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Original Research Article

Retention of polyphenols in blueberries (*Vaccinium corymbosum*) after different cooking methods, using UHPLC–DAD–MS based metabolomics

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ABSTRACT

This study investigated the impacts of baking, boiling and microwaving methods on polyphenols in blueberries, using ultra-high performance liquid chromatography (UHPLC) with a photodiode array detector (PDA) and mass spectrometer (MS). Twenty-eight characteristic peaks were found in blueberries of which 25 were significantly affected by cooking. The retention of each compound was calculated based on its peak areas in PDA chromatograms and expressed as ratio (%) of its peak area in cooked to fresh blueberries. The retention of total anthocyanins ranged from 74.3–76.4%, 52.9–77.4%, and 58.0–72.3%, and the values for other polyphenols ranged from 77.1–88.7%, 76.0–86.7%, 66.6–76.8%, respectively, after baking, boiling, and microwaving treatments. Caffeoylquinic acid was the predominant peak in both cooked and fresh blueberries. Its concentrations in blueberries baked for 5 min or boiled for 1, 3, and 10 min were not significantly different from those in fresh samples ($p > 0.05$). An A-type procyanidin trimer was found to be the most unstable polyphenol; its concentrations decreased to 53.0%, 42.8%, and 36.1% of that in fresh blueberries, respectively, after 15-min baking, 10-min boiling, and 45-s microwaving. Compared to other polyphenols, caffeoylquinic acid, catechin and quercetin glycosides were the most stable. In general, microwaving led to the highest losses of polyphenols when cooking blueberries.

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1. Introduction

Blueberries (*Vaccinium corymbosum*) are one of the most popular berries consumed in the United State. Blueberries contain large amounts of polyphenols and are either consumed fresh or used in different foods, such as muffins, scones, salads, bread, syrup, smoothies, and so on. Due to seasonal availability, they are subject to various types of processing treatments. Blueberries are very popular fruits, of which the consumption has increased rapidly over the past decades. The United States blueberries total production in 2014 was 5.67 million pounds. The intake of blueberries or a blueberries-enriched diet has been associated with anti-inflammatory (Coban et al., 2015) and antioxidant (Huang et al., 2012; Castrejon et al., 2008) activities, protective effects on neuronal stress (Poulose et al., 2014), diabetes intervention (Liu et al., 2015), benefit to cardiovascular health (Basu et al., 2010), improvement in cognitive performance (Whyte and Williams, 2015), modulation of intestinal microbiota (Vendrame et al., 2011), and improvement of renal function (Nair et al., 2014). Increasing evidence suggests that different categories of polyphenols contained in blueberries are responsible for such beneficial effects (Basu et al., 2010; Howard et al., 2012; Lachance and Das, 2007). These polyphenols include anthocyanins (delphinidin, cyanidin, petunidin, peonidin, malvidin and their *O*-glycosylated saccharides), flavonols (quercetin, syringetin and their *O*-glycosides), catechin and proanthocyanins, as well as hydroxycinnamic acid derivatives (Gavrilova et al., 2011; Li et al., 2013).

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Generally, blueberries (fresh or frozen ones) are popularly consumed as fruits, added to cereals, salads, smoothies, yogurts, and so on, eaten directly without cooking. They are also widely consumed after being cooked, such as those used in pizza, cakes, cookies and microwavable foods. However, thermal treatments usually have impacts on polyphenols and therefore on blueberries' health-promoting properties (Brownmiller et al., 2008).

The retention of polyphenols after cooking has been studied in food science. It was reported that baking, boiling, and microwaving treatments could lead to different degrees of reduction of polyphenols in different vegetables (Price et al., 1997; Tudela et al., 2002; Vallejo et al., 2003). As for the studies on blueberries, thermal processing resulted in loss of polyphenols and lowered antioxidant activities (Brownmiller et al., 2008; Arancibia-Avila et al., 2012). However, these antioxidant activity values were not specific for polyphenols and the pH differential method does not serve well for anthocyanin profiling. Another study reported that a 32% degradation of anthocyanins was observed after heating blueberries in a thermostatted water-bath or oil-bath for 20 min at 100 °C at atmospheric pressure, and that 50% of total anthocyanins were lost in blueberry juice after being heated for 20 min at 100 °C at 600 MPa (Buckow et al., 2010).

Metabolomics is an emerging field of "omics" research that focuses on high-throughput data on small molecules in complex matrices. Chemometrics is generally used to help explain high-throughput data obtained from such matrices, e.g., petroleum products (Eide and Zahlsen, 2005), herbal medicines (Donno et al., 2016) and fruits (Eisenstecken et al., 2015). The combination of the two methods serves as a perfect tool to explore the internal relevance of high-throughput data. So far, most of the studies on metabolomics combined with chemometrics have focused primarily on clinical or pharmaceutical applications, such as drug discovery and development (Kell, 2006), drug safety assessment and disease diagnosis (Lindon et al., 2004), clinical toxicology (Griffin and Bollard, 2004) and clinical chemistry (Moolenaar et al., 2003). Over the past few years, metabolomics combined with chemometrics has also emerged as a field of interest to food and nutrition scientists (Gibney et al., 2005), who have used the technique widely in food component analysis (Ehrman et al., 2007), food quality/authenticity assessment (Aursand et al., 2007), food consumption monitoring (Gibney et al., 2005), and physiological monitoring of diet and nutrition studies (German et al., 2005; Wishart, 2008).

