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Research Article

Effect of vacuum blending on antioxidant activities of apple juice and blueberry juice

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Abstract The present study aimed to assess and compare the quality characteristics and antioxidant activities of apple juice and blueberry juice at hourly intervals over a period of time based on the presence or absence of vacuum blending (0 and 800 hPa) using a household blender. Measurement of the dissolved oxygen content revealed that the removal rates of dissolved oxygen were approximately 83% and 86% in the apple and blueberry juice samples, respectively, after vacuum blending. Moreover, compared with general blending, there was little change in the antioxidant property and degree of browning over time with vacuum blending. Furthermore, hourly assessments revealed that the decrease in the polyphenol and flavonoid contents in the apple and blueberry juice samples was significantly lesser with vacuum blending for 3, 6, and 12 h than with general blending. Assessment of the change in ferric reducing antioxidant power (FRAP) activity over time revealed that the rate of decrease in FRAP activity over time was lower with vacuum blending than with general blending for both juice samples. ABTS and DPPH radical scavenging assays performed to determine the change in free radical scavenging activity revealed inhibitory activity at 0, 3, 6, and 12 h and confirmed that vacuum blending resulted in long-lasting antioxidant activities in both apple and blueberry juice samples. Taken together, the present results confirmed that vacuum blending is associated with superior quality maintenance and antioxidant properties in comparison with general blending.

Keywords Vacuum blending, Apple juice, Blueberry juice, Antioxidant, Free radical

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Introduction

Rapid development of living standards has resulted in increased awareness of health and well-being, with a focus on the physiological effects in the human body, such as disease prevention and suppression of aging (Choi et al. 2011). Free radicals, which are always generated by oxidative stress, cause various diseases including aging and cancer by putting stress on physiological functions in the body, thus interest in antioxidant nutrients is increasing (Shulz 1994).

Antioxidants play a role in protecting the human body from cell damage caused by oxidative stress. Functions of reactive oxygen species (ROS) generated in the body include energy metabolism, immune response, and electrical signal transmission of nerves. Excessive generation of ROS causes modification of lipid, protein, and DNA as an adverse reaction in the living body; it also causes damage to biological membranes and tissues, leading to aging, metabolic disease, and cancer (Valko et al. 2007).

Research on natural antioxidants that are capable of maintaining the antioxidant system in the body has recently been conducted, with development of products such as juice, beverages, and drinks with palatability, convenience and health functionality (Park et al. 2011). Fruit juices containing a large amount of functional ingredients good for maintenance of health are preferred over carbonated drinks (Lee et al. 2008). In the current market, development of products such as extracts, concentrates, and juices from natural products using natural plants is underway (Kalt 2006). Development of these various products has resulted in the existence of secondary metabolites such as vitamin C, tocopherol, carotenoids, and phenolic compounds in fruits and vegetables; these metabolites exhibit high antioxidant activity (Byers and Perry 1992), which not only prevents oxidative damage, but also acts on the inflammatory response and the immune system, exhibiting various physiological activities, including inhibition of cellular aging and improvement of cardiovascular diseases

(Klimczak et al. 2007).

However, due to contact with oxygen during the juice processing of fruits and vegetables, there is a problem resulting from deterioration of the product, including changes in color and physiologically active ingredients. This phenomenon results in release of the polyphenol oxidase (PPO) enzyme (a mixture of monophenol oxidase and catechol oxidase) present in the chloroplasts and cytoplasm of many plant tissues. In the presence of oxygen, this enzyme converts phenolic compounds into o-quinones, which then polymerize to form insoluble brown pigments. In general, contact with oxygen causes oxidation and browning of fruits and vegetables such as apples, bananas, grapes, and blueberries. In addition, antioxidant activity is reduced.

Study of the juicing method examines the chemical properties of compressed and centrifugal citron juice (Lee et al., 1994), and the quality and fragrance of citron juice using rotary grinding, pressing and belt-type juicers, and squeezing, centrifugation, belting, such as proteolytic enzyme and antioxidant activity (Park et al. 2016) according to the low-temperature storage conditions of pineapple and kiwi juice using a low-speed press-type juicer, and antioxidant activity of vegetable and fruit juice according to the juice manufacturing device (Choi et al. 2014). Research is conducted using a mass compression method such as formula; most studies have focused on antioxidant activity. As such, with the recently increased interest in health, the market for juicers for home use where fruits and vegetables are juiced and eaten at home is expanding. However, few comparative studies of the juicing method in general households have been reported, and no studies comparing the technical differences based on the vacuum extraction method and the effective ingredients of nutrients have been reported, so that consumers who use the juicer have not received adequate information.

