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Total polyphenol and ferulic acid analysis of a new variety of corn, Bandiburichodang, according to steaming time and roasting temperature

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Abstract Bandiburichodang (BDC) is a new variety of *Zea mays* L. Total polyphenol content (TPC) assay and quantitative analysis of ferulic acid (FA) were performed to determine the steaming, roasting conditions of BDC kernels that lead to the highest content. TPC levels increased after roasting under all conditions. TPC levels in samples steamed at 115 ºC for 25 min were 3.157 mg/g before roasted, and increased to 3.825 and 4.739 mg/g after roasting at 160 and 200 ºC, respectively. Whether BDC kernels were roasted was relevant with TPC content. BDC kernels were extracted to perform quantitative analysis of FA. Roasting temperature affected FA content: the higher the temperature, the lower the content. BDC kernels that were steamed at 115 ºC for 25 min had 0.178 mg/g of FA content before roasting, and levels decreased to 0.132 and 0.115 mg/g after roasting. Under different roasting conditions, FA content decreased 15 to 50%. We hypothesize that this phenomenon is due to a breakdown of phenolic compounds or cell wall disruption.

Keywords Bandiburichodang · Ferulic acid · High-performance liquid chromatography/Photodiode array detector · Roasting · Total polyphenol content · *Zea mays*

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Introduction

Zea mays L., otherwise known as maize, is a crop that is cultivated worldwide. *Z. mays* is a staple food in many continents including Africa, Asia, and Latin America [1]. Approximately 60% of the global yield of *Z. mays* is produced by USA and China [2]. *Z. mays* is beloved all over the world for its rich carbohydrate content and its numerous uses in animal feeds [3]. It is an excellent source of bioactive compounds, such as polyphenols, phenolic acids, flavonoids, anthocyanins, and carotenoids [4].

Ferulic acid (FA) is a phenolic compound in *Z. mays* kernels [5]. It is found in the seeds and leaves of numerous plants [6]. FA was isolated from *Ferula foetida,* where its name originated. FA is known for its various therapeutic activities: antioxidative effects against diabetes, cancer, and other diseases and for its superior cytoprotective ability [7,8]. In fact, FA is frequently a component of Chinese phytomedicine, and awareness of FA is high in China because of this connection [9]. In addition to anticancer, antiproliferative, and antibacterial effects, FA has also been observed to promote wound healing in studies with diabetic rats. Wounds treated with FA re-epithelialized in a shorter time, compared with untreated wounds [10]. Studies have shown that *Z. mays* has a high FA content. A study by Adom and Liu (2002), showed that corn had higher free FA content than other grains such as rice, oats, and wheat [11].

Bandiburichodang (BDC), a new variety of *Z. mays*, was first developed by Mr. Gunhwa Park, Agricultural Corporation Company Maru, Pyeongtaek, Korea. BDC is a yellow chodang, or super sweet corn, species of *Z. mays* [12]. Super sweet corn is often harvested as green corn and used as a snack food [13].

Since it is a novel variety, studies regarding BDC is relatively scant. FA has been found to be one of the abundant compounds in corn [14]. Hence, this study speculated that it could also be detected well in the new variety. The existence of a correlation between how BDC kernels are pretreated (steaming time, steaming temperature, and roasting temperature) and FA contents of the kernels has been suggested.

This study hypothesized that in addition to steaming, the roasting of BDC kernels might influence the phenolic content. Hence, a quantitative analysis of FA by high-performance liquid chromatography (HPLC) and total polyphenol content (TPC) assays with BDC kernel samples was performed.

Materials and Methods

Plant materials

BDC (National Seed Resource Variety Protection No. 5008) was cultivated by Mr. Gunhwa Park, Agricultural Corporation Company Maru, Pyeongtaek, Korea (Fig. 1). Kernel samples were made using different steaming and roasting conditions. BDC plants were grown according to the instructions of the Rural Development Administration, Korea. Mr. Gunhwa Park modified the spacing of individual plants, such as 15 plants per square foot with distances of 30 cm between stems and 70 cm between rows.

