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**Research Article**

# Effect of Ultrasound on Polyphenols Extraction, Microstructure and Antioxidant Activity of Plum (*Prunus salicina* Lindl.)

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**Abstract:** The effects of ultrasonic treatment on the extraction and antioxidant capacity of polyphenols from plum fruit (*Prunus salicina* Lindl.) were evaluated. The microstructure of the extracted residue was observed by scanning electron microscope. The extraction efficiency of polyphenols increased gradually with the increase of ultrasonic power from 0 W to 420W and the extraction efficiency of total phenol at 420W of ultrasonic power was increased by 22.25% compared with that of without ultrasonic treatment. After ultrasonic treatment, the microstructure of the plum material had significant changed and there were more severe wrinkles of the cell wall and hollow structure in the microstructure of plum. Furthermore, ultrasound did not affect the DPPH scavenging capacity and hydroxyl radical scavenging capacity of plum fruit polyphenols. Therefore, ultrasound was suitable for the extraction of polyphenols from plum fruit.

**Keywords:** DPPH scavenging capacity, hydroxyl radical scavenging capacity, microstructure, polyphenols, scanning electron microscope; ultrasound

## INTRODUCTION

Ultrasound is an emerging technology in food science and technology. Meanwhile, ultrasonic technology is a kind of environmental friendly technology and has been widely used in the extraction of natural products (Rastogi, 2011; Chemat *et al*., 2011). Ultrasound technology had been applied to the polyphenols extraction of plant (Chemat *et al*., 2011; Vilkhu *et al*., 2008; Corbin *et al*., 2015), but most studies mainly focused on optimum extraction parameters and there were few studies reports yet about the impact of ultrasound on the cell microstructure of plant materials and antioxidant capacity of polyphenols.

Plum (*Prunus salicina* Lindl.) is a nutritionally important fruit planted commercially in China, Japan, the United States, Europe and South African (FAO, 2015; Rajchl *et al*., 2013). In addition, plums have a fairly high phenolic content in comparison to some other fruits such as grapes, apple, orange, banana and kiwifruit (Perez-Jimenez *et al*., 2010). Although plum fruits are popular and widely consumed, few studies report on their polyphenols extraction and there are no data about effects of ultrasound on their polyphenols. In the present article, we studied the effects of ultrasound on the extraction efficiency of the polyphenols, the microstructure of plum and the antioxidant capacity of the polyphenols, in order to provide a reference for the application of ultrasonic technology in the extraction of plum polyphenols.

## MATERIALS AND METHODS

**Plant materials:** The plums of *Prunus salicina* Lindl. cv.Furong were collected from Yongtai County of Fujian Province, China. After picking and cleaning, the plum were dried with hot air at 50°C and stored at -20°C refrigerator in the dark. Before extraction processes, they were milled and sieved (0.5 and 2.0 mm particle size).

**Chemicals:** Gallic acid were purchased from Shanghai Yuanye Biotechnology Co., Ltd.. All other organic solvents used in the study were analytical grade.

**Ultrasound-assisted extraction process:** Five grams of samples were placed in a capped triangular flask (250 mL) and mixed with ethanol. The extraction process was conducted in an ultrasonic bath (KQ-600DV, 40 kHz, 300W, Kunshan Ultrasonic Instrument Co. Jiangsu, China). After ultrasonic extraction, the

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mixture was filtered (Whatman No.1 paper). Then the filtrate was collected in volumetric flask and used for the determination of the total polyphenol content. The filtrate was evaporated by a rotary evaporator (at 0.09MPa, 45°C) and the extracts were then lyophilized. The plum extracts were stored at-20°C until further use.

**Effect of ultrasound treatment on polyphenols extraction:** The efficiencies of ultrasonic power of 0, 180, 240, 300, 360 and 420 W, respectively on the yield of polyphenols from plum were investigated. Ultrasonic extraction was performed at temperature of 50°C and a sonication time of 30 min and 60% ethanol was used as extracting solvent.

**Determination of total polyphenol content:** Total polyphenol content in the extracts was determined by colorimetry, using the Folin-Ciocalteu method (Singleton and Rossi, 1965). Briefly, 2.5 mL diluted Folin-Ciocalteu reagent (10%, v/v) was added to 200 μL of sample in a capped glass tube. After 2 min of incubation in the dark at room temperature, 2 mL of aqueous sodium carbonate (7.5%, w/v) was added to the mixture. And then it was made up 10 ml by adding distilled water. After gentle vibration, the mixture was placed in a water bath at 50°C for 30 min and then rapidly cooled down to room temperature. Absorbance was measured at 765 nm using a UV-vis spectrophotometer (756 P, Shanghai Spectrum Instruments Co., Ltd., China). Total polyphenol content was expressed as mg GAE (Gallic Acid Equivalent) per gram of dried plum.