Therefore, in this study, a comparison and analysis of hourly quality characteristics and antioxidant activity of juiced apple and blueberry juices was performed over time based on the presence or absence of the vacuum (0 and 800 hPa) of a home blender. The vacuum blender was used under conditions that allowed blending at home.

Materials and Methods

Experimental materials and preparation

Apples (domestic) and blueberries (Chile) were purchased at a local market in Cheonan, Chungcheongnam-do. Apples were cut into quarters by removing the skin from the water to minimize contact with oxygen. In blueberries, non-edible parts such as stems and seeds were removed, washed first in tap water, and then washed in tertiary distilled water and dried. After the water was gone, it was used as a blending sample. Juicing method is by adding 2 times water and using ultra-high-speed vacuum blender (Kuvings Chef CB 1000, NUC Electronics Co., Ltd., Daegu, Korea). Juice was prepared under the conditions of general blending (0 hPa) and vacuum blending (800 hPa). The prepared juice was measured for antioxidant activity at 0, 3, 6, and 12 h, respectively.

Dissolved oxygen (DO) measurement

The DO content of apple and blueberry juices under normal blending (0 hPa) and vacuum blending (800 hPa) conditions was measured using a dissolved oxygen meter (MW600, Milwaukee Instruments, Inc. NC, USA).

Juice properties and degree of browning

The change over time of apple and blueberry juice according to vacuum was confirmed through visual observation. The degree of browning of apple juice over time was measured by absorbance at 420 nm (UVIKON 922, Kontran Co., Milan, Italy).

Total polyphenol and flavonoid content

For the total polyphenol content, Folin-Denis method (Folin and Denis, 1912) was applied to 1 mL of each 1 mg/mL sample solution and 1 mL of Folin-Ciocalteau's phenol reagent (Sigma Co., MA, USA) diluted twice, and reacted at room temperature (RT) for 3 min. 1 mL of a 10% Na_2CO_3 (Sigma Co.) solution was added, mixed, and reacted at room temperature for 1 h, and absorbance was measured at 700 nm (xMarkTM, BIO-RAD, USA). As a standard material, a standard curve for each concentration was prepared using gallic acid (Sigma Co.), and the total polyphenol content was calculated. The total flavonoid content was obtained by modifying the method of Nieva Moreno et al. (2000) and adding 10% aluminum nitrate (Sigma Co.) and 1 M potassium acetate (Sigma Co.) to 0.5 mL of a mixture of 0.1 mL of sample and 0.9 mL of 80% ethanol. After adding 0.1 mL and 4.3 mL of 80% ethanol, reacting at RT for 40 min, absorbance was measured at 415 nm. As a standard material, a standard curve was prepared using quercetin (Sigma Co.), and the total flavonoid content was calculated.

Ferric reducing antioxidant power (FRAP) measurement

FRAP was measured by applying the method of Benzie and Strain (Benzie and Strain, 1996). FRAP reagent was prepared by preparing a 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution and 20 mM $FeCl_3$ solution dissolved in 300 mM sodium acetate buffer (pH 3.6) and 40 mM HCl in a ratio of 10:1:1. was mixed and used immediately before the experiment. 10 μL of 20-fold diluted juice sample and 200 μL of FRAP reagent were mixed and mixed, reacted at 37°C. for 5 min, and absorbance was measured at 593 nm using microplate spectrophotometer (BIO-RAD) equipment. The FRAP level of the sample was obtained by applying the absorbance value to a standard calibration curve prepared with 0-5 mM $FeSO_4.H_2O$, and the result was expressed as $FeSO_4$ eq mM/mL.

