



# Changes in physicochemical properties of mulberry fruits (*Morus alba* L.) during ripening

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## ABSTRACT

Mulberry fruits contain various substances known to have physiological activities in human health. The aim of this study was to investigate changes in physicochemical properties of mulberry fruits at seven maturity stages during ripening. As the fruits were ripening, lightness and yellowness decreased. Firmness rapidly decreased when the fruits fully matured, changing from 12.7 to 1.1 kgf. Soluble solids and pH tended to increase, while acidity, crude ash, dietary fiber, protein, minerals, and amino acids decreased during ripening. Free sugars of the fully matured fruits were ten times higher than those of the immature fruits. Content of  $\gamma$ -aminobutyric acid tended to decrease, changing from 113.2 to 17.1 mg/100 g during ripening. Free phenolic acids, quercetin-3-rutinoside, and 1-deoxynojirimycin decreased during ripening, especially over six times more chlorogenic acid in the immature fruits than in the fully matured fruits. Total phenolics, anthocyanins, and antioxidant activities increased during ripening.

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## 1. Introduction

Mulberry belongs to the genus *Morus* of the family Moraceae, which grows widely throughout the area ranging from the tropics to the subarctic (Machii et al., 2000). Mulberry fruits are commonly consumed as fresh fruits, juice, and alcoholic beverages like wine. In addition, immature and mature mulberry fruits are used as traditional medicines for tonics, antidiabetics, and immunostimulants (Tang and Eisenbrand, 2011; Kim et al., 2013; KFDA, 2012). Chemical components in mulberry fruits, including phenolics,  $\gamma$ -aminobutyric acid (GABA), amino acids, organic acids, minerals, and sugars, have been studied (Lee et al., 2004; Kim et al., 2004; Ercisli and Orhan, 2007; Pawlowska et al., 2008; Song et al., 2009; Kim et al., 2010). It has been reported that the physiological functions of mulberry are related to various chemical constituents such as alkaloids (Kim et al., 2013), 1-deoxynojirimycin (Kong et al., 2008), and phenolic compounds (Bae and Suh, 2007; Zhang et al., 2008; Wang et al., 2013).

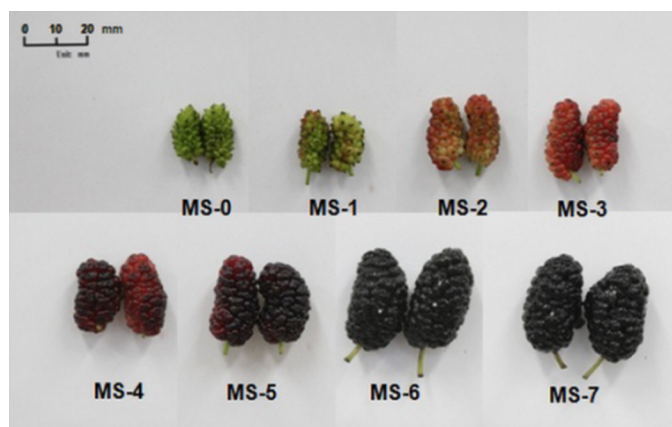
However, there are several disadvantages of using mulberry fruits. Mulberry fruits are prone to bruising during storage and distribution because of their perishable characteristics. Mature mulberry fruits are softer and are more susceptible to mold growth

than immature fruits, causing their quality to deteriorate (Park et al., 2013). Therefore, the physicochemical properties of mulberry fruits at different maturity stages (MS) have been studied in order to enhance their potential utilization. Most researchers have mostly studied mature mulberry fruits. There have been a few studies regarding immature mulberry fruits or physicochemical changes in the fruits during ripening. Lou et al. (2012) reported the assessment of total phenols, total flavonoids, sugars, and antioxidant activity in mulberry fruits with different skin color intensities, which correspond to different development levels of the fruits. Oki et al. (2006) investigated anthocyanins, phenolics, and DPPH radical scavenging activity of mulberry fruits at four different MS, reporting they are the fruit's major DPPH radical scavenging compounds. Lin and Lay (2013) studied chemical constituents including soluble solids, pH, water content, flavonoids, and DPPH radical scavenging activity of mulberry fruits at three different MS. Although these studies were conducted to determine some physicochemical characteristics of the fruits at different MS, a comprehensive study regarding the physicochemical and functional composition of mulberry fruits during ripening is needed to promote their potential availability.