**Scanning electron microscopy:** Extraction residues were dried with hot air at 50°C and further observed their microstructure. Morphological characteristics of the plum powders before and after extraction were observed using a JSM-6380LV scanning electron microscope (Japan).

**DPPH free radical scavenging capacity:** DPPH assay was done according to a method (Yu *et al*., 2005; Dudonné *et al*., 2009) with some modifications. A 100 μM solution of DPPH in methanol was prepared and 5 mL of this solution were added to 1 mL of extract re-suspended in distilled water. The mixtures were vigorously shaken and allowed to stand in the dark for 30 min at 25°C. The absorbance was measured using a spectrophotometer at 517 nm against a blank sample without DPPH. The ability to scavenge the DPPH radical was calculated by using the equation below:

$$\text{Antioxidant capacity (\% inhibition)} = [(A_{control}-A_{sample})/A_{control}]\times 100$$

where $A_{control}$ is the absorbance of the control (DPPH solution without sample) and $A_{sample}$ is the absorbance of the test sample.

**Hydroxyl radical scavenging capacity:** Hydroxyl radicals scavenging capacity was determinated according to previously reported methods (Sun *et al*., 2010; Damien Dorman *et al*., 1995) with some slight modifications. The stoppered test tube added 4.7mL of ultrapure water, 1.0 mL of phosphate buffer 0.15 M pH 7.4, 2 mL of the saffron solution 0.52 mg/mL, 1.0 mL of EDTA-$Na_2$-$Fe^{2+}$ 6 mM, 0.5 mL of sample solution. The mixtures were shaken well and added 0.8 mL of 0.3% $H_2O_2$, then immediately placed in 40°C water bath for 30min. The blank group was replaced by an equal volume of ultrapure water instead of the sample solution and the control group was replaced by an equal volume of ultrapure water instead of the sample solution and $H_2O_2$ solution. Then absorbance was measured at 520 nm (756P spectrophotometer, Shanghai Spectrum Instruments Co., Ltd., China). The capability of scavenge hydroxyl radicals was calculated using the following equation:

$$\text{Hydroxyl radical scavenging ability (\%)} = [(A_{sample}-A_{blank})/(A_{control}-A_{blank})]\times 100$$

where $A_{blank}$, $A_{control}$ and $A_{sample}$ are the absorbance of the blank group, the control group and the test sample respectively.

## RESULTS AND DISCUSSION

**Effect of ultrasonic treatment on the extraction yield of polyphenols:** The effect of ultrasonic treatment on the extraction efficiency of plum fruit polyphenols was shown in Fig. 1. The total phenol content increased with the increase of the power within the range of 0-420W. This is similar to the research results of the Wang *et al*. (2013). The extraction efficiency of the polyphenols in the ultrasonic treatment of 420W was increased by 22.25% compared with that of without ultrasound. Therefore, ultrasound-assisted extraction is a simple and efficient technology.

**Microstructure analysis of the residue after extraction of polyphenols from plum fruit:** In order to investigate the changes in cell wall microstructure of extracted materials, a scanning electron microscope was used to obtain pictures of samples before and after extraction. In Fig. 2A, the cell tissue surface of the plum powder before extraction was smooth and the structure was compact and complete. After variant extractions, the microstructure of the plum was changed obviously, the cell walls were damaged and wrinkled and hollow cell structure existed. Moreover, there were apparently more severe wrinkles of the cell wall and hollow structure in Fig. 2C than that of in Fig. 2B. Therefore, ultrasonic treatment destroyed more seriously the plum cell than that of without ultrasound.

