpha MOS) were used.

![Fig. 1 HPLC chromatogram and absorption spectrum of black pepper pericarp](image-url)
Minerals
Ca, Cu, Fe, K, Mg, Mn, Na and Zn were identified by the procedure developed by Jung et al. [24]. Aliquot of the sample of 0.6 g was prepared in a Teflon digestion vessel with concentrated nitric acid of 7 mL, and it was digested for 40 min under incrementally increasing pressure heating at 1000 W. The digested sample was diluted with water in flasks of 50 mL for analysis of the minerals using an inductively coupled plasma atomic emission spectrometer (ACTIVA-M, HORIBA Jobin–Yvon, Longjumeau, France) with a charge-coupled device. Argon gas plasma was used (flow rate of 13 L/min, sheath flow of 1.5 L/min). The absorption wavelengths were 317.933, 324.754, 238.207, 766.490, 285.213, 275.610, 589.592 and 213.857 nm for Ca, Cu, Fe, K, Mg, Mn, Na and Zn, respectively. A multi-element standard solution was diluted for calibration. The determined concentrations were shown as milligrams/100 g FW.

Statistical analysis
All experiments were conducted triplicately. Data were presented as mean ± standard deviation using Microsoft Office Excel 2010 (Microsoft Corporation, Redmond, WA, USA). Analysis of variance was performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA) for the statistical analysis of each experiment. Significance was determined by Duncan’s multiple range test with p < 0.05.

Results and discussion
Bioactive compounds and antioxidant activity of pepper pericarp
The total phenols, total flavonoids and piperine are important antioxidants in plants, including black pepper [11, 25, 26]. Pericarp contained total phenol, total flavonoid and piperine contents of 1421.95 ± 22.35 mg GAE/100 g, 983.82 ± 8.19 mg CE/100 g and 2352.19 ± 68.88 mg/100 g, respectively (Table 1). These data were compared with the corresponding

Table 1 Concentrations of total phenol, total flavonoid and piperine in black pepper pericarp

| Type                         | Total phenol (mg GAE/100 g) | Total flavonoid (mg CE/100 g) | Piperine (mg/100 g) | References |
|------------------------------|-------------------------------|-------------------------------|---------------------|------------|
| Black pepper pericarp        | 1421.95 ± 22.35              | 983.82 ± 8.19                 | 2352.19 ± 68.88     | This study |
| Methanol extract of black pepper | 172.8                       | 108.7 (μg QE/100 g)          |                     | [37]       |
| White pepper, dried          | 447.23 ± 10.38               |                               |                     | [38]       |
| Black pepper                 | 654                          | 2149 (μg QE/100 g)           |                     | [39]       |
| Black pepper                 | 338 ± 1.414                  |                               |                     | [40]       |
| Black pepper                 | 11.9 ± 0.1                   | 2.1 ± 0.2 (μg QE/100 g)      |                     | [41]       |
| Black pepper                 |                               |                               | 3950–7990           | [42]       |
| Black pepper                 |                               |                               | 5700–7800           | [43]       |
| Black pepper                 |                               |                               | 3566.1              | [44]       |

GAE gallic acid equivalents, CE catechin equivalents, QE quercetin equivalents
literature values for black and white pepper and black pepper extract. Total phenols were from 11.9 to 654 mg GAE/100 g in black pepper and 447.23 mg GAE/100 g in white pepper. Total flavonoids were from 0.002 to 0.109 milligrams of quercetin equivalents (mg QE)/100 g in black pepper and methanol extracts of black pepper (Table 1). The concentration of total phenols was higher in pericarp than black pepper, white pepper and the methanol extract of black pepper, whereas pericarp contained less piperine compared with black pepper. However, the concentrations of total flavonoids in black pepper and the black pepper extract were determined as quercetin equivalents, so a direct comparison with pericarp was not feasible.