Instruments and reagents

Quantitative analysis was performed using the Waters Alliance 2695 Separations Module (USA), and Water 996 Photodiode Array detector (USA). INNO C_{18} columns (4.6×250 mm, 5 μm) for high-performance liquid chromatography (HPLC) were purchased from Youngjin Biochrom Co., Korea, and acetic acid (glacial, 100%) from Sigma-Aldrich, Germany. HPLC-grade water, methanol (MeOH), and acetonitrile (ACN) were obtained from J. T. Baker (Phillipsburg, PA, USA). Acetic acid (AA), tannic acid (TA) and FA (Fig. 2) were provided by Natural Product Institute of Science and Technology (www.nist.re.kr), Anseong, Korea.

Extraction of BDC kernels

BDC kernels were extracted with ethanol (EtOH) using a Soxhlet reflux evaporator. Ten grams of dried BDC kernel samples were measured and then ground into fine powder before extraction with 300 mL for 3 h. Each experiment was performed in triplicate. Dehydrated extracts were collected after evaporation with a rotary vacuum evaporator.

Total polyphenol content (TPC)

One gram of each sample was dissolved in 10 mL distilled water, and sonicated for 30 min. After centrifugation at 4,000 rpm for 10 min, the supernatant liquid was collected and filtered through 0.45 μm polyvinylidene fluoride (PVDF) membrane for testing. TA was used as a standard of the TPC assay. One milligram of TA was dissolved in 1 mL distilled water and sequentially diluted for testing. TPC was measured by adding 7.5 mL distilled water to each test tube, followed by 1 mL each standard and sample solutions, 0.5 mL of Folin-Denis reagent, and 1 mL 35% sodium carbonate. Samples were incubated in a dark room for 1 h and then the absorbance at 760 nm was measured with a microplate reader.

Preparation of standard and sample solutions for HPLC

BDC extracts (20 mg) were dissolved in 1mL of MeOH (20 mg/mL), and sonicated. FA (1 mg) were dissolved in 1 mL MeOH, and then sequentially diluted to produce a calibration curve. Both the standard and the sample solutions was filtered through 0.2 μm PVDF membrane filter.

HPLC conditions

A wavelength of detector was set to 324 nm. The mobile phase consisted of 0.3% AA in water (A) and ACN (B). The gradient conditions were as follows; 90% of A at 0 min, 70% of A at 20 min, 50% of A at 25 min, 0% of A at 30 min, and 90% of A at 40 min. The injection volume was set to 10 μL, flow rate to 1 mL/min, and column temperature to 35 °C.

Calibration curve

When forming the calibration curve, the value of the X axis (μg/mL) signifies the concentration of FA, and the Y axis value (mAU) indicates the area of FA (Fig. 3). To generate a calibration curve, seven FA solutions with different concentrations (100-1.56

Fig. 1 Image of BDC kernels

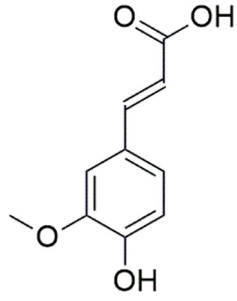


Fig. 2 Chemical structure of FA

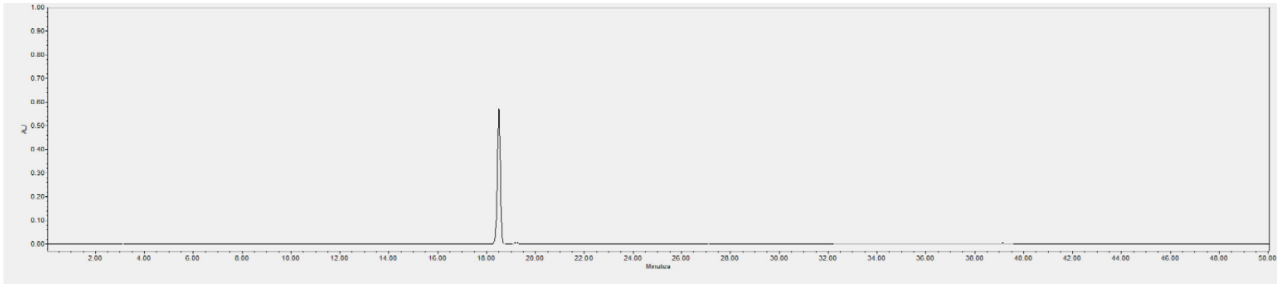


Fig. 3 HPLC chromatogram of FA

Table 1 Calibration curve of FA

Compound	t_R	Calibration equation[a]	Correlation factor, r^2 [b]
FA	18.5	Y = 44170 X - 13156	0.9998