Radical scavenging activity

The DPPH radical scavenging activity of the sample solution was determined by adding 200 μL of 0.15 mM DPPH solution to 800 μL of juice diluted 20-fold with methanol according to the method of Blois (1958), mixing, and leaving it at room temperature for 30 min, and then measuring the absorbance at 517 nm. ABTS radical scavenging activity was measured by modifying the method of Re et al. (1999). 7 mM ABTS and 2.45 mM potassium persulfate (Sigma Co.) were mixed (v/v) to a final concentration and left in the dark at RT for 24 h. After that, add distilled water and dilute so that the absorbance value at 732 nm becomes 0.70 (±0.02), take 990 μL, add 10 μL of the juice diluted 20 times with PBS, and leave at RT for 1 min, then absorb the absorbance value at 732 nm was measured.

Statistical analysis

All experimental results were expressed as mean and standard deviation, and ANOVA was used to verify significance using the statistical program SPSS statistics (ver. 25, IBM Co., Armonk, NY, USA). The statistical significance of the mean value was analyzed using Duncan's multiple range test at $p < 0.05$ level.

Results and Discussion

Dissolved oxygen (DO) content of apple and blueberry juice by vacuum blending

All food quality changes and decreases due to the reproduction of microorganisms, the activity of enzymes, physical changes, and biochemical reactions during the distribution period. The main cause of this is the interaction of oxygen in the air with oxygen dissolved or contained in the food. Considering the oxidation rate of oxygen, it can be confirmed that if oxygen is reduced to a concentration of almost zero state, it can effectively exert a great effect in suppressing biochemical changes through biological stability.

After blending apples and blueberries under vacuum and non-vacuum conditions, measured using a dissolved oxygen meter. In the case of apple juice, the vacuum blended sample showed about 83% DO removal rate compared to normal blending at 1.2 mg/L, Blueberry juice showed a DO removal rate of about 86% compared to general blending at 1.3 mg/L in the vacuum blending sample (Table 1).

DO is oxygen dissolved in water, and when DO is increased in processed foods, various biochemical reactions cause food quality deterioration. On the other hand, the browning reaction of food is non-enzymatic or enzymatic. Enzymatic browning requires oxygen, such as bananas, apple slices exposed to air, fruits such as apricots and strawberries, vegetables such as potatoes and lettuce, and seafood. These reactions are related to catalytic polyphenol oxidase, phenolic substances, and oxygen (Martinez et al., 1995). Therefore, the rapid browning of apple juice during general blending is considered to be enzymatic browning by oxygen.

Changes in properties over time and degree of browning by vacuum blending

As shown in Fig. 1, the change in the properties of apple and blueberry juice according to vacuum blending is confirmed that the color change proceeds more rapidly than vacuum blending in general blending over time. In the case of blueberry juice, the phenomenon was conspicuously visible (Fig. 1A, B). The degree of browning of apple juice was measured using absorbance. As a result, in the case of general blending, the absorbance values were significantly higher for 3, 6, and 12 h compared to immediately after blending. On the other hand, in the case of vacuum blended apple juice, there was browning in the upper layer after 6 h, but there was no

Table 1 Comparison of dissolved oxygen contents in apple juice and blueberry juice with and without vacuum blending

Sample	Dissolved oxygen content	
	Control (0 hPa)	Vacuum (800 hPa)
Apple juice	7.0 mg/L	1.2 mg/L
Blueberry juice	9.2 mg/L	1.3 mg/L