The DPPH and ABTS antioxidant activities were higher than those in black pepper by 42.5.2% and 41.9%, and ABTS radical scavenging activities of pericarp were 225.33 ± 25.14 VCE/100 g FW for black pepper, respectively. The DPPH and ABTS radical scavenging activities of pericarp were higher than those in black pepper by 42.5.2% and 41.9%, respectively.

**Flavor compounds by GC/MS**

Thirty-eight volatile flavour compounds were characterised in pericarp, and their peak areas were determined (Table 2). The main components were monoterpenes, sesquiterpenes and their oxygenated forms [27]. Most of the monoterpenes, except for linalool and 3-carene, were more volatile than the sesquiterpenes, with earlier retention times. The principal monoterpene compounds were α-pinene (9.2%), 2-β-pinene (14.3%), δ-3-carene (21.5%) and δ-limonene (18.8%), and the primary sesquiterpenes were α-copaene (5.1%) and caryophyllene (17.2%). β-Pinene, δ-3-carene, limonene and caryophyllene are abundant volatile flavour compounds in black, white and green peppers [28]. Pericarp contained a higher percentage of α-pinene and α-copaene than peppers. α-Pinene imparts a terpenic odour, according to Murthy and Bhattacharya [29]. Therefore, the pericarp has a more terpenic odour than black, white and green peppers. β-Mycene and linalool are known as the most potent odorants of black pepper, although they are relatively minor flavour compounds in black pepper [15, 17], as confirmed in the current work. Therefore, pericarp would have a more potent odour compared with black pepper.

**Flavor compounds by an electronic nose**

Black pepper, pericarp and inner kernel after removing the skin were analysed by an e-nose. Some of the discriminative volatile compounds were identified by the AroChemBase database (Table 3). Figure 3 shows the GC/FID of discriminative volatile compounds of black pepper, pericarp and inner kernel. 1R-(+)-α-pinene, α-chlorotoluene, cyclopentane, psi-cumene, heptyl mercaptan, dipentene, terpine, limonene, 3-methyldeca-1, 8-octanethiol, heptyl benzene and methyl undecanoate were identified as the main volatile compounds by an e-nose with two different columns. Although

