



# Chemical composition and antioxidant and anti-inflammatory potential of peels and flesh from 10 different pear varieties (*Pyrus* spp.)



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## ABSTRACT

This study was performed to compare the contents of total phenolics, total flavonoids, and total triterpenes between peel and flesh of ten different pear cultivars. The monomeric compounds were analyzed by HPLC, their antioxidant and anti-inflammatory activities were also measured. Peel and flesh from Yaguang, Hongpi, Qingpi and Guifei varieties contained relatively more total phenolic, total flavonoids and total triterpene, and showed stronger antioxidant and anti-inflammatory activities, while Lvbaoshi and Youran appeared to be weakest among them. All the chemical components found in the pear peel were approximately 6–20 times higher than those in the flesh of pear. For the monomeric compounds, arbutin, oleanolic acid, ursolic acid, chlorogenic acid, epicatechin, and rutin were the dominant components contained in the ten pear cultivars both in peel and in flesh. All of the analyses suggested that the peel of pear might be an excellent polyphenol and triterpenes source.

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## 1. Introduction

Pear (*Pyrus* spp.) fruit is one of the most widely consumed fruits through the whole world, and it is commonly found in processed products such as drink, candy, preserved fruit and jams. Pears have also been used as a traditional folk remedy in China for more than 2000 years because of their antitussive, anti-inflammatory, antihyperglycaemic and diuretic activities. There is a great diversity of pear varieties in China due to its widespread consumption. Each kind is characterized by different appearances and contents of phenolic compounds, nutritional ingredients, antioxidant and anti-inflammatory activities and some other properties.

During recent years, some researchers have been focused on analyses and comparison of the nutritional components contained in the edible part of pear fruit such as total sugars, vitamins, organic and fatty acids, amino acids, volatiles, polyphenols, minerals and so on (Kahle, Preston, Richling, Heckel, & Schreier, 2005; Tanrıöven & Ekşi, 2005). Apart from some common reported compounds such as arbutin, chlorogenic acid, catechin, quercetin, kaempferol, various hydroxycinnamoylmalic acids and their ethyl esters, hydroxycinnamoyl malates, procyanidins and triterpenes compounds have also been found in the peel of pear (Lee et al., 2011a; Lee et al.,

2011b; Ma et al., 2012). In addition, 108 volatile compounds were identified by headspace solid-phase microextraction (HS-SPME) with gas chromatography-mass spectrometry (GC-MS) from 33 cultivars of the Chinese pear *Pyrus ussuriensis* (Qin et al., 2012). Furthermore, the changes of the aroma volatile components, sugars, organic acids and phenol acids of Yali (*Pyrus bretschneideri* Rehd.) during storage have also been studied (Chen, Yan, Feng, Xiao, & Hu, 2006).

Phenolics, important bioactive compounds, possess strong antioxidant activity. Vegetables and fruits are good sources of phenolic compounds. It is widely believed that ingestion of fresh fruits and vegetables related to the reduction of cardiovascular and cancer diseases (Jaramillo et al., 2010). Increasing researches also showed that the higher intake of vegetables and fruits could reduce the risk of getting immune-dysfunctions (Gibson et al., 2012). Phenolics are correlated to resistance to pathogens environment, and contained in all the part of plant including root, stem, leaf, and fruit (peel, flesh, seeds). Compared to flesh, many fruits such as apple, hawthorn, and pomegranate, their peels contain more phenolics (Li et al., 2013). However, it is well known that peels are often discarded during the manufacture. For this reason, research for the active compounds and their bioactive effects contained in the peel of fruit will be of great importance and necessity. Although the chemical and nutritional components and their bioactivities contained in peel have already been substantially reported (Fischer, Carle, & Kammerer, 2011; Lin & Harnly, 2008), it is still

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remained unclear about the difference of polyphenolic, triterpenes contents and their antioxidant and anti-inflammatory activities between peel and flesh in Chinese different pear fruits. In our previous work, the phenolic acid, flavonoids, triterpenes contents and their antioxidant, anti-inflammatory effects of some pears distributed in China were compared (Li, Zhang, Gao, & Wang, 2012), and seven compounds were isolated and identified from Xuehua pear, including two sterols compounds ( $\beta$ -sitosterol, daucosterol), four triterpenes compounds (oleanolic acid, ursolic acid, 2 $\beta$ , 19 $\alpha$ -dihydroxy ursolic acid and  $\alpha$ -amyrin) and one quercitrin compound. In order to make further development from the available pear source, we determined the phenolic acid, flavonoids, triterpenes contents in peel and flesh of ten common pear fruits grown in China. Their major phenolic acid, flavonoids, triterpenes compounds were identified by high performance liquid chromatography (HPLC), and the antioxidant and anti-inflammatory activities of peel and flesh in pears were assessed and compared, respectively.

## 2. Materials and methods

### 2.1. Reagents

Arbutin, catechin, epicatechin, rutin, chlorogenic acid, gallic acid, ferulic acid, vanillic acid, *p*-coumaric acid, oleanolic acid and ursolic acid (reference standard, purity  $\geq 99.0\%$  each) were purchased from the Natl. Inst. for the Control of Pharmaceutical and Biological Products (Beijing, China). High-performance liquid chromatography (HPLC) grade acetonitrile and glacial acetic acid were purchased from Concord Technology Co., Ltd. (Tianjin, China). Folin–Ciocalteu reagent (FC reagent, puriss.,  $\geq 99.0\%$ ) and xylene were purchased from Guangfu Fine Chemical Research Institute (Tianjin, China). The 1,1-diphenyl-2-picrylhydrazyl (DPPH puriss.,  $\geq 98.0\%$ ) and Evan's blue were purchased from Sigma Company (St. Louis, MO), while dexamethasone (DMX) was purchased from Lisheng Pharmaceutical Company, Ltd. (Tianjin, China). Other chemical reagents including Vanillin, NaNO<sub>2</sub>, NaOH, AlCl<sub>3</sub>, KCl, HCl, HClO<sub>4</sub>, methanol and so on, were of analytical grade (purity  $>90\%$ ) and purchased from Tianjin Jiangtian Chemical Technology Co., Ltd. (Tianjin, China).