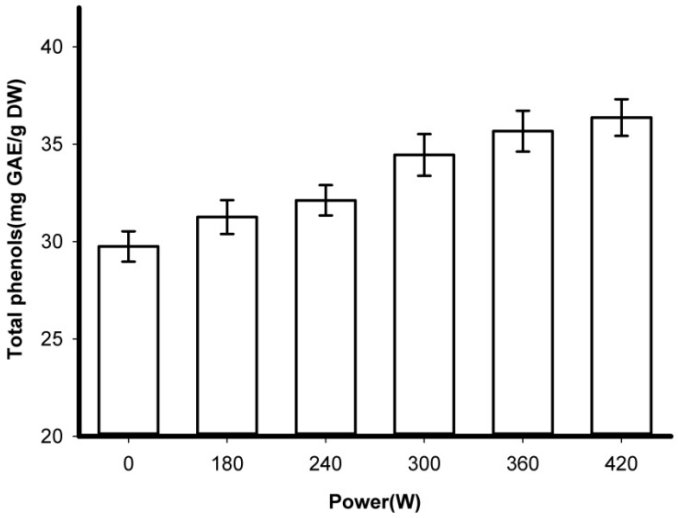


Fig. 1: The effect of ultrasonic treatment on the extraction efficiency of polyphenols

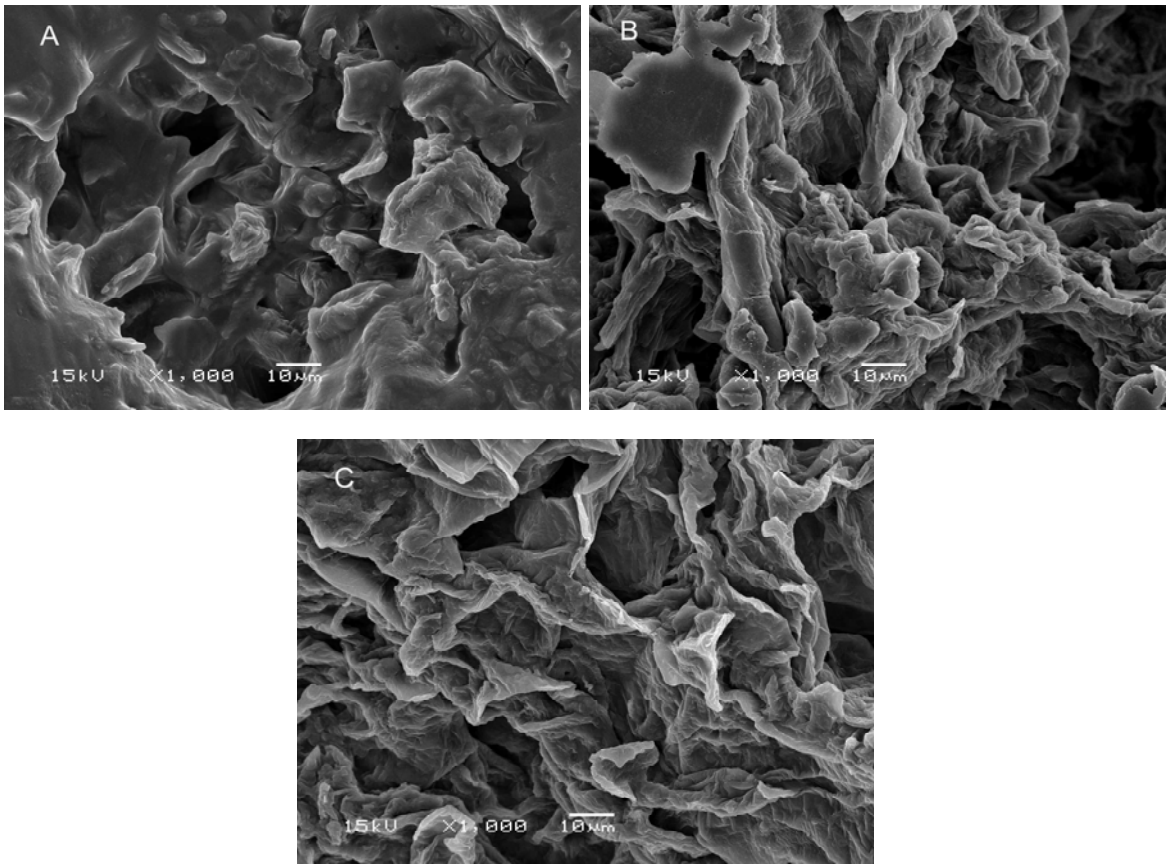


Fig. 2: Scanning electron microscope pictures (15.0 kV) of plum before and after extraction. A: raw powder before extraction; B: residue after water bath extraction (not using ultrasound); C: residue after ultrasonic treatment

The reason may be that ultrasonic cavitation destroys the cell walls of the plant matrix and its content can be released into the medium. Many reports presented that ultrasound waves after interaction with subjected plant material alter its physical and chemical properties and their cavitational effect facilitates the release of extractable compounds and enhances the mass transport by disrupting the plant cell walls (Chemat *et al*., 2011; Vilkhu *et al*., 2008). The phenomenon of ultrasonic destruction of cell wall structure was also accordant with the extraction yield in Fig. 1.