| Flavour compound | Retention time (min) | Peak area (×10^4), (%area) | Type |
|------------------|----------------------|---------------------------|------|
| 1 α-Pinene       | 9.4                  | 231.65 ± 56.69 (0.2)      | m    |
| 2 α-Fenchene     | 9.0                  | 5.00 ± 1.55 (0.2)         | m    |
| 3 Camphene       | 10.0                 | 5.17 ± 1.68 (0.2)         | m    |
| 4 2-β-Pinene     | 11.0                 | 333.21 ± 81.81 (14.3)     | m    |
| 5 Sabinene       | 11.4                 | 14.45 ± 4.81 (0.6)        | m    |
| 6 α-Terpine      | 11.6                 | 0.70 ± 0.21 (0.0)         | m    |
| 7 δ-3-Carene     | 12.2                 | 499.25 ± 19.74 (21.5)     | m    |
| 8 β-Mycene       | 12.6                 | 57.78 ± 19.44 (2.5)       | m    |
| 9 1-Phellandrene | 12.7                 | 40.88 ± 11.36 (1.8)       | m    |
| 10 Sabinene      | 12.8                 | 1.02 ± 0.35 (0.0)         | m    |
| 11 α-Terpine     | 13.1                 | 2.97 ± 1.04 (0.1)         | m    |
| 12 α-Limonene    | 13.7                 | 437.14 ± 99.26 (18.8)     | m    |
| 13 β-Phellandrene| 14.0                 | 7.94 ± 2.35 (0.3)         | m    |
| 14 1,8-cineole   | 14.1                 | 2.04 ± 0.51 (0.1)         | o.m  |
| 15 α-Thujene     | 14.9                 | 1.35 ± 0.39 (0.1)         | m    |
| 16 γ-Terpine     | 15.0                 | 3.40 ± 0.97 (0.1)         | m    |
| 17 α-Cymene      | 15.7                 | 3.44 ± 1.11 (0.1)         | m    |
| 18 p-Cymene      | 15.8                 | 50.36 ± 15.23 (2.2)       | m    |
| 19 α-Terpinolene | 15.9                 | 2.57 ± 0.85 (0.1)         | m    |
| 20 α-Terpinolene | 16.1                 | 5.32 ± 1.72 (0.2)         | m    |
| 21 α-Cubebene    | 20.8                 | 10.19 ± 3.06 (0.4)        | m    |
| 22 δ-Elemene     | 21.2                 | 16.02 ± 4.83 (0.7)        | m    |
| 23 α-Amorphene   | 21.6                 | 1.64 ± 0.37 (0.1)         | m    |
| 24 α-Copaene     | 21.8                 | 120.72 ± 30.75 (5.1)      | m    |
| 25 α-Gurjunene   | 22.8                 | 2.78 ± 0.68 (0.1)         | m    |
| 26 β-Cubebene    | 23.0                 | 7.45 ± 1.99 (0.3)         | m    |
| 27 Linalool      | 23.1                 | 13.36 ± 3.49 (0.6)        | m    |
| 28 germacrene A  | 24.3                 | 7.87 ± 2.68 (0.3)         | m    |
| 29 Caryophyllene | 24.6                 | 399.78 ± 89.37 (17.2)     | m    |
| 30 α-Humulene    | 26.3                 | 19.30 ± 5.11 (0.8)        | m    |
| 31 Linalyl propionate | 26.9 | 2.12 ± 0.66 (0.1) | m    |
| 32 germacrene D  | 27.2                 | 1.53 ± 0.47 (0.1)         | m    |
| 33 β-bisabolene  | 27.4                 | 2.06 ± 0.90 (0.1)         | m    |
| 34 β-selinene    | 27.5                 | 11.58 ± 4.57 (0.5)        | m    |
| 35 α-selinene    | 27.6                 | 6.85 ± 1.95 (0.3)         | m    |
| 36 δ-Cadinene    | 28.2                 | 8.97 ± 2.70 (0.4)         | m    |
| 37 3-Carene      | 28.5                 | 1.09 ± 0.38 (0.0)         | m    |
| 38 Caryophyllene oxides | 33.5 | 5.18 ± 1.51 (0.2) | a.s. |
| Sum              | 232.61 ± 575.42 (100)|                        | m    |

m monoterpenes, a.o.m oxygenated monoterpenes, s sesquiterpenes, a.o.s oxygenated sesquiterpenes
some volatile compounds, such as pinene, terpene and limonene, were identified by GC/MS, most of the volatile compounds identified by an e-nose were not identified by GC/MS. Therefore, GC/MS is not suitable as the sole method to identify volatile flavour compounds in black pepper, and an e-nose should be simultaneously used. Furthermore, cyclopentane, pentyl and dipentene/terpene/limonene were identified by only column MTX-1701. Column MTX-1791 is better for e-nose analysis than column MTX-5. Pericarp contained higher peak areas of heptyl benzene and methyl undecanoate compared with black pepper.

Figure 4 shows an odour map based on PCA of all volatile compounds of black pepper, pericarp and inner kernel. PC1 and PC2 accounted for major differences (99.379%) and minor differences (0.5935%), respectively.