In this study, therefore, we specifically collected mulberry fruits at seven different MS, and investigated changes in color, proximate composition, and various chemical constituents including sugars, minerals, amino acids, phenolic compounds, and 1-deoxynojirimycin, and antioxidant activity. Furthermore, we would propose the potential availability of immature mulberry fruits as well as mature fruits as functional food sources.

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**Fig. 1.** Image of mulberry fruits at seven different maturity stages (MS). MS-0, four weeks elapsed after flowering; MS-0, 1, 2, 3, 4, and 5, immature phases; MS-6, mature phase; and MS-7, fully mature phase.

## 2. Materials and methods

### 2.1. Plant materials

Mulberry fruits (*Morus alba* L.) were grown on a farm (Yangpyeong Mulberry Farming Association Corporation, Yangpyeong, Korea) in 2014 and hand-harvested at seven different MS based on their fruit color and sizes (Fig. 1): the fruits at MS-0 were green, at which stage four weeks elapsed after flowering, and those at MS-1, 2, 3, 4, and 5 were green to red and reddish black, whereas those at MS-6 and 7 were black as they were fully matured. The fruits at MS-7 could be picked more easily from the stems, while those at MS-6 adhered quite firmly to the stems. The fruits were collected, weighed, and measured for skin color, moisture content, and firmness. The collected fruits were immediately frozen in a box with liquid nitrogen. The frozen fruits were lyophilized (Alpha 1–2 LD plus, CHRIST, Osterode, Germany), milled into powder (Artlon Gold Mixer, Daesung Artlon Co., Paju, Korea), and stored at  $-22^{\circ}\text{C}$  until used for analysis.

### 2.2. Determination of color, weight, firmness, soluble solid, pH, and acidity of mulberry fruits

The skin color of the fresh mulberry fruits was measured by using a CR-400 Chromameter (Konica Minolta, Osaka, Japan) and recorded as Hunter's L, a, and b values. Ten fresh fruits at each MS were randomly weighed with a digital balance (OHAUS Explorer, Daejeon, Korea). The soluble solid content of extracted fruit juice was determined using a refractometer with a digital thermometer (ATAGO Co. Ltd., Tokyo, Japan) at  $20^{\circ}\text{C}$ . pH and acidity were measured using a digital pH meter (Orion 3 Star pH Portable, Thermo Scientific Inc., Waltham, MA). Acidity was determined using method AOAC 942.15 (AOAC, 2005). The diluted fruit juice was titrated to pH 8.2 using 0.1 N sodium hydroxide (Wako Pure Chemical Industries, Osaka, Japan), which was expressed as mg citric acid/100 mL fruit juice. The firmness of fresh fruits at different MS was analyzed by a texture analyzer (TA XT plus, Stable Micro System, London, England) using a 25 mm cylinder probe. The center of flatly laid fruits was compressed using a 5.0 g trigger force to a 60% strain, recording firmness as the force ( $\text{kgf}$ ) at the peak.

### 2.3. Proximate analysis

The proximate compositions of dried mulberries, including crude ash, protein, and fiber, were determined using methods AOAC 930.05, AOAC 2001.11, and AOAC 2002.04 (AOAC, 2005),

respectively. Moisture content was determined by two-stage drying method (AACC, 1995). Crude fat was determined with ethyl ether (Fisher Scientific Korea Ltd., Seoul, Korea) using an automated Soxhlet extraction apparatus (Büchi Extraction System B-811, Büchi, Flawil, Switzerland).

