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# The effect of grinding at various vacuum levels on the color, phenolics, and antioxidant properties of apple

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ABSTRACT

The purpose of this study was to evaluate the effect of grinding at different vacuum levels (2.67, 6.67, 13.33, 19.99, and 101.33 kPa) on key quality factors of apple. In the control apple, ground at atmospheric pressure of 101.33 kPa, the antioxidant activities rapidly decreased within the first 30 min, then plateaued thereafter, while enzymatic browning increased. When apples were ground and held under vacuum, changes in color and antioxidant activity were much less, and the least change was measured in samples prepared at the lowest pressure. Model fitting of the data showed that antioxidant activity decreased as a function of the logarithm of the absolute pressure. The results from analysis for key phenolic compounds including chlorogenic acid, procyanidin B2, and epicatechin indicated that these compounds were least changed at vacuum grinding at 2.67 kPa, compared to atmospheric grinding.



## 1. Introduction

Fruit and vegetable consumption is important to maintaining human health and reducing the risk of several diseases. Most fruits and vegetables contain necessary nutrients, as well as fiber, antioxidants, phytochemicals, and other bioactive compounds. Several plant antioxidants may mitigate the consequences of oxidative stress in chronic disease development and aging (Devasagayam et al., 2004; Valko et al., 2007; Zhang & Tsao, 2016). Fruits and vegetables contain a variety of bioactive substances including phenolic compounds (tocopherols, flavonoids and phenolic acids), nitrogenous compounds (alkaloids, chlorophyll derivatives, amino acids and amines), carotenoids, and ascorbic acid (Hall & Cuppett, 1997; Larson, 1988). In addition to health promoting effects, several studies have shown that natural antioxidants can help limit lipid and protein oxidation, help preserve color and flavor, and generally extend the shelf life of many fruits and vegetables (Halliwell, 1997).

Apples are a popular fruit worldwide and are a good source of vitamin C as well as fiber. In addition, many apples are a rich source of phenolics such as chlorogenic acid, epicatechin, procyanidin B2, phloretin, and quercetin, which contribute to a relatively high level of antioxidant activity as compared to other fruit (Escarpa & Gonzalez, 1998; Boyer & Liu, 2004; Tsao, Yang, Young, & Zhu, 2003). Several studies have shown that apple antioxidants may help lower the risk of prostate, liver, colon, and lung cancers (Eberhardt, Lee, & Liu, 2000; Le-Marchand, Murphy, Hankin, Wilkens, & Kolonel, 2000; Xing, Chen, Mitchell, & Young, 2001; Zhang & Tsao, 2016). Other studies suggest that apple consumption can help reduce the risk of cardiovascular disease (Hyson, Studebaker-Hallman, Davis, & Gershwin, 2000; Knekt, Jarvinen, Reunanen, & Maatela, 1996).

However, apples are susceptible to browning which is a major cause of deterioration in apple products. This is especially true for apples that are sliced or ground into smaller particles. These actions release polyphenol oxidase (PPO) enzymes– typically a mixture of monophenol oxidase and catechol oxidase– present in the chloroplasts and cytoplasm of many plant tissues. In the presence of oxygen, these enzymes convert phenolic compounds into *o*-quinones, which undergo subsequent polymerization to form insoluble brownish pigments. The main factors that determine the rate of browning in apples are the concentration of PPO enzymes and phenolic compounds, as well as the pH, temperature, and oxygen concentration. Browning in apple usually impairs the sensory properties because of the associated change in color, flavor, and antioxidant activity (Martinez & Whitaker, 1995).

PPO enzymes can be inactivated by heat treatment (Sulaiman, Soo, Farida, & Silva, 2015; Vámos-Vigyázó & Haard, 1981) and to some extent by high-pressure processing (Terefe, Buckow, & Versteeg, 2014; Terefe, Matthies, Simons, & Versteeg, 2009).

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However, these processes can have a detrimental effect on the nutrients and bioactive compounds found in foods. A particular challenge is that many consumers demand high quality fruit and vegetable products with natural flavor, texture, and appearance. As many apple products are developed through slicing, dicing or grinding it is important to develop processing operations that limit deterioration. Grinding is an important unit operation used to substantially reduce particle size, leading to increase in particle surface, and is required in apple processing when producing sauces and purees. However, the influence of grinding on fruit and vegetable antioxidants and phenolic compounds has not been studied.

The first objective of this study was to evaluate the effect of grinding on the enzymatic browning, phenolic compounds, and antioxidant activity in apples. In addition, as oxygen is required for apple browning, a second objective was to investigate the effect of grinding under different vacuum levels on the quality characteristics and antioxidant activity in ground apples. This was accomplished using a specially designed vacuum-grinding system.

## 2. Materials and methods

### 2.1. Apples

Commercially matured 'Fuji' apples (*Malus pumila*) were harvested in Geochang County (Gyeongsang Province) in South Korea in 2015, and stored at −4 °C in the dark until subsequent analysis. Analyses of the fresh apples showed an average pH of 4.14 ± 0.15, Brix level of 13.32 ± 1.16°, and pulp content of 30.5 ± 0.96%.

### 2.2. Chemicals

All chemicals used in this study were of high purity "Reagent" grade. Folin-Ciocalteu's reagent, 2,2-diphenyl-1-picrylhydrazyl, and 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) were purchased from Fluka Sigma-Aldrich (Seoul, South Korea).

