

## SHORT COMMUNICATION

# Quality of extracts from blueberry pomace by high hydrostatic pressure, ultrasonic, microwave and heating extraction: A comparison study

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## ABSTRACT

The objective of this study was to compare the efficiency of high hydrostatic pressure, ultrasonic, microwave, and heat extraction techniques on the blueberry pomace extract characteristics. The results showed that the highest extraction yield of anthocyanins and total phenols were obtained by high hydrostatic pressure assisted extraction. The difference of extraction yield of anthocyanins was significant ( $p < 0.05$ ) between high hydrostatic pressure assisted extraction and heating extraction. Furthermore, significant difference of total phenols extraction yield was obtained between high hydrostatic pressure assisted extraction and the other three extraction methods. The total color difference was significant for the four extraction methods. High hydrostatic pressure assisted extraction had the highest chroma and least browning index. Moreover, high hydrostatic pressure assisted extraction was found to have the highest hydroxyl radical scavenging activity. In summary, high hydrostatic pressure assisted extraction was revealed to be the suitable technique for phenolic extraction of blueberry pomace.

**Keywords:** Anthocyanins; Antioxidant activity; Ccolor

## INTRODUCTION

A number of beneficial effects are attributed to anthocyanins, which is an important bioactive compound found in blueberries, including improving visual acuity, antioxidant, anticancer (Shim et al., 2012; Faria et al., 2010). There are many anthocyanins residues that remain after blueberries have been processed for juice, wine or other products (Lee, 2002; Khanal et al., 2010), the anthocyanins extracted from blueberry pomace is a potential source for natural colorants and nutraceuticals. Anthocyanins is sensitive to many factors, including pH, metal ions, light, enzyme, oxygen (Zheng et al., 2003; Castañeda-Ovando et al., 2009) especially, it is unstable during heating. Traditional extraction method contained heating processing will degrade anthocyanins. Many researchers have demonstrated that the heat degradation of anthocyanins aggravates in many products along with the temperature rising (Brownmiller et al., 2008; Sarkis et al., 2013).

A suitable method of anthocyanins extraction is necessary, there are some researches on blueberry anthocyanins extraction by ultrasound (He et al., 2016), microwave (Zheng et al., 2013) and heating (Barnes et al., 2009).

In this years, high hydrostatic pressure (HHP) is recognized as a non-thermal and environment-friendly technology widely used in food industry. High hydrostatic pressure extraction (HHPE) as a technique was first reported by Shouqin et al (2004) is used for extraction active compound from plant materials. HHP can enhance the mass transfer, damage cell membrane, increase permeability, it had been successfully used for extraction carotenoid from tomato puree (Sánchez-Moreno et al., 2004), flavonoids from propolis (Shouqin et al., 2005), anthocyanins from grape skins (Corrales et al., 2009), catechins from green tea (Jun et al., 2010), bioactive compounds from *Deodeok* (He et al., 2011), anti-obesity substance from garlic (Joo et al., 2013).

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Their results indicated HHPE could improve yield, shorten time, and enhance biological activity. However, any study has been conducted on anthocyanins extraction of blueberry by HHPE.

Therefore, the aim of this study was to compare the differences in yield, color, browning index and antioxidant capacity of extracts, obtained from blueberry pomace by different methods such as HHPE, ultrasonic assisted extraction (UE), microwave assisted extraction (ME) and heating extraction (HE).

## MATERIALS AND METHODS

### Plant material

Blueberry pomace were obtained after blueberry wine fermentation, the fresh Rabbiteye blueberries (*Vaccinium ashei* Reade, Gardenblue) were picked from a plantation in Lishui District (China). Blueberries were broken using a presser, after enzymolysis 1.5h at 35°C, blueberries were fermented at 21°C for 7 days. The wine pomace was collected after squeezing and drying to a constant weight at 40°C in a hot-air drying oven. The dried pomace was powdered used a grinder. The powders were stored at -20°C until used.

### Chemicals

All chemicals used in the study were analytical grade and was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

### Anthocyanins extraction preparation

Extraction were conducted by 5.00 g blueberry pomace in a 150 mL extraction solvent which contained 60% ethanol and 12M HCl (V/V=99/1).

### Heating extraction

Packaged samples in polythene bags were cooled immediately after incubated in a water bath at 60°C for 1 h.

### Ultrasonic assisted extraction

Packaged samples in polythene bags were extracted in a Ultrasonic bath equipment (Wuxi Fanbo Biological Engineering Co. Ltd, Wuxi, China) with ultrasonic power of 50w/L water (the device power is 300W, 6L water in the treatment chamber) and ultrasonic frequency of 28KHz for 1h.

### Microwave assisted extraction

Samples in a tub were extracted in a LG W900 microwave oven (Tianjin, China) with microwave power 360W for 150s. The samples were cooled rapidly when extraction completed.