### 2.4. Analysis of free sugars

Dried mulberries (0.5 g) were mixed with 10 mL 50% (v/v) ethanol (Fisher Scientific Korea Ltd.) and centrifuged (Combi-514R, Hanil Science Industrial Co., Incheon, Korea) at  $1717g$  for 5 min. The residue was re-extracted. The combined supernatant was made up to 25 mL with 50% ethanol. The extract was used for HPLC analysis after being filtered through a  $0.45\text{-}\mu\text{m}$  nylon filter (Chemco Scientific Co., Osaka, Japan). Free sugars were analyzed by HPLC (Agilent Technologies 1200 Series, Agilent Technologies) with an evaporative light scattering detector (Agilent Technologies). The chromatographic separation was performed on a carbohydrate column ( $5\ \mu\text{m}$ ,  $4.6\ \text{mm} \times 250\ \text{mm}$ , Agilent Technologies) with a mobile phase made up of 75% (v/v) acetonitrile (Merck Chemicals, Darmstadt, Germany) at a flow rate of 1.0 mL/min for 25 min. The column oven was set at  $40^{\circ}\text{C}$ .

### 2.5. Analysis of minerals

Dried mulberries (0.5 g) were dissolved in 10 mL nitric acid (Wako Pure Chemical Industries) and wet-digested using a microwave digestion system (MARS 5, CEM Co., Matthews, NC). The conditions of the wet-digested system were as follows: Temperature was increased linearly to  $190^{\circ}\text{C}$  for 15 min and held at  $190^{\circ}\text{C}$  for 1 h. The digested solution was cooled to room temperature. The minerals were determined by an inductively coupled plasma optical emission spectrometer (ICP-OES, Varian 730-ES, Melbourne, VIC, Australia) after appropriate dilution. Certified reference material (CRM-3244, ephedra-containing protein powder, obtained from NIST, Gaithersburg, MD) was also analyzed at the same time in order to calculate recoveries of minerals.

### 2.6. Analysis of ascorbic acid

Ascorbic acid was determined using an HPLC (Agilent 1200 Series, Agilent Technologies, Waldbronn, Germany) with a Quicksorb column (Chemco Pak,  $3\ \mu\text{m}$ ,  $4.6\ \text{mm} \times 150\ \text{mm}$ , Chemco Scientific Co.). Dried mulberries (0.5 g) were soaked in 2 mL 10% (v/v) metaphosphoric acid (Wako Pure Chemical Industries) and then mixed with 10 mL 5% (v/v) metaphosphoric acid in an ultrasonic bath (Branson Ultrasonics Co., Danbury, CT) for 10 min. After the extract was centrifuged at  $1717g$  for 5 min, the residue was re-extracted. The combined supernatant was made up to 25 mL with 5% metaphosphoric acid and used for HPLC analysis after being filtered using a  $0.45\text{-}\mu\text{m}$  nylon filter. The separation was operated using acetonitrile/0.05 M potassium phosphate (30:70, v/v) (Wako Pure Chemical Industries) at a flow rate of 0.8 mL/min and an oven temperature of  $30^{\circ}\text{C}$ . Ascorbic acid was detected at 254 nm.

### 2.7. Analysis of GABA and amino acids

GABA and amino acids were analyzed using an amino acid analyzer (Hitachi L-8800, Hitachi Ltd., Tokyo, Japan). Approximately 0.5 g of dried mulberries was mixed with 10 mL 3% (v/v) trichloroacetic acid (Wako Pure Chemical Industries) with vigorous shaking and ultrasonication for 1 h at  $40^{\circ}\text{C}$ , and then centrifuged at  $1717g$  for 5 min. The residue was re-extracted and the combined supernatant was made up to 25 mL with 3% trichloroacetic acid. The extract was used for GABA analysis after filtration through a  $0.45\text{-}\mu\text{m}$  nylon filter.

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