### 2.3. Device for grinding under controlled vacuum level

The apples were ground using a specially designed device equipped with ports for introducing nitrogen gas or vacuum conditions (Fig. 1). The device consisted of a sample cylindrical chamber (1) constructed from stainless steel, equipped with a sealable top (7) that could be used to maintain an airtight headspace. The diameter and height of the device was 40 and 48 cm, respectively. The device had two valves on the top side walls for nitrogen (9) and vacuum (10) attachments, and a release valve (11) on the bottom side wall. The chamber lid was fitted with a pressure gauge (6) to measure the absolute pressure, and used to adjust the desired pressure inside of the chamber. A grinder (HWF-630WG, Hanil Electric, Wonju, Korea) (2) equipped with 4 stainless steel blades and a grinder lid (3) with a hole (1 mm diameter) was placed in the inner of the device. The grinder could be operated by a controller (8) placed outside of the sealed chamber. The system allowed samples to be ground at pressures ranging from 101.33 to 2.67 kPa, and to be opened without exposure to oxygen by introducing nitrogen gas.

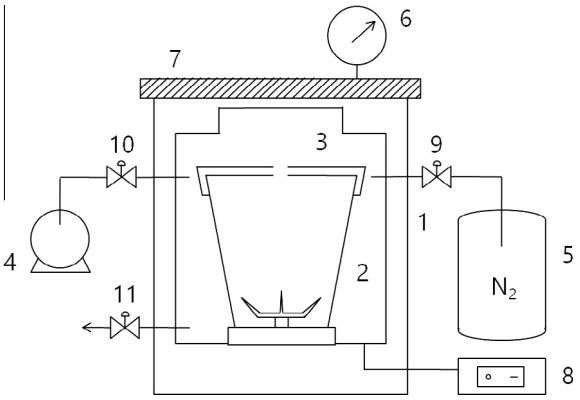


**Fig. 1.** Schematic diagram of a device used for grinding apples under vacuum, equipped with vacuum pump and nitrogen cylinder. The system consisted of: (1) sealable outer sample chamber, (2) blade grinder, (3) lid with a hole, (4) vacuum pump, (5) nitrogen gas, (6) vacuum/pressure gauge, (7) airtight detachable lid, (8) external power controller, and (9–11) valves.

### 2.4. Apple grinding

The apples were cored, peeled, and cut into 8 pieces using a sharp stainless steel knife. The operation took 2 min, and was conducted under water to prevent enzymatic browning by limiting oxygen diffusion. Approximately 100 g of the slices were immediately transferred to the grinder (2), the top lid placed on the chamber, the vacuum-side valve (10) opened and the vacuum pump (4) initiated. Vacuum pumping proceeded until the desired vacuum level attained, then all valves closed to maintain that level. Grinding was accomplished at absolute pressures (and percent vacuum) of 2.67 kPa (97.4%), 6.67 kPa (93.4%), 13.33 kPa (86.8%), 19.99 kPa (80.3%), and 101.33 kPa (0%, atmospheric pressure) and continued for 30 s at room temperature (~18 °C) in the dark condition, initiated by the external power controller (8). Grinding at atmospheric pressure, namely 101.33 kPa, served as the control. All samples were then kept at the prevailing vacuum pressure for up to 12 h in the chamber at room temperature. The ground apples pulled at specified intervals for subsequent analyses. After being held at the set vacuum level and time, the valve (9) was only opened and then the chamber was flushed with nitrogen gas (5) at flow rate of 5.25 m/s when vacuum gauge backed to atmospheric pressure to allow opening the chamber, but limiting exposure to oxygen.

Several physical and chemical properties of the ground samples were measured including pH, °Brix, pulp content, and degree of browning. In addition, particle size distribution was assessed using light scattering. For other chemical analyses, the ground apple samples were first diluted 5 times with methanol–water (80:20, v/v) and blended at 10,000 rpm for 1 min using a D-500 homogenizer (Wiggen Hauser, Berlin, Germany). The homogenized samples were shaken overnight at 200 rpm at ambient temperature. The samples were then filtered using 0.45 μm PTFE filters (Sigma-Aldrich, Seoul, South Korea). The filtrates were analyzed for antioxidant activity (DPPH radical scavenging activity, ABTS radical scavenging activity, and FRAP assay) and degree of browning. In addition, individual compounds were characterized using ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-Q-TOF-MS) as described below.

### 2.5. pH, brix, pulp content, degree of browning, and color

Several chemical and physical measurements were made on the ground apple samples including pH, brix, pulp content, degree of browning, and color. The pH was measured using an Istek Model 735P pH meter (Istek, Seoul, Korea) without further dilution. The Brix was measured using an Atago digital refractometer (Pal-1, Atago Co., Ltd., Tokyo, Japan). The pulp content of the ground apple samples was determined by separating the pulp after centrifugation at 5000*g* for 1 h. The pulp percentage was expressed as the relationship between the weight of pulp sediment to the initial sample weight (Qiu & Rao, 1988). The degree of browning was measured by the absorbance at 420 nm using a spectrophotometer (UV–1800, Shimadzu Corporation, Kyoto, Japan) as described by