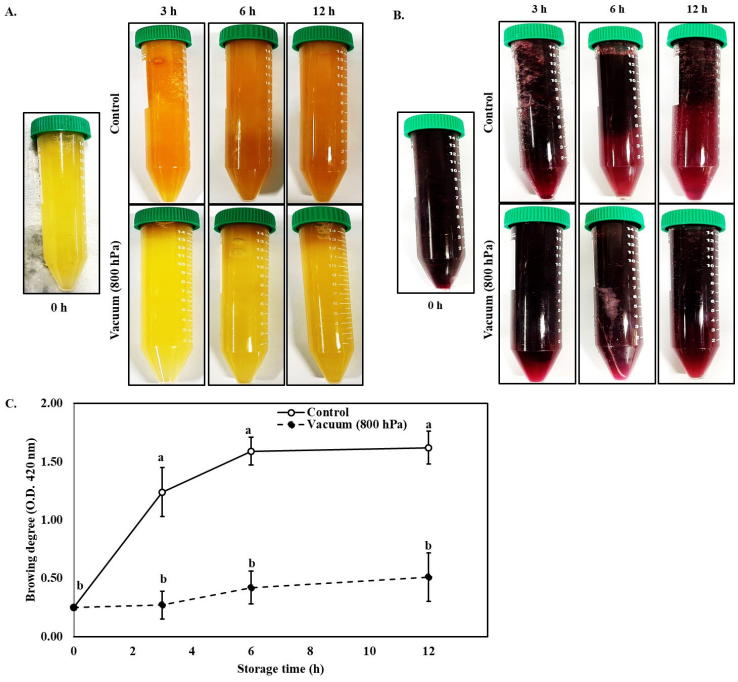

Fig. 1 Changes in (A) apple juice appearance, (B) blueberry juice appearance, and (C) the degree of browning of apple juice with and without vacuum blending. Data are expressed as the means ± standard deviations (n = 3). Different superscripts in a column indicate significant differences at $p < 0.05$ using Duncan's multiple range test

significant change compared to 0 h (Fig. 1C).

Change of polyphenol and flavonoid content with time by vacuum blending

The results of time-dependent polyphenol and flavonoid contents of apple and blueberry juice according to vacuum blending are shown in Fig. 2. After 3 hours, the polyphenol and flavonoid content of both apple and blueberry juice decreased. During vacuum blending, it was confirmed that the amount of decrease was significantly less than that of general blending for 3, 6, and 12 h.

These results were similar to those of Kim et al. (2017), which showed that polyphenol content was higher when blending in vacuum mode among various juicing methods using various carrots and blueberries. The hydroxyl group (OH-) of the phenolic compound exhibits various physiological actions and effects such as antioxidant, anti-aging and anti-obesity while reducing unstable electrons to water (Kim et al., 2014). Also, because it has a phenolic hydroxyl group, it has the property of binding to proteins, in a chain reaction, hydrogen is donated to an alkyl radical or an alkylperoxy radical to remove the radical, thereby inhibiting oxidation (Labuza and Dugan Jr 1971). Therefore, it is thought that a vacuum blending method with a high polyphenol content will be more helpful in intake of antioxidant nutrients.

Changes in FRAP activity over time by vacuum blending

The FRAP is a method for measuring the degree of reduction of ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to ferrous tripyridyltriazine (Fe^{2+}-TPTZ) colored by antioxidants. The reducing principle of antioxidants is to suppress the chain reaction of free radicals by donating hydrogen atoms, thereby exhibiting antioxidant activity (Duh et al. 1999). The FRAP activity of apple and blueberry juice according to vacuum blending over time is shown in Fig. 3. It was confirmed that FRAP activity was rapidly decreased in apple juice than in blueberry juice. In particular, in apple juice, FRAP activity was reduced by more than 2 times after 3 h of general blending. On the other hand, it was confirmed that the rate of decrease in FRAP activity over time in both apple juice and blueberry juice during vacuum blending was lower than that of general blending.

Free radical scavenging activity change with time by vacuum blending

In order to measure the free radical scavenging activity of apple and blueberry juice according to vacuum blending, the inhibitory activity was measured for 0, 3, 6, and 12 h of 20-fold diluted juice using ABTS and DPPH radicals.

