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Influence of Additions of Seeds and Stems on the Properties of Processed Campbell Grape Juice

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Campbell 포도주스의 제조에 있어서 포도 씨와 줄기의 첨가가 포도주스의 품질에 미치는 영향

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Abstract

Grape juice, one of the most valuable fruit derived product has large amounts of phenolic compounds which provide many of the health benefits. Campbell grape juice, divided into Sample A (without seeds and stems), Sample B (with seeds only), Sample C (with seeds and stems) were processed and assessed for its components, sensorial attributes and functional properties as influenced by the addition of seeds and stems. The presence of seeds and stems in the processing of Campbell grape juice has significant effects on pH and total soluble solids (°Brix) but showed no significant effects on other physicochemical and color properties and corresponds to an increase in total phenolics, radical scavenging activity, total anthocyanin and total flavonoid. The sensory evaluation showed that there is no significant differences among the samples but were all liked moderately to very much by the panelists. The result of this study demonstrated that seeds and stems can be included in the processing of Campbell grape juice as it enhances the juice functional properties without altering its physicochemical and sensorial properties.

Key words : grape juice, antioxidant, quality, total phenol, total anthocyanin, total flavonoid

Introduction

Nowadays, consumers are particularly concerned with the health benefits of the different food products such as fruit and vegetable derived products. Grape is one of the most important fruit crops of the world(1) and one of its valuable components is phenolics, also known as polyphenols. Grape juice possesses antioxidant properties and is an excellent nonalcoholic alternative to red wine(2).This can be supported by the recent studies that grape juice indeed has the highest antioxidant capacity out of a group of commercial fruit juices including grape, grapefruit, orange, tomato and apple(3). It was also found that purple grape juice inhibited or decreased platelet aggregation (4) enhanced nitric oxide release(5), and improved endothelial function(6). Further researches showed that grape juice and red wine inhibited platelet activity(7) and reduced LDL oxidation in humans(8).

Many studies are devoted on how to increase the antioxidant capacity and total phenolics of grape juice. Morris et al.(9) reported that maturity, extraction temperature, storage time and temperature have an effect on color degradation and quality of grape juice. Revilla et al.(10) noted that in maceration processes, the phenols are extracted from the seed and can increase the phenolic content of the grape juice that is used for wine making. Accordingly, Auw et al.(11) determined the effect of several presses, hot presses and skin fermentation on the phenolic composition of some red wines and juices. They noted that high pressure during pressing and skin fermentation promoted the extraction of some phenols.

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The grapes in general are composed of 2-6% stems, 5-12% skins, 80-90% juice and 0-5% seeds(12). Prior to processing all the undesired materials considered as waste like leaves and stems are usually removed because it may cause manufacturing problems and can affect the quality of the juice and wine. Many researches have been made on the phenolic composition of grape berries, skins and seeds(13-15). Furthermore, Huang et al.(16) analyzed the effects of stems, petioles, and leaves on the total phenolic, flavonoid, and tannin content of Concord and Aurora Blanc wine and juice. These authors reported that grape stem contained an appreciable amount of phenolic components as supported by the study made by some authors(15). In addition, Souquet et al.(17) and Cantos et al.(18) reported that grape skin and berry stem contains major flavonols such as quercetin-3-glucoronide, quercetin-3-rutinoside, quercetin-3-glucoside, kaemferol-3-galactoside and flavan-3-ols which include gallocathecin, procyanidin B1, procyanidin B2, procyanidin B4, procyanidin C1, catechin, and epigallocatechin. Moreover, the phenolic compounds that can be derived from leaf, stem, petioles, skin and seeds depends on the different factors such as concentration, chemical and physical properties of phenolic compounds, level of tissue damage, variety, maturity, seasonal factors during growth, and the processing methods used(19,20). In literature, not so many studies are available concerning the effects of seeds and stems on the quality of processed grape juice thus, the aim of this study was to determine the influence of seeds and stems on the compositional properties, sensorial attributes and functional properties of processed Campbell grape juice.

Materials and Methods

Sample preparation

Campbell grapes which were obtained from a local grower in Sang Ju, South Korea were used for the production of grape juice. To determine the influence of seeds and stems on the Campbell grape juice the grapes were divided into three groups Sample A (without seeds and without stems), Sample B (with seeds) and Sample C (with seeds and with stems). In the juice industry, seeds are usually included in processing grape into juice which is the sample B in this study. The grapes to be used as sample A were manually separated from the stems and seeds and in sample B, the stem is also manually separated from the grape. All the grape samples were crushed individually before heating to 80°C for 30 minutes and added with pectinase (Sigma-Aldrich, Germany) to hydrolyze the pectins. The samples were then pressed while hot. The juice samples were placed in a container which were covered and refrigerated at -5°C overnight for tartarate precipitation (cold stabilization). The juice samples after the cold stabilization process were then filtered, pasteurized and analyzed. The results of the analyses were expressed as means which correspond to the three analytical replicates.

Chemical analyses

The pH of the processed grape juice was measured using the pH meter (Metler Toledo model Delta 320, China). The Total Soluble Solids (°Brix) were measured using an ATAGO hand refractometer (model N-1E, Japan). Titratable acidity was measured by adding 10 mL of grape juice sample to 100 mL of distilled water and titrating with 0.1 N sodium hydroxide to an endpoint of pH 8.2(21). The results were expressed as milligrammes per 100g tartaric acid.

Color measurement

Color of the processed grape juice was determined using a Commision Internationale de l'Eclairge illuminant (CIE), L* (lightness), a* (green to red) and b* (blue to yellow) tri-stimulus values and were obtained by using a Hunter colorimeter (Minolta model CR 200, Japan). These values were used to calculate chroma ($C=\sqrt{a^2+b^2}$), which indicates the intensity or color saturation and hue angle ($H=\tan^{-1}\frac{b}{a}$).

