

Research Article

Measurement and Analysis of Calcium Content in Wild Chinese Dwarf Cherry (*Prunus humilis*) Fruits by Inductively Coupled Plasma-optical Emission Spectrometer

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Abstract: Different forms of calcium in wild Chinese dwarf cherry (*Prunus humilis*) fruits (two genotypes, small-fruit-type and big-fruit-type) were sequentially extracted and measured by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES). The calcium content variation after postharvest storage was also studied, to provide the theory basis for the active nutritional ingredient evaluation of Chinese dwarf cherry (*Prunus humilis*). The results showed that, wild Chinese dwarf cherry (*Prunus humilis*) fruits in mature stage were rich in calcium nutrition element and mainly composed by water soluble calcium and calcium phosphate which absorbable to human. Absorbable calcium (including water soluble calcium and calcium phosphate) content in small-fruit-type (63.79%) was significantly higher than that of big-fruit-type (54.97%). After postharvest storage, the water soluble calcium content was increased while the calcium pectate and calcium oxalate content dropped. Especially to the big-fruit-type, the absorbable calcium content increased to 75.49% compared with just picking 54.97% (small-fruit-type increased from 63.79% to 68.86%). The study suggested that Chinese dwarf cherry (*Prunus humilis*) was a natural and biological calcium supplement to people; meanwhile, small-fruit-type was more suitable, especially after postharvest storage.

Keywords: Calcium composition, calcium content, chinese dwarf cherry (*Prunus humilis*), fruit, ICP-OES

INTRODUCTION

Chinese dwarf cherry (*Prunus humilis*) is a kind of Chinese endemic wild species, which extensively distributed in north of China (Wang *et al.*, 2005; Zhao *et al.*, 2010). Their seeds are widely used in medicine as laxatives, diuretic and detumescence. Their fruits are rich in sugar, amino acid, vitamin C and several kinds of trace elements, especially the calcium element (Cao *et al.*, 1999; Ma *et al.*, 2007). It has been reported that the calcium content in Chinese dwarf cherry (*Prunus humilis*) is higher than many other fruits (Matkovic and Heaney, 1992; Weaver, 2000; Cashman, 2002).

It is well known that calcium as the essential element to human body is closely related to growth and development. So, we took the Chinese dwarf cherry (*Prunus humilis*) as materials to study the different forms of calcium contents in mature fruits as well as the calcium contents variation after postharvest storage, to provide a scientific basis for their further development and utilization.

EXPERIMENTS

Apparatus and work conditions: Optima 2100DV Inductive coupled plasma optical emission spectrometer

(PerkinElmer Instruments Co., Ltd./America). ICP-OES optimal working conditions were determined by orthogonal experiments and the results were as followed: plasma flow rate: 16.0 L/min; auxiliary flow rate: 0.20 L/min; nebulizer flow rate: 0.70 L/min; RF generator power: 1200 w; observation methods: radial; observation height: 15 mm; solution uptake rate: 1.50 mL/min; washing time: 30s; integral time: 2-5s.

Reagents: NaCl, HCl, HClO₄, SrCl₂ and CH₃COOH were of GR, high purity argon (99.999%).

Ultrapure water (18 mΩ) was obtained from MILLI-Q Biocel ultrapure water instrument (Millipore Company/America). A series of standard solution 0, 25, 50, 100 mg/L were prepared by 2% HNO₃ and 1000 mg/L Ca standard solution (provided by China national standard research center). All the vessels used were firstly soaked with 10% nitric acid solution for 24 h, ultrapure water flushed 3-4 times and dried.

Materials: Chinese dwarf cherry (*Prunus humilis*) were obtained from the wild population of YanShan mountains in Hebei province, north of China, about ten years old. According to the morphological differences, they can be divided into two genotypes, small leaf and small fruit type (small-fruit-type for short) and big leaf

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and big fruit type (big-fruit-type for short). The former leaves light green, single fruit weight about 5 g, the latter leaves dark green and single fruit weight about 10 g.