### High hydrostatic pressure assisted extraction

Packaged samples in polythene bags were pressed under 500 Mpa for 3min at room temperature using a high hydrostatic pressure machine (Intelligent Super High Pressure Food Processing Device, Jiangsu University, China).

### Total anthocyanins extraction

The total anthocyanins extraction was carried out UE. The extraction solution was filtered using vacuum filtration equipment (RE-2000B, Shanghai Yarong Biochemical Instrument Factory, China), residue was extracted again using the extraction solvent until the absorbance of the extraction solution was less than 0.005. The filtrate was merged and used to determine the content of anthocyanins and total phenols.

### The extraction yield of Anthocyanins and total phenols

The anthocyanins content of extracts was determined according to Giusti and Rrolstad, 2001. The total phenols content was determined as described by Sellappan et al., (2002). The extraction yield of anthocyanins and total phenols were calculated as follows:

$$\text{Extraction yield} = \frac{\text{the content of different extraction method}}{\text{the content of total extraction}}$$

### Sample determination preparation

The extracted sample was prepared by the method of Prasad et al (2010) with some modifications. 100 mL extraction solution was filtered using vacuum filtration equipment. The filtrate was evaporated to remove the extraction solvent by a rotary evaporator under vacuum at 40°C. The concentration solution was dried using a vacuum freeze dryer, 0.1g dried extracts was diluted with sodium citrate buffer (pH3.0, 0.1M) to 100mL. The sample solution was stored at 4°C until used.

### Browning Index (BI)

The browning index of blueberry extracts was determined according to Buglione and Lozano (2002).

### Color measurements

A DC-P3 colorimeter (Beijing Xingguang Color-Measurement Instrument Company Ltd., China) was used to determine the lightness ( $L^*$ ), Hue (H), Chroma (C) and total color difference ( $\Delta E$ ).

### Hydroxyl radical ( $\cdot\text{OH}$ ) scavenging activity

The antioxidant activity was determined as described by Wang et al., 2008.

### Statistical analysis

All experiments were conducted in triplicate. The statistical analyses were performed using software Excel 2003 and

SPSS 17.0. The Duncan's test was used to determine the differences of means at the 5% confidence level.

## RESULTS AND DISCUSSION

In this research total anthocyanins content reached  $208.53 \pm 20.38$  (mg/100 g sample powders) and total phenols content reached  $2298.99 \pm 16.99$  mg gallic acid equivalents/100 g of sample powders. The extraction yield of anthocyanins and total phenols by different extraction methods were showed in Fig. 1 and Table 1. HHPE had the highest extraction yield of anthocyanins, but the difference between HHPE and HE was significant ( $p < 0.05$ ). Meanwhile, the highest extraction yield of total phenols was obtained by HHPE, the difference was significant between HHPE and the other three extraction methods. The extraction yield of total phenols obtained by UE was higher than HE and ME, but the difference was not significant.

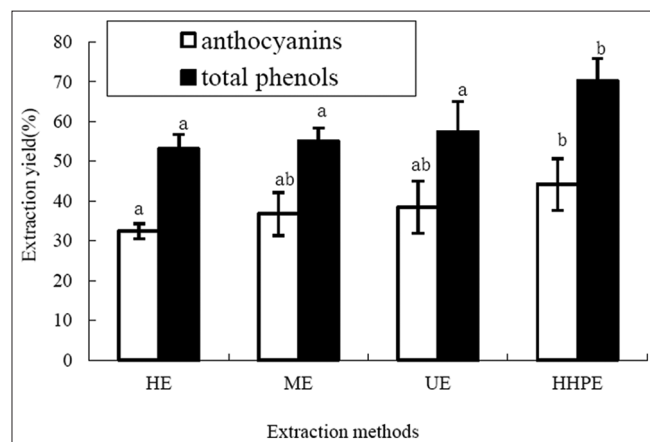
The result was similar to Corrales et al., 2009 who reported that the total anthocyanins content extracted from grape pomace using HHPE was higher than HE and UE, and the difference was significant.

The color parameters is an important factor on the quality of products, the color parameters and browning index of extracts by different extraction methods were measured and

**Table 1: Extraction yield of anthocyanins and total phenols by four extraction methods**

|      | Anthocyanins extraction yield | Total phenols extraction yield |
|------|-------------------------------|--------------------------------|
| HE   | $32.43 \pm 1.91^a$            | $53.18 \pm 3.61^a$             |
| ME   | $36.76 \pm 5.44^{ab}$         | $55.07 \pm 3.32^a$             |
| UE   | $38.44 \pm 6.54^{ab}$         | $57.35 \pm 7.68^a$             |
| HHPE | $44.2 \pm 5.76^b$             | $70.26 \pm 5.63^b$             |

The same letter in the same column indicates that the difference of the means is not significant at the level 0.05