To the best of our knowledge, no comprehensive and systematic study has been reported on retention of individual polyphenols after fresh blueberries are cooked in different ways. Therefore, the objective of the present study was to investigate the effects of baking, boiling and microwaving methods for different cooking times on retention of different categories of polyphenols in blueberries, based on a modern ultra-high-performance liquid chromatography diode array detection high-resolution mass spectrometry (UHPLC–PDA–HRMS) method. After cooking and preparing the samples, we acquired chemical profiles for blueberries by both PDA and MS. Then the data were processed and analyzed in two steps: 1) metabolomics combined with chemometrics was used, based on total ion current chromatograms (TIC), to discover marker compounds which were statistically different between fresh and cooked blueberries; and 2) retention of the marker compounds was calculated based on their peak areas in PDA chromatograms.

Consumers may consider using frozen or cooked blueberries when fresh blueberries are not in season. The results obtained in the present study will enable consumers to make decisions about how to maximize the retention of nutrients during cooking.

2. Materials and methods

2.1. Chemicals and fruit samples

Formic acid for mass spectrometry, ~98% pure, was purchased from Sigma-Aldrich (St. Louis, MO). Optima® LC/MS grade methanol and acetonitrile were purchased from Thermo Fisher Scientific (Fair Lawn, NJ). HPLC-grade water was prepared from distilled water using a Milli-Q system (Millipore Laboratories, Bedford, MA). Ten lots of blueberries were purchased from a Giant Food Store (Beltsville, MD). The labels indicated that they were products of USA.

2.2. Sample preparation

Ten lots of fresh blueberries were fully mixed and prepared using 3 different cooking methods in the present study. The selection of cooking methods and cooking times was based on traditional recipes.

1) *Baking method:* 20 g of fresh blueberries was accurately weighed and baked at 350 °F using a Modern Maid oven (Whirlpool, Benton Harbor, MI) for 3 different cooking times (5/10/15 min).

2) *Boiling method:* 20 g of fresh blueberries was accurately weighed and put into 30 mL of boiling water in a 100-mL beaker and boiled for 3 different cooking times (1/3/10 min).

3) *Microwaving method:* 20 g of fresh blueberries was accurately weighed and microwaved using a Panasonic NN-SN651 B Genius 1.2 cubic feet 1200-W microwave for 3 different cooking times (15/30/45 s).

Fresh and cooked blueberries were transferred into a Waring Commercial BB190 NuBlend Elite Blender with 60 mL of methanol. After being blended for 30 s, 60% methanol was added into the slurry mixture to make a constant volume at 150 mL which was then centrifuged at 5,000*g* for 15 min. Five milliliters of supernatant for each sample was filtered through a 17-mm PVDF syringe filter (VWR Scientific, Seattle, WA) and transferred into a 2-mL HPLC vial for analysis.

2.3. UHPLC–PDA–HRMS conditions

The UHPLC–PDA–HRMS system consisted of an LTQ Orbitrap XL mass spectrometer with an Accela 1250 binary pump, a PALHTC-Accela1-TMO autosampler, an Accela PDA detector (Thermo Fisher Scientific, San Jose, CA), and a G1316A column compartment (Agilent, Santa Clara, CA). The separation was carried out on a Hypersil Gold C18 column (200 mm × 2.1 mm, 1.9 mm; Thermo Fisher Scientific, San Jose, CA) with a flow rate of 0.3 mL/min. The mobile phase consisted of a combination of **A** (0.1% formic acid in water) and **B** (0.1% formic acid in acetonitrile). The linear gradient was from 4% to 20% **B** (*v*/*v*) at 40 min. The column temperature was set at 50 °C, and PDA spectra were recorded from 200 to 700 nm. The MS conditions were set as follows: sheath gas, 70 (arbitrary units); aux and sweep gas, 10 (arbitrary units); spray voltage, −2.5 kV (−)/3.0 kV (+); capillary temperature, 250 °C; capillary voltage, −50 V (−)/50 V (+); tube lens, −150 V (−)/120 V (+). The most intense ion was selected for the data-dependent scan with normalization collision energy at 35%. Dynamic exclusion was used for the ion triggered data-dependent scan for 3 times in 15 s and the exclusion time was 30 s. The injection volume was 5 μL.

2.4. Metabolomics data pretreatment and analysis

The raw data acquired by LTQ Orbitrap XL mass spectrometer were converted to mzXML files by MSConvert in centroid mode, which were compatible with centWave method for feature