[a]Y = peak area, X = concentration of standards (μg/mL)
[b]r^2 = correlation coefficient based on three data points in the calibration curves

ppm) and associated peak areas (4420304-75203 mAU). The total FA content (mg/g) was calculated by multiplying C, V, D, P and dividing by W (C: concentration of standard, V: total volume of the test solution, D: dilution factor, P: standard purity, W: sample weight.). The calibration curve of FA showed good linearity, with the correlation factor (r^2) of 0.9998 (Table 1).

Results and Discussion

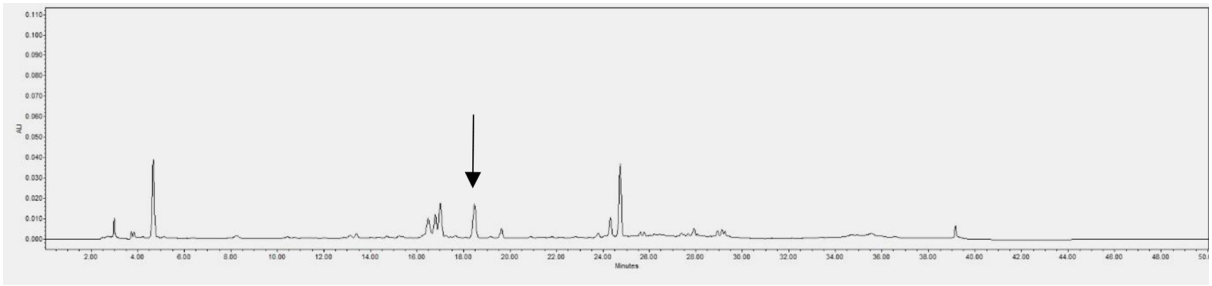
Z. mays is used in diverse food items, such as chips, tortillas, and bread [15]. Getting a healthy dose of functional food has become a concern for consumers [16]. Our goal was to identify treatment conditions that isolate the highest quantity of functional phenolic compounds from BDC kernels. Therefore, we evaluated different steaming and roasting conditions and measured the yields of TPC. After extraction with EtOH, TPC assays on each sample and quantitative analysis using HPLC to measure content of FA, a marker component of BDC kernels, were performed.

We found that TPC increased after roasting. One proposed reason is that roasting improves extraction efficiency. TPC levels increased after roasting under all conditions. TPC levels in samples steamed at 115°C for 25 min were 3.157 mg/g before roasted and increased to 3.825 and 4.739 mg/g after roasting at 160 and 200 °C, respectively, and TPC levels in samples steamed at a same temperature for 50 min were 2.916 mg/g before roasted and increased to 6.535 and 4.834 mg/g after roasting at 160 and 200 °C, respectively. When steamed at 121 °C for 25 min, TPC levels were 3.342 mg/g before roasted and elevated to 4.375 and 5.399 mg/g after roasting at 160 and 200 °C, respectively, and for samples steamed at a same temperature for 50 min were 3.918 mg/g before roasted and increased to 8.336 and 7.574 mg/g after roasting at 160 and 200 °C, respectively (Table 2). Boateng et al. (2008) explained that the heating process improves the extractability of these substances by disrupting the cell wall and degrading insoluble phenolic compounds [17]. Thermal processing is studied to weaken the cell wall and cause unwanted color changes, because of the heat [18]. Kim et al. (2014) discovered that carrot cell walls that were treated with heat more than 5 min were broken and irregularly shaped [19]. However, in 50 min of steaming conditions, certain decrease in TPC levels has been observed when roasting temperature increased. We assumed this phenomenon was a result of extreme heating conditions. Yadev et al. (2012) found out that as steaming time increased, tannin content decreased [20].