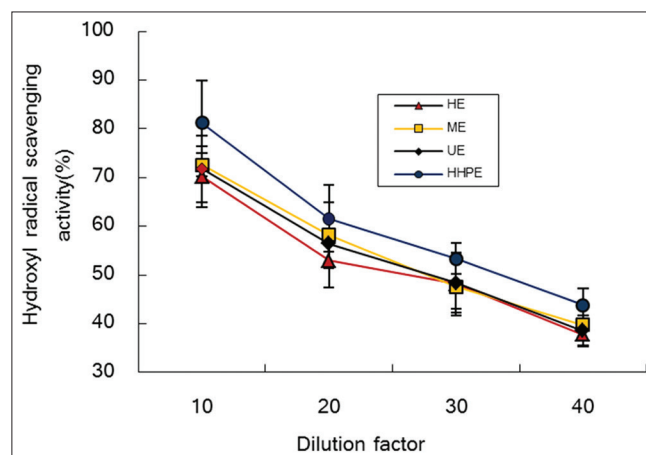


**Fig 1.** Extraction yield of anthocyanins and total phenols by four extraction methods

showed in Table 2. HHPE treated sample had the maximum  $L^*$  and H, but the difference was not significant during four extraction methods. Significant difference of  $\Delta E$  and C were observed in Table 2, HHPE had the highest  $\Delta E$  and C. This may be due to the difference on anthocyanins content, anthocyanins was the main color substance in fruits (He and Giusti, 2010), the most anthocyanins was obtained by HHPE. Hyoung reported  $\Delta E > 2$  was considered a noticeable difference in the visual perception of the samples (Lee and Coates, 2003), therefore, the noticeable difference was likely to be exist between HHPE sample and HE sample which was accordance with the difference of anthocyanins yield. Wrolstad et al. (2005) considered the C increased with pigment concentration increasing within certain limits (Wrolstad et al., 2005), the results also showed some level of positive correlation between C and the content of anthocyanins, the C reflected the content of anthocyanins to some extent.

ME had the highest BI, and HHPE had the least, the difference was significant between HHPE and the other three methods. BI was related to the extraction temperature and extraction time, higher BI was obtained by HE and ME because HE had the longest extraction time (40°C/1 h) and ME had the highest extraction temperature (exceed 60°C), nevertheless, HHPE had the least BI with ambient temperature extraction of 3 min. This may be because the high temperature (heating and microwave) accelerated anthocyanins degradation and Maillard reaction, which enhanced BI. However, the non-significant of HHP treatment under 600 Mpa on anthocyanins degradation or Maillard reaction has been reported by Torres et al. (2011) and Schwarzenbolz et al. (2002).

·OH scavenging capacity of an extracts is directly related to its antioxidant activity (Kim et al., 2011). HHPE revealed a higher ·OH scavenging activity (Fig. 2) than HE, ME and



**Fig 2.** Hydroxyl radical scavenging activity of extracts by four extraction methods.

**Table 2: The color quality and browning index of extracts by four extraction methods**

|      | L*                      | ΔE                     | H                       | C                       | BI                      |
|------|-------------------------|------------------------|-------------------------|-------------------------|-------------------------|
| HE   | 28.86±1.11 <sup>a</sup> | ---                    | 23.42±1.24 <sup>a</sup> | 43.42±0.63 <sup>a</sup> | 1.06±0.01 <sup>bc</sup> |
| ME   | 28.65±0.18 <sup>a</sup> | 1.45±0.52 <sup>b</sup> | 23.95±1.06 <sup>a</sup> | 43.78±0.63 <sup>a</sup> | 1.08±0.01 <sup>c</sup>  |
| UE   | 28.86±1.27 <sup>a</sup> | 0.69±0.16 <sup>a</sup> | 23.22±1.26 <sup>a</sup> | 43.57±0.67 <sup>a</sup> | 1.04±0.02 <sup>b</sup>  |
| HHPE | 30.20±1.06 <sup>a</sup> | 2.95±0.34 <sup>c</sup> | 24.23±1.31 <sup>a</sup> | 45.85±0.52 <sup>b</sup> | 0.99±0.01 <sup>a</sup>  |

The same letter in the same column indicates that the difference of the means is not significant at the level 0.05, Lightness (L\*), Total color difference (ΔE), Hue (H), Chroma (C), Browning index (BI)

UE, it indicated more antioxidant compounds extracted by HHPE because HHP could enhance mass transfer, increase cell permeability and accelerate the movement of water and metabolites. It was in accordance with the extraction yield of anthocyanins and total phenols. The results were similar with some researchers who found the antioxidant activity from green tea, aloe vera, strawberry and blackberry improved under HHPE (Xi et al., 2011; Vega-Gálvez et al., 2011; Patras et al., 2009).

## CONCLUSION

Four extraction methods were used to extract active compounds from blueberry pomace. Compared to HE, ME and UE, HHPE revealed a good effect on blueberry pomace extraction, including higher extraction yield, better color quality, shorter extraction time and higher hydroxyl radical scavenging activity.

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## Authors' Contributions

Yongkun Ma conceived and designed the study, Haining Zhang performed the experiments and analyzed the data, William Tchabo reviewed and edited the manuscript.

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