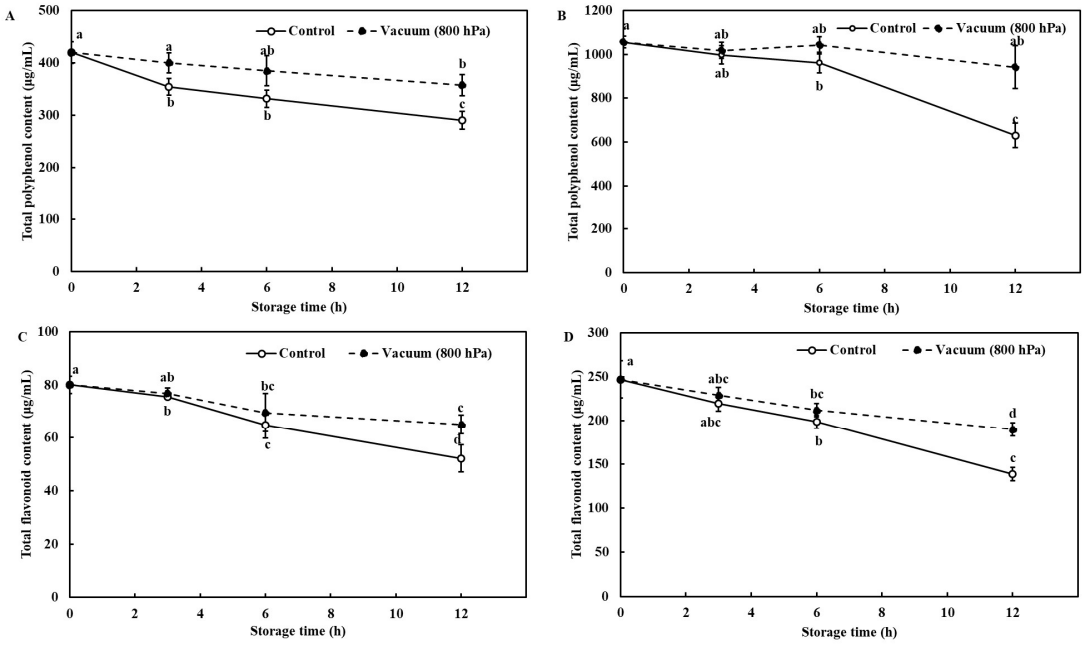

Fig. 2 Changes in the total polyphenol and total flavonoid contents in apple juice and blueberry juice with and without vacuum blending. A, C: apple juice; B, D: blueberry juice. Total polyphenol content, analyzed as μg gallic acid equivalents (GAE)/mL of juice. Total flavonoid content, analyzed as μg quercetin equivalents (QE)/mL of juice. Data are expressed as the means ± standard deviations (n = 3). Different superscripts in a column indicate significant differences at $p < 0.05$ using Duncan's multiple range test

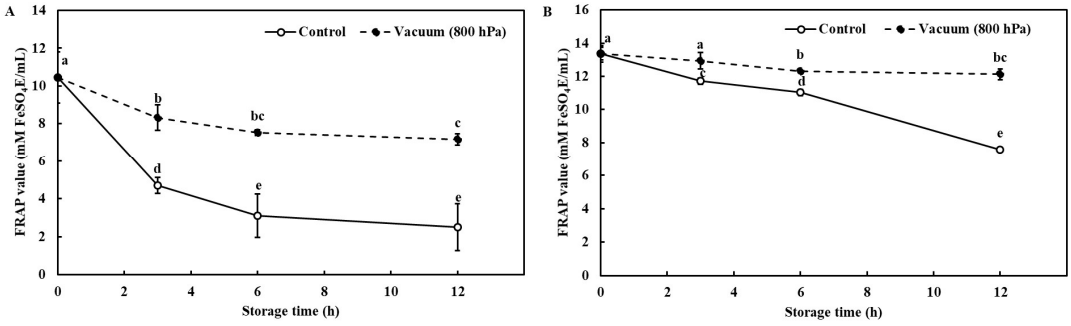

Fig. 3 Changes in the reducing power of apple juice and blueberry juice with and without vacuum blending. A: apple juice; B: blueberry juice. FRAP value, analyzed as mM $FeSO_4$ equivalents ($FeSO_4E$)/mL of juice. Data are expressed as the means ± standard deviations (n = 3). Different superscripts in a column indicate significant differences at $p < 0.05$ using Duncan's multiple range test

Immediately after blending apple and blueberry, ABTS radical scavenging activity was measured to be 78.53% and 92.88%, respectively. In the case of normal blended apple juice, the inhibitory activity was significantly reduced after 6 h compared to vacuum blending, and in the case of blueberry juice, it was confirmed that the scavenging activity was significantly reduced after 12 h. In the case of DPPH radical scavenging activity, 82.85% and 91.01% of the juice immediately after blending apple and blueberry were measured, respectively, and it was confirmed that the DPPH radical scavenging activity decreased sharply from 3 h in the juice of normal blending of apple and blueberry. It was confirmed that the vacuum blended blueberry juice maintained high activity for up to 6 h, and then decreased to 71.36% at 12 h. As a result, it was confirmed that the antioxidant activity lasted longer during vacuum blending than with general blending.