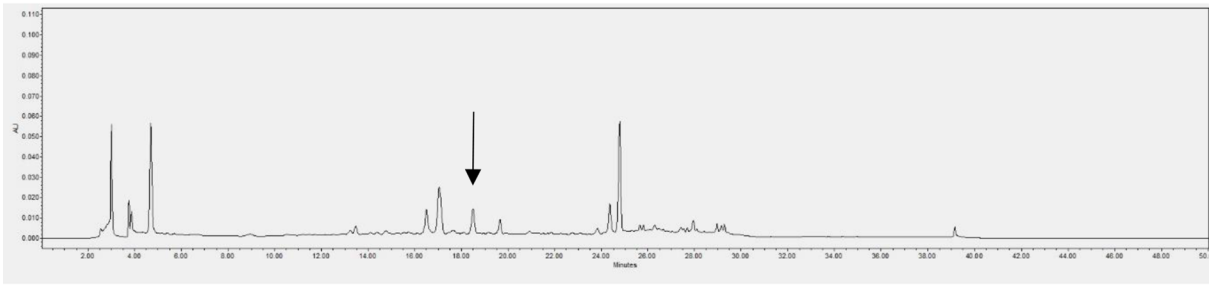
Because there are many phenolic compounds, there are diverse functionalities. As secondary metabolites, phenolic compounds affect plant color, volume, and resistance against infectious agents [21]. In humans, they are associated with pharmaceutical functions, such as cancer suppression [22]. Phenolic compounds are anticipated to have utilizations in various industries, such as food and cosmetics. For example, phenolic compounds have been

Table 2 TPC in distilled water extracts of BDC kernels, according to steaming conditions and roasting temperature

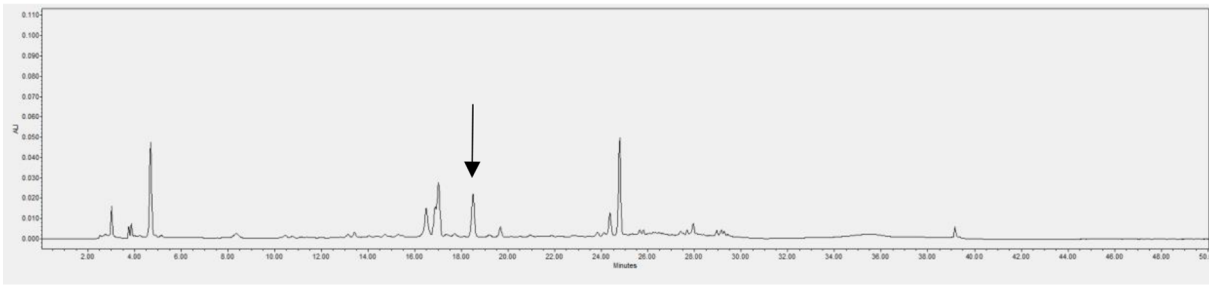
Steaming time (min)	Steaming temperature	Roasting temperature		
		Raw (mg/g extract)	160 °C (mg/g extract)	200 °C (mg/g extract)
25	115 °C	3.157±0.199	3.825±0.403	4.739±0.148
	121 °C	3.342±0.136	4.375±0.173	5.399±0.027
50	115 °C	2.916±0.041	6.535±0.448	4.834±0.049
	121 °C	3.918±0.033	8.336±0.207	7.574±0.260