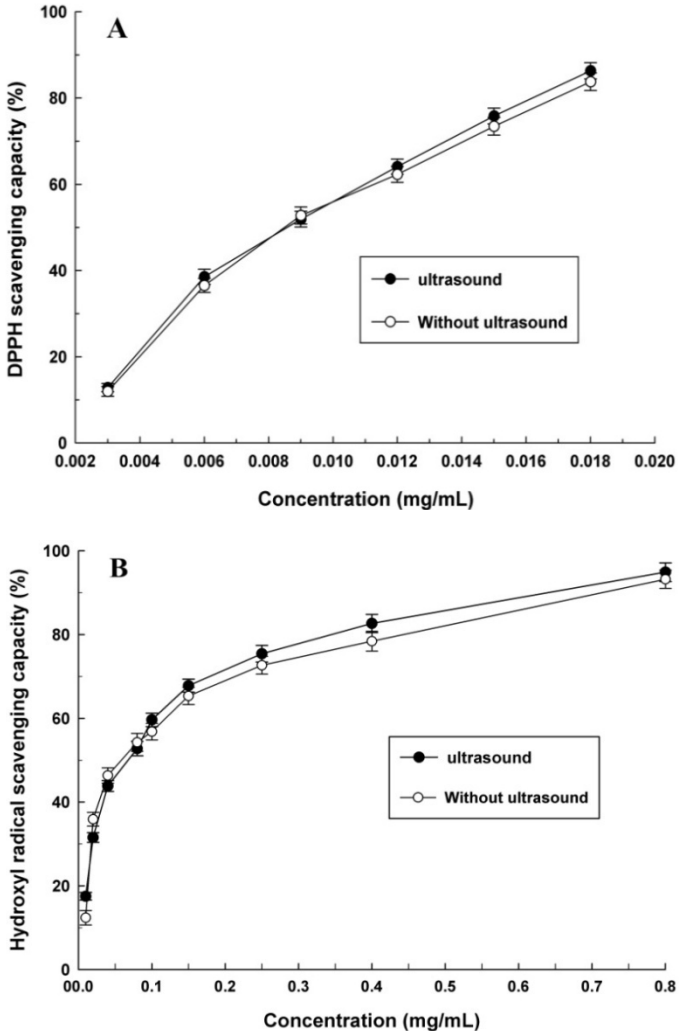


Fig. 3: Effect of ultrasound on antioxidant capacity of polyphenols from plum fruit: A: DPPH scavenging capacity; B: hydroxyl radical scavenging capacity

**Effect of ultrasound on antioxidant capacity of polyphenols from plum fruit:** The effect of ultrasonic on the antioxidant capacity of polyphenols from plum fruit was shown in Fig. 3. As can be noted in Fig. 3A, the effect of ultrasonic treatment on the DPPH scavenging capacity of polyphenols from plum fruit was not significantly. In the range of 0.003 mg/mL to 0.018 mg/mL, the DPPH scavenging capacity increased obviously with the increase of phenolic concentration. There is a dose effect relationship between DPPH scavenging capacity and concentration. From Fig. 3B, it can be seen that the ultrasonic assisted extraction has no significant effect on the hydroxyl radical scavenging capacity of the plum extracts. The hydroxyl radical scavenging ability of plum polyphenols rose sharply to 60% when polyphenol concentrations from 0.01 mg/mL to 0.1 mg/mL. Therefore, the antioxidant capacity of the plum extracts from the ultrasonic assisted extraction was not significantly different from that of the plum extracts from without ultrasonic extraction.

## CONCLUSION

The extraction efficiency of polyphenols from plum fruit increased with the increase of ultrasonic power from 0W to 420W and the extraction efficiency of 420W was increased by 22.25% compared with that of without ultrasonic treatment. After ultrasonic treatment, the microstructure of the plum material had significant changed, cell surface wrinkled more severe and more cavities, which may be caused by ultrasonic cavitation. In addition, ultrasound had no significant effect on the DPPH scavenging capacity and hydroxyl radical scavenging capacity of polyphenols extract of plum fruit. Therefore, ultrasound can improve the extraction efficiency of *Prunus salicina* Lindl, but does not affect its antioxidant effects.

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