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Preparation of resveratrol-enriched grape juice from ultrasonication treated grape fruits

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ABSTRACT

Grape (*Vitis* spp.) is a major source of resveratrol that can be eaten directly or after making jam, jelly, wine and juice. Resveratrol (3,5,4′-trihydroxystilbene) has a profound positive influence on human health, including anti-carcinogenic, anti-cancer, anti-inflammatory, and anti-ageing effects and the ability to lower blood sugar. During industrial production of grape juice, resveratrol is lost because of the use of clarifying agents and filtration; therefore, commercial grape juice contains very low amounts of resveratrol. In this study, we investigated the accumulation of resveratrol in grape juice prepared from three varieties of grape, *viz.* Campbell Early, Muscat Bailey A (MBA) and Kyoho, following post-harvest ultrasonication cleaning for 5 min and 6 h of incubation in the dark at 25 °C. This process resulted in the amounts of resveratrol increasing by 1.53, 1.15 and 1.24 times in juice prepared from Campbell Early, MBA and Kyoho, respectively, without changing the amounts of total soluble solids. Overall, our results indicate that ultrasonication treatment of post-harvested grape fruits can be an effective method for producing resveratrol-enriched grape juice as well as cleaning grapes thoroughly.

1. Introduction

Grape (*Vitis* spp.) is an important fruit that can be eaten raw or after making jelly, jam, juice, wine, etc. Grape juice is prepared from grapes by crushing and blending the fruit into liquid, while wine is produced by fermentation of this liquid. An inverse relationship between moderate wine consumption and coronary heart diseases has been reported [1], and consumption of moderate amounts of wine has been shown to have a beneficial effect on human health, including the prevention of different kinds of cancer [2] and degenerative disorders such as dementia or Alzheimer's disease [3]. However, these beneficial effects are not only achieved by consuming wine. Indeed, regular consumption of grape juice may also diminish the threat of coronary heart disease and severe platelet thrombus formation [4]. Grape juice also reduces platelet aggregation [5] and atherosclerosis and improves lipid and antioxidant potential [6]. Additionally, grape juice has been shown to improve human memory and motor function, suggesting that foods supplemented with grape juice may have beneficial effects against ageing [7]. Moreover, grape juice and wine have been found to have similar antioxidant capacities [8].

Grape fruits or foods produced from the fruit, including wine and grape juice, contain high amounts of different phenolic compounds, such as stilbenes, anthocyanin, catechins, flavonols and proanthocyanidins [9]. Foods or beverages enriched with phenolic compounds are beneficial to human health in that they prevent cardiovascular diseases and different kinds of cancer [2]. Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene), a stilbene compound found in grapes, wine and grape juice, prevents cardiovascular diseases and different types of cancer, [10]. In addition, resveratrol increases resistance to stresses and extends the life of many organisms, including yeast [11] and vertebrates [12].

Resveratrol contents in the skin of ripening grape fruits vary greatly depending on grape variety or cultivar and growth stages of the fruit [13]. Furthermore, climate, ecological area of cultivation, growing conditions, post-harvest operations, etc. have strong effects on the accumulation of resveratrol [14]. The amounts of resveratrol in grapes were shown to increase in response to treatment of the fruit with UV-C illumination [15] and methyl jasmonate [16]. The increased amount of resveratrol in grape skin can be directly linked to the production of resveratrol-enriched grape products.

In addition to the original contents of resveratrol in grape skin, processing and manufacturing parameters can significantly affect resveratrol contents in grape fruit products. Vinification techniques have been shown to have profound effects on resveratrol in wine, especially during maceration of the grape skins [17].

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During the wine making process, the amount of resveratrol can be decreased by clarifying agents and the filtration process [18]. However, UV-C treated grape berries coupled with maceration for two hours during juice production were found to increase the amount of resveratrol 24 times relative to a control [19].

Many fruits and vegetables are cleaned by applying ultrasonication cleaners. Such cleaners use ultrasound (20–400 kHz) and a suitable cleaning solvent, usually tap water for fruits and vegetables. Ultrasonic cleaners have been effectively employed to generate contamination-free fruits and vegetables [20]. Additionally, ultrasonic cleaners ensured elimination of all contaminants present in objects, including sludge, worms, mold, bacteria, fungi and agrochemicals. Ultrasonication treatment at 40 kHz on post-harvested strawberry fruits significantly reduced the decay incidence and infection of microorganisms, but maintained the fruit quality [21]. In addition to the effects of thorough cleaning, plant secondary metabolites were also induced by ultrasonication. Examples of the metabolites elicited by ultrasonication included ginsenoside saponins by 75% in ginseng cell [22], shikonin by 60–70% in *Lithospermum erythrorhizon* cell culture [23], taxol by 3 times in *Taxus baccata* cell culture [24] and resveratrol by 8–143 times in whole or sliced peanut kernel [25,26].

The major source of resveratrol in grape juice is the skin of grape fruits; therefore, increasing the amount of resveratrol in grape skin can have a practical importance in the grape juice industry. Accordingly, in this study, we investigated the effects of ultrasonication on the amounts of resveratrol in grape juice prepared from three different grape cultivars. To the best of our knowledge, this study is the first to report increased amounts of resveratrol in grape juice following ultrasonication of post-harvested grape fruits.