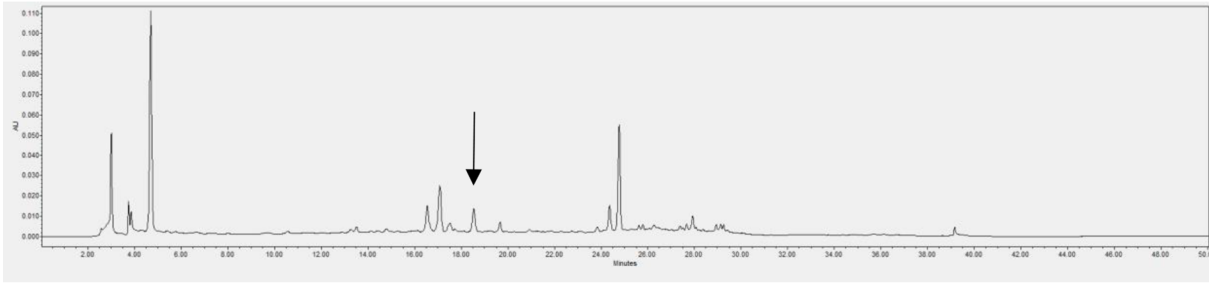
(A)



(B)



(C)



(D)

Fig. 4 HPLC chromatograms of 115 ºC/25 M/Raw (A), 115 ºC/25 M/160 ºC (B), 121 ºC/25 M/Raw (C) and 121 ºC/25 M/160 ºC (D): steaming temperature/steaming time/roasting temperature treatments of BDC kernels

added to processed foods to intensify antioxidative activities. Using phenolic compounds in the dyeing of fabric has been proposed to improve environmental friendliness [23].

Previous studies reported correlations between roasting and chemical compositions within plants. Jannat et al. (2013) found that TPC and γ-tocopherol content in Iranian sesame seeds increased after roasting. The reason proposed was that γ-tocopherol disconnected from membrane proteins or phospholipids during roasting [24].

On the other hand, we found that roasted samples had a lower FA content relative to unroasted samples (Fig. 4). BDC kernels that were steamed at 115 ºC for 25 min had 0.178 mg/g FA content before roasting, and levels decreased to 0.132 and 0.115 mg/g after roasting. Under different roasting conditions, FA

Table 3 Content of FA in EtOH extracts of BDC kernels, according to steaming time and roasting temperature

Steaming time (min)	Steaming temperature	Roasting temperature		
		Raw (mg/g extract)	160 °C (mg/g extract)	200 °C (mg/g extract)
25	115 °C	0.178±0.001	0.132±0.000	0.115±0.001
	121 °C	0.222±0.001	0.120±0.001	0.118±0.000
50	115 °C	0.222±0.000	0.190±0.001	0.111±0.001
	121 °C	0.232±0.000	0.103±0.000	0.118±0.000

content decreased 15 to 50% (Table 3). There is an inverse relationship between roasting temperature and FA content. However, this case wasn't true for the kernels steamed at 121 °C for 50 min as FA content decreased when the roasting temperature increased from 180 to 200 °C. Therefore, the authors concluded that it is hard to find a definite relationship between roasting temperature and FA content since the samples had minute amount of FA.

According to an analysis of free phenolic compounds by Samaras et al. (2005), FA was shown to be degraded in heavily roasted malts [25]. Previous studies mention that FA has anti-inflammatory and anticancer properties, with low toxicity [8]. An experiment on rats by Wang et al. (2018) suggested that FA helps reduce obesity and the symptoms associated with obesity; they discovered that FA-treated mice fed high-fat meals gained less weight and had substantial blood sugar increases than control mice [26].

FA is used in multiple packaging and cosmetics applications because of its antimicrobial and other effects [27]. Sharma et al. (2020) studied the impacts of FA on food films, such as antibacterial efficiency and temperature equilibrium, and proposed that FA could be used in food packaging [28]. Peres et al. (2018) performed *ex vivo* antioxidant, *in vivo* sun protection factor, and *in vitro* UVA protection factor assays and proposed that FA has a synergistic effect with UV filters in sunscreens [29]. Park et al. (2018) investigated the skin-whitening and anti-wrinkle activity of FA as a food additive [30].

We observed no significant relationship between the two steaming conditions and FA content in BDC kernels; however, we found that roasting increased the TPC. We propose that the increase in TPC is not related to FA; rather, the TPC increase is likely due to better extractability of phenolic compounds as a result of roasting. We hypothesize that additional studies of FA might reveal more industrial applications for FA.

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