Sample preparation: In maturation period (13th August), randomly collected fully ripened Chinese dwarf cherry (*Prunus humilis*) fruits without mechanical wound and diseases. Each genotype selected three plants, each plant collected 45 fruits, repeated 3 times and then stored at room temperature about 25°C. In 13th (picking day), 21st (stored for a week) and 29th (stored for two weeks), tested calcium content, respectively.

Different forms calcium was sequentially extracted according to Konishi and Kasai (1963) and improved as: deionized water, 1 mol/L NaCl, 0.348 mol/L CH₃COOH and 1.625 mol/L HCl as extraction solution to extract water soluble calcium, calcium pectate, calcium phosphate and calcium oxalate, respectively. After smashed, accurately weighed about 10 g homogenate, mixed with the extracted solvent mentioned above in a centrifuge tube and kept in a thermostat water bath about 25°C for 1 h, then centrifuged under 6000 r/min for 10 min. The supernatant solution was transferred to a 100 mL volumetric flask. The precipitate was washed by the extracted solvent 3~4 times, then mixed the washing liquid with supernatant solution, added some SrCl₂ solution to exclude the interference factor, at last added

extracted solvent to 100 mL. The residue which the main component was calcium silicate (referred as residual calcium), was acid digested by the HNO₃:HClO₄ (V: V = 1: 5). The calcium content was measured by the ICP-OES.

All the data were analyzed by the SPSS software.

RESULTS AND DISCUSSION

The calcium composition of Chinese dwarf cherry (*Prunus humilis*) fruit: The extracted calcium in Chinese dwarf cherry (*Prunus humilis*) fruits including water soluble calcium, calcium pectate, calcium phosphate and calcium oxalate, which amount to more than 98% of the total calcium (big-fruit-type 98.43%, small-fruit-type 98.76%). The residual calcium was less than 2% (big-fruit-type 1.57%, small-fruit-type 1.24%). And, the calcium content in small-fruit-type (202.30 mg/kg) was obviously higher than that of big-fruit-type (163.65 mg/kg) ($p < 0.05$) (Table 1). The contents of different forms calcium were different in two genotypes fruits. To small-fruit-type, the water soluble calcium, calcium phosphate and calcium oxalate content were significantly higher than that of big-fruit-type ($p < 0.01$), while in big-fruit-type fruits the calcium pectate content was higher ($p < 0.01$). The absorbable calcium including water soluble calcium and calcium phosphate (Bao *et al.*, 2007; Wang *et al.*, 2010) in big-fruit-type fruits accounted for 54.97% of the total calcium and in small-fruit-type that was 63.79%.

Table 1: The content of diverse forms calcium in *Prunus humilis* fruits (X±S.D.)

Calcium forms	Big-fruit-type (n = 3)		Small-fruit-type (n = 3)	
	Content (mg/kg·FW)	% Total	Content (mg/kg·FW)	% Total
Soluble calcium	61.69±1.61	37.70	91.59±2.38 ^a	45.27
Calcium pectate	51.41±2.49	31.41	37.46±2.19 ^a	18.52
Calcium phosphate	28.27±1.71	17.27	37.46±2.34 ^a	18.52
Calcium oxalate	19.71±1.90	12.04	33.29±3.95 ^a	16.46
Residual calcium	2.57±0.47	1.57	2.50±0.47	1.24
Total calcium	163.65±10.28	100.0	202.30±16.65 ^b	100.0