2. Materials and methods

2.1. Grapes and chemicals

Three different grape cultivars selected based on their availability in September were used for this experiment. Specifically, Campbell Early (*Vitis* hybrid) grapes were purchased from a supermarket located in Gyeongsan, while Muscat Bailey A (Muscat Hamburg × Bailey; MBA) and a Concord-like cross Kyoho (*Vitis vinifera* L. × *Vitis labrusca* L.) grapes were purchased from a local provider in the middle of September, 2012. Standard *trans*-resveratrol was obtained from Sigma–Aldrich (St. Louis, MO, USA), and stock *trans*-resveratrol solution was kept at −20 °C. The chemicals and solvents used for analysis in this study were of analytical or high performance liquid chromatography (HPLC)-grade and purchased from Sigma–Aldrich (St. Louis, MO. USA) or Merck (Darmstadt, Germany).

2.2. Ultrasonication treatment

Similar-sized grape bunches were selected for treatment with or without ultrasonication. The grape bunches were cleaned by ultrasonication at 40 kHz using an ultrasonic cleaner bath (Branson 8510, overall dimensions: 24″ × 18″ × 14.5″, tank dimensions: 19.5″ × 11.5″ × 6″, weight: 36 lbs, Fredericksburg VA, USA). The treatment was applied by submerging the bunches of grape fruits in sterile ddH_2O in an ultrasonic tank as described by Lin et al. [22]. The fruit was subjected to ultrasonication for 5 min, followed by incubation for 6 h at 25 °C in the dark based on previous study [27]. Following treatment, the grape bunches were dried using a paper towel. Same procedures were followed for the control samples, excluding the ultrasonication treatment. Application of ultrasonication treatment and all subsequent analyses were conducted in dark to avoid isomerization of *trans*-resveratrol to *cis*-resveratrol as described by Trela et al. [28].

2.3. Preparation of grape juice

After treating the grape bunches with or without the ultrasonication treatment, a total of 300 g of grape fruits were collected from the outside of each bundle and washed two-times with distilled water. The grape fruits were then blended for 10 s using a blender (HM-3310, Hanil, Seoul, Korea). The total extracts were subsequently filtered through two layers of cheesecloth and centrifuged at 3500*g* for 20 min at 4 °C, after which the supernatant was collected to obtain the clear part of the grape juice.

2.4. Measurement of pH, titratable acidity, and total soluble solids

The pH of the grape juice was measured using a pH meter (LE438, Mettler-Toledo, Switzerland). Before measuring the pH, the pH meter was calibrated with commercial buffer solutions of pH 7.0 and 4.0. The titratable acidity of the grape juice was measured by titrating 10 mL of juice with 0.1 N NaOH to pH 8.3 and then expressed by tartaric acid (TA) equivalents as a percentage. The contents of total soluble solids in the grape juice were measured using a refractometer (N-1E, Atago, Tokyo, Japan) and expressed in Brix.

2.5. Analysis of anthocyanin contents

The amounts of anthocyanin pigments in filtered grape juices were analyzed using the pH differential absorbance method [29]. Briefly, the absorbance of the grape juices was measured at 510 nm and 700 nm at pH 1.0 (hydrochloric acid–potassium chloride, 0.2 M) and pH 4.5 (acetic acid–sodium acetate, 1.0 M). The amounts of anthocyanins were then calculated on the basis of cyanidin-3-glucoside (cyd-3-glu) using the following formula:

$$\text{anthocyanin pigments(cyd-3-glu; mg/L)} = (A \times \text{MW} \times \text{DF} \times 10^3)/(\varepsilon \times l)$$

where $A = (A_{510\text{nm}} - A_{700\text{nm}})$ pH 1.0 $- (A_{510\text{nm}} - A_{700\text{nm}})$pH 4.5; MW(molecular weight) = 449.2 g/mol for cyd-3-glu; DF = dilution factor established in D; l = path length in cm; ε = 26,900 M extinction coefficient in $L \times mol^{-1} \times cm^{-1}$ for cyd-3-glu; and 10^3 = factor for the conversion from g to mg.

2.6. Analysis of total phenolic compounds

The amount of total phenolic compounds was measured according to the Folin–Ciocalteu method [30], with slight modification. In a Falcon tube, 2.4 mL of juice sample was mixed with 0.15 mL of Folin–Ciocalteu reagent. After the addition of 0.45 mL of 20% $NaCO_3$, the mixture was kept at room temperature for 1 min and then vortexed. The absorbance was measured at 750 nm after incubation of the mixture for 30 min at room temperature. The total amount of phenolic compounds was calculated from a calibration curve using gallic acid as a standard and expressed as mg gallic acid equivalents per liter (mg/L).

2.7. Analysis of resveratrol contents

The grape juices were further filtered through a 0.45 μm syringe filter and injected in duplicate into an HPLC system. The reversed-phase HPLC system consisted of a pump equipped with a Denali C-18 column (5 μm, 4.6–250 mm, Grace Davison Discovery Sciences, Deerfield, IL, USA) and a UV detector (Young-Lin, YL9100, Seoul, Korea). The isocratic mobile phase was used with