Active oxygen is oxygen with free radicals, and broadly includes lipid peroxide, lipid peroxy radical, peroxynitrite and hydroxy radical. Free radicals attack living cells, destroying lipids, nucleic acids (DNA, RNA), and proteins, inhibiting various enzyme functions, destroying cell membranes by generating lipid peroxide, causing cancer, and promoting diseases and aging (Cho et al. 2008). When a protein is attacked, a carbonyl group is created and cell is destroyed, and

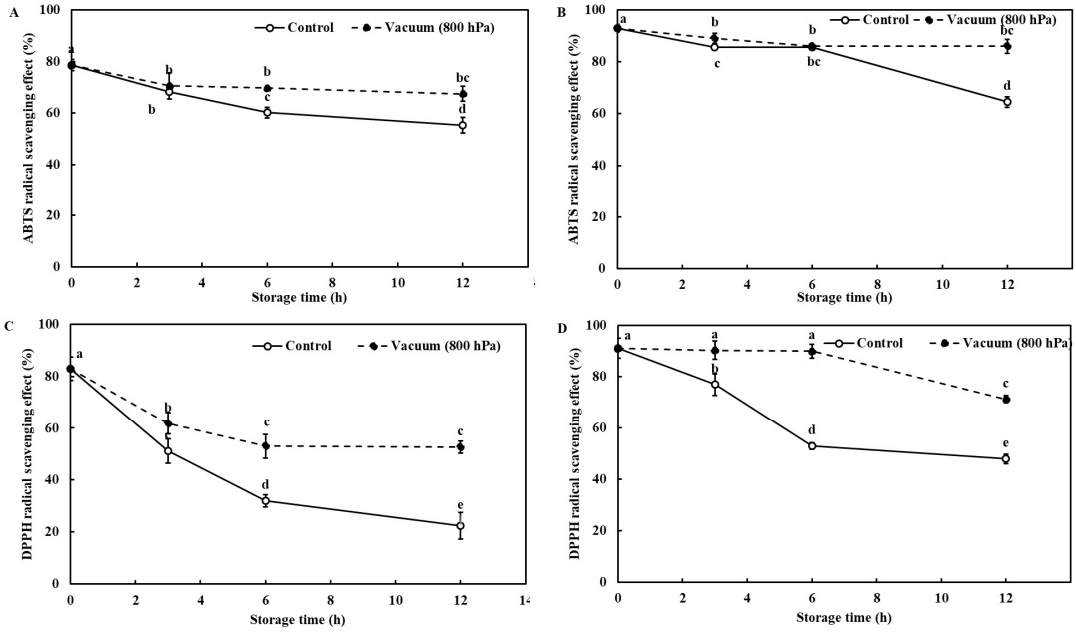

Fig. 4 Changes in the $ABTS^{+}$ radical and DPPH radical scavenging activities of apple juice and blueberry juice with and without vacuum blending. Data are expressed as the means ± standard deviations (n = 3). Different superscripts in a column indicate significant differences at $p < 0.05$ using Duncan's multiple range test

when it attacks DNA, various mutations are induced. It was reported that the brown pigment (lipofusein), an aging pigment, increases in the skin with increasing age. Also, it has been reported that it also affects the neurotransmitters dopamine, serotonin, and acetylcholine, and inhibits enzymes such as acetylcholine esterase (Kang et al. 1996). Fresh vegetable and fruit juices are considered to be beneficial to health because they have very good antioxidant effects (Jirum et al. 2013). Although the nutritional superiority and high antioxidant activity of fresh juice are beneficial, it is very important to minimize the loss of nutrients during processing to maintain this antioxidant activity (Song et al. 2006). However, the reduction of polyphenol components and antioxidant activity is accompanied by contact with oxygen during juice production at home (Arena et al. 2001; Van der Sluis et al. 2002; Sanchez-Moreno et al. 2003). As shown in the results of this study, when blending apples and blueberries, the decrease in antioxidant activity is suppressed even when contact with oxygen is minimized by vacuum blending.

Summarizing the above results, vacuum blending has better quality maintenance and antioxidant nutrients than general blending. These results suggest that it would be beneficial to health to choose a vacuum blended product rather than a general blended product to consume nutrient-rich juice when making juice using various fruits at home.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interest.

Acknowledgments

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