a: $p < 0.01$, b: $p < 0.05$

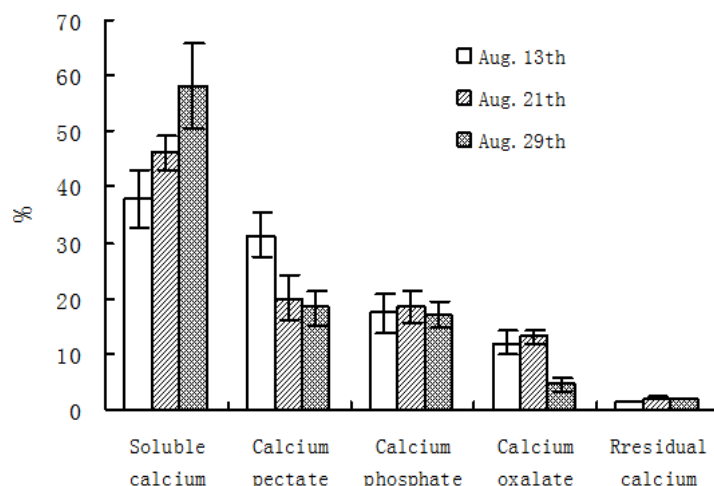


Fig. 1: The composition percentage change of diverse form calcium of *Prunus humilis* large fruits during storage period

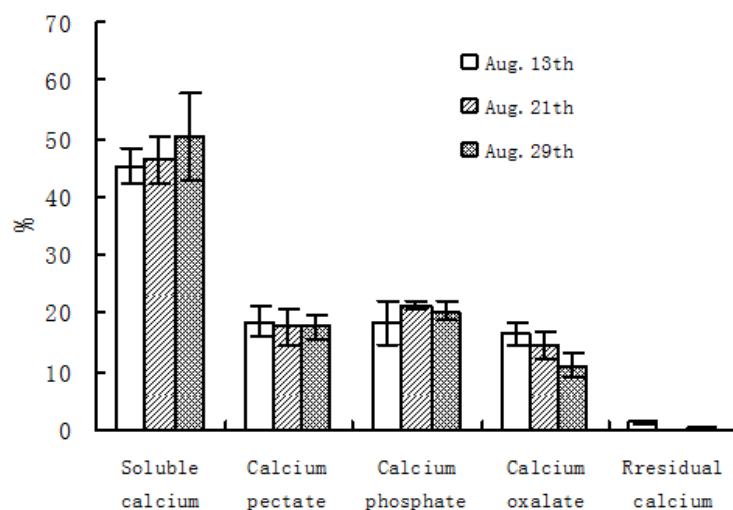


Fig. 2: The composition percentage change of diverse form calcium of *Prunus humilis* small fruits during storage period

The calcium composition percentage variation after storage: The calcium composition percentage variation after storage was shown in Fig. 1 and 2. To two genotypes fruits, the trend was identical, that was water soluble calcium content increased and calcium pectate and calcium oxalate contents were distinctly decreased, meanwhile, the calcium phosphate and residual calcium content had no significant change. However, in terms of two genotypes fruits, the different forms calcium variation range was different. After stored two weeks, the water soluble calcium in big-fruit-type and small-fruit-type were increased by 20.52% ($p < 0.01$) and 5.07% ($p > 0.05$), calcium pectinate were decreased by 13.18% ($p < 0.01$) and 0.72% ($p > 0.05$), calcium oxalate state were decreased by 7.37% ($p > 0.05$) and 5.53% ($p > 0.05$), respectively. The results indicated that, during the storage, different forms of calcium reciprocally converted and the conversion degree had to do with the genotypes.

CONCLUSION

Chinese wild dwarf cherry (*Prunus humilis*) fruits in mature stage were rich in calcium nutrition element and mainly composed by water soluble calcium and calcium phosphate. The calcium content especial the absorbable calcium content in small-fruit-type (63.79%) was significantly higher than that of big-fruit-type (54.97%). After storage, the absorbable water soluble calcium content was increased while the calcium pectate and calcium oxalate contents dropped. Especially to the big-fruit-type, the absorbable calcium content rose to 75.49% compared with just picking 54.97% (small fruit increased to 68.86% from 63.79%), moreover calcium and phosphorus ratio is suitable (Guo and Wang, 2010). Therefore, after storage, Chinese dwarf cherry (*Prunus humilis*) fruits

are appropriate for children and elder people to supplement calcium.

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