### 2.2. Materials

Ten pear cultivars were used in this study. Among them, Laiyang (*P. bretschneideri* Rehd. cv., LYL), and Guifei (*Pyrus communis*, GFL) were collected from Yantai and Liaocheng, Shandong Province, respectively. The other two *P. communis* species, Qingpi and Hongpi were cultivated in South Africa and collected from the supermarket in Tianjin. Yaguang belongs to the *P. ussuriensis* Maxim species, was collected from Langfang, Hebei Province, while Jinqiu was collected from Shaoyang, Hunan Province. Lvbaoshi (LBS), a hybrid kind of Xinshiji and Zaosu, was collected from Liaocheng, Shandong Province. Mantianhong (MTH) and Meirensu (MRS) are two new varieties with red color peels, which are hybrid varieties produced by crossing Kingshui and Huoba pear about ten years ago and were widely planted in southwest China in recent years. The two samples were picked from Kunming, Yunnan Province. Youran (YRL), an import pear species from Korea, was collected from the supermarket in Tianjin, China. The ten pear cultivars without disease symptoms were selected and then peeled. The fresh pericarp and flesh tissues were collected, respectively, then lyophilised in liquid nitrogen and finally stored at  $-20\text{ }^{\circ}\text{C}$  for further extraction and analysis.

### 2.3. Methanol extraction procedure

The frozen peel and pulp of each pear cultivar were ground into powders (10 g) and then extracted with methanol: water (6:4,

100 mL  $\times$  3) (KH-250B, 120 W, 50 kHz), each time lasted 30 min. Afterwards, the suspension was with medium speed filter paper (15–20  $\mu\text{m}$ ) and then evaporated in vacuum below  $-50\text{ }^{\circ}\text{C}$  to obtain the solid extract. The extraction was prepared at a 0.1 g/mL of the freeze-drying pear concentration in methanol, and the solution was stored at  $4\text{ }^{\circ}\text{C}$  until used for chemical analysis.

The methanol extract samples for anti-inflammatory activity tests were prepared same as the procedure described by Huang, Gao, Li, and Zhao (2010).

### 2.4. Animals

Kunming mice (about 18–22 g) were purchased from the Institute of Tianjin Laboratory Animal Center, Tianjin. These mice were kept at  $25 \pm 1\text{ }^{\circ}\text{C}$  under a 12 h light/dark cycle environment and allowed free access to food (standard pellet diet) and water ad libitum. All experiments were performed according to the approved protocols of the Animal Ethics Committee, China Pharmaceutical University, China. These mice were free access to water and standard diet and were fasted for 10 h before each experiment. This study was carried out comply with the "Regulation for the Administration of Affairs Concerning Experimental Animals" (State Council of China, 1988).

### 2.5. Determinations of total phenolics content

The Total phenolics (TPs) content in the peel and pulp extract of those ten pear cultivars was determined using Folin–Ciocalteu's reagent as described in Singleton and Rossi (1965) with minor modification (Cui, Nakamura, Ma, Zhong, & Kayahara, 2005). The absorbance of each sample was measured at 765 nm with a spectrophotometer after incubating at  $25\text{ }^{\circ}\text{C}$  for 2 h. The TPs content was calculated from a calibration curve, using gallic acid as standard (40–900  $\mu\text{g}$  per 100 g of dry weight).

### 2.6. Determinations of total flavonoids content

The total flavonoid (TFs) content was measured as aluminum chloride colorimetric method which described by Allothman, Bhat, and Karim (2009). Rutin was chosen as the standard. The TFs was determined at 506 nm with spectrophotometer. The data were expressed as milligram rutin equivalents (RE)/100 g dry weight and the calibration curve was ranged from 40 to 840  $\mu\text{g}$ .

### 2.7. Determination of total triterpenes

The quantitative analysis of total triterpenes (TTs) was tested by the pH differential method which described by Chen, Xie, and Gong (2007b). The mixture solution, contained 0.2 mL of 5% Vanillin-glacial acetic acid solution and 1 mL of perchloric acid, was used as chromogenic agent, and the absorbance was measured at 550 nm by spectrophotometer. TTs content was determined using colorimetric technique and expressed as oleanolic acid equivalents (oleanolic acid/mg sample) through the calibration curve and the calibration curve ranged from 1.42 to 98.26  $\mu\text{g}/\text{mL}$ .

### 2.8. HPLC analysis of monomeric compounds

Phenolic compounds were determined using a Waters 1525–2998 HPLC-PAD (photodiode array detector) system. The Kromasil C18 column (250  $\times$  4.6 mm i.d., 5  $\mu\text{m}$  particle size, Phenomenex, USA), preceded by a 4  $\times$  3.0 mm i.d. security guard column with the same material. The methanol solution was filtered through a 0.22  $\mu\text{m}$  filter (Agilent Technologies, Beijing, China) and 20  $\mu\text{L}$  of the standard and sample solution was injected into the HPLC system. The mobile phases were 0.5% (v/v) aqueous acetic

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