### Table 3 Main volatile compounds identified by electronic nose in black pepper pericarp

| Flavor compounds | Retention time (s) | Peak area ($\times 10^3$, (%area)) | Sensory description | Peak area comparison |
|------------------|-------------------|----------------------------------|--------------------|-------------------|
|                  | MXT-5  | MTX-1701 | MXT-5  | MTX-1701 |                  |
| 1 1R- (+)-α-pinen | 68.68 | 64.74  | 21.6 (4.6) | 20.1 (4.8) | Aromatic, harsh, minty, pine, terpenic | B > P > I |
| 2 o-chlorotoluene | 76.33 | 74.05  | 67.6 (14.4) | 52.0 (12.3) | Aromatic       | B > P > I |
| 3 Cyclopentane, pentyl | –    | 76.05  | 11.7 (2.8) | –     | –                | B > P > I |
| 4 Psi-cumene      | 81.65 | 78.89  | 104.1 (22.1) | 94.7 (22.5) | Aromatic herbaceous plastic | B > P > I |
| 5 Heptyl mercaptan| 84.55 | 82.91  | 106.1 (22.5) | 81.8 (19.4) | Onion sulphurous | B > P > I |
| 6 Dipentene/terpene/limonene | –   | 85.82  | 12.2 (2.9) | Citrus green pine | B > P > I |
| 7 3-methyldecane  | 94.30 | 92.96  | 4.9 (1.0) | 1.1 (0.3) | Balsamic mild phenolic | B > P > I |
| 8 1-octanethiol   | 99.89 | 102.01 | 1.5 (0.3) | 1.2 (0.3) | Mild sulphurous | B > P > I |
| 9 Heptyl benzene  | 137.47| 137.15 | 34.5 (7.3) | 28.7 (6.8) | –                | P > I > B |
| 10 Methyl undecanoate | 146.25 | 146.82 | 130.7 (27.7) | 117.7 (27.9) | Bland fatty fruity oily sweet waxy winey | P > I > B |

B: black pepper; P: pericarp of black pepper; I: inner kernel of black pepper after removing the pericarp
The flavour compounds in the various parts of black pepper had different flavour profiles, with the pericarp exhibiting higher minor and tiny differences. It shows that pericarp has a fruitful flavour than the other parts, as determined by GC/MS analysis.

Minerals
Based on the mineral composition of pericarp, K was the most abundant, followed by Ca, Mg, Fe, Mn and Na (Table 4). Comparatively lower concentrations of K, Ca, Mg, Fe, Mn, Zn and Cu have been reported in black pepper (Table 4) [13, 30]. Ca and Mg are important minerals in forming bone, strengthening heart functions, relaxing muscle, conducting memory and metabolizing glucose [31–33]. Black pepper pericarp would have stronger health effects than black pepper because of its higher concentrations of minerals. Moreover, it contained a much higher level of K relative to white bean (463.1 mg/100 g), one of the best sources of K. Black pepper contained higher concentrations of Ca compared with yoghurt (181.6 mg/100 g) and milk (120 mg/100 g), which are known to contain high levels of Ca. The Mg level in black pepper pericarp was more than that mentioned in spinach (67 mg/100 g), tuna (64 mg/100 g) and brown rice (44 mg/100 g), and only slightly lower than that in almonds (270 mg/100 g) [34–36].

Table 4 Mineral contents in black pepper pericarp

| Mineral | Concentration (mg/100 g) | This study | Al-Jasass and Al-Jasser [13] | Özcan and Akbulut [30] |
|---------|--------------------------|-----------|-----------------------------|------------------------|
| Mn      | 24.52±2.80               | 3.5±0.2   | 12.97±2.28                  |
| Zn      | 1.02±0.01                | 0.9±0.1   | 0.93±0.09                   |
| Mg      | 209.97±0.61              | 52.0±8.0  | 148.69±21.58                |
| Cu      | 0.90±0.02                | 1.3±0.1   | 0.48±0.06                   |
| Ca      | 549.73±4.61              | 195.0±15.0| 264.00±30.24                |
| Fe      | 42.44±1.08               | 20.5±0.5  | 8.92±1.14                   |
| K       | 2377.03±13.68            | 663±25.0  | 1000.60±49.75               |
| Na      | 7.44±0.11                | –         | 189.21±27.95                |

Fig. 4 An odour map of black pepper, its pericarp and its inner kernel based on principal component analysis.

Abbreviations
DPPH: 2,2-Diphenyl-1-picrylhydrazyl; ABTS: 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid).

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Authors’ contributions
JGL analyzed data and wrote the manuscript and YS analyzed the antioxidant ability. YC analyzed the flavours with e-nose analyzer. YJK organized this study and manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data analysed during this study are included in this published article.

Competing interests
The authors declare that they have no competing interests.

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