Total phenolics analysis

The total phenolic content was determined by the Folin-Ciocalteu method(22) which was previously modified(23) to reduce the assay volume. 0.1 of the sample (10% v/v grape juice) was added to 3.90 of H_2O followed by 0.5 mL of Folin-Ciocalteu reagent (Junsei Chemical Co., Ltd., Japan). After 3-6 min, 0.5 ml of saturated sodium carbonate (20 g of Na_2CO_3 in 100 mL of H_2O) (Yukuri Pure chemicals Co, Ltd., Kyoto, Japan) was added. After 30 minutes of vigorous mixing, optical density was measured at 725 nm (Optizen 2120UV spectrophotometer, Korea). The results were expressed as gallic acid equivalents (GAE) using a calibration curve with gallic acid (Sigma-Aldrich Chemical Co., Germany) as the standard (mg/L).

Free radical scavenging activity

The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH(24). 1 mL diluted sample (10% v/v grape juice) was placed in a test tube and 4 mL of $6x10^{-5}$ mol/L ethanolic solution of DPPH (Sigma-Aldrich Chemical Co., Germany) was added. The mixture was shaken vigorously for 40 seconds and then absorbance measurements commenced immediately. The decrease in absorbance at 517 nm was determined using Optizen 2120 UV spectrophotometer, Korea. The absorbance of the DPPH radical ethanolic solution was measured daily. All evaluations were made in triplicate. The % DPPH radical scavenging activity of the sample was calculated according to Blois' formula(25).

Total flavonoid analysis

Zhishen et al.(26) outlined the procedures to determine the total flavonoid concentrations of the grape juice samples. One milliliter of diluted sample (1 mL juice/ 5 mL distilled H_2O) was placed in a 10 mL flask. Four milliliter distilled water and 0.3 mL of $NaNO_2$ (5 g/100 mL distilled water) was added to the diluted sample. After 5minutes, 0.3 mL of $AlCl_3$(10 g/100 mL distilled H_2O) was added then after another 6minutes, 2 mL of 1 N NaOH was also added. The solution was diluted to a total volume of 10ml with distilled water. The absorbance of the solution was measured at 510 nm and flavonoid concentration was determined by using a rutin standard curve.

Total anthocyanin analysis

The total anthocyanin contents were determined using the pH-differential method(27). Grape juices were diluted with potassium chloride buffer solution, pH 1.0 so that the absorbance reading at 520 nm, which is the wavelength of maximum absorption for anthocyanins, was less than 1.0 absorption units. Two dilutions of the grape juice samples, one with potassium chloride buffer, pH 1.0 and the other with sodium acetate buffer are allowed to equilibrate for 15 min. The absorbance of each equilibrated solution was then measured at the wavelength of maximum absorption (λ_{max}) and 700 nm for haze correction, against a blank cell filled with distilled water. Malvidin-3-glucose was used as a reference compound with a molar absorbance of 28,000 and molecular weight of the pigment (493.2 g)to calculate the concentration of monomeric anthocyanin pigments (mg/L) in the juices.

Color density, browning, and polymeric color analysis

Grape juices were diluted with distilled water using appropriate dilution factor already determined. About 2.8 mL of the diluted samples was transferred each to two cuvettes, one cuvette added with distilled water while the other one added with bisulfite solution and are allowed to equilibrate for 15 min. The absorbance of each equilibrated solution was then measured at the wavelength of maximum absorption (λ_{max}), 420, and 700nm (for haze correction), against a blank cell filled with distilled water. The measured absorbance was used to determine browning, color density, polymeric color and % polymeric color(27).

Sensory evaluation

The sensory evaluation was conducted in the Department of Food Science at Kyungpook National University, Republic of Korea. Grape juices were presented in glasses with three-digit numbers. The five trained judges scored each attribute on a scale of 1-9, in which 1 denotes like extremely and 9 denotes dislike extremely.

Statistical analysis

Analysis of Variance and Duncan's multiple range tests were performed using the SAS program to determine the differences among the processed juice samples. The level of significance was set at $P < 0.05$.

Results and Discussion

Physicochemical properties

The mean values of the measured properties of the processed Campbell grape juice are presented in Table 1. It can be observed that the three processed grape juice samples have significant differences in pH and total soluble solids (°Brix) but no significant differences in titratable acidity and sugar -acid ratio. The grape juice samples showed an increasing trend in pH from 3.38 to 3.50, with the addition of seeds and stems whereas, the total soluble solids showed a decreasing trend from 17.0 °Brix to 16.60 °Brix. Sample C has the highest pH of 3.5 and the lowest total soluble solids of 16.60 °Brix. It is also shown in the study that titratable acidity and sugar-acid ratio has an inverse relationship thus, sample B showed the highest titratable acidity but the lowest sugar - acid ratio, 0.86 mg/100 g tartaric acid and 19.47, respectively. The result clearly indicates that even there is no significant difference in some properties of the juice still the seeds and stems have an effect on the physicochemical properties of the grape juice.

Table 1. Physicochemical and color properties of the three Campbell grape juice samples

Sample[1)]	Physicochemical properties			
	pH	°Brix	TA	Sugar-acid ratio
A	3.38±0.00	17.00±0.00^{a}	0.85±0.02^{a}	19.96±0.44^{a}
B	3.44±0.00	16.80±0.00^{b}	0.86±0.02^{a}	19.47±0.56^{a}
C	3.50±0.00	16.60±0.00^{c}	0.85±0.01^{a}	19.61±0.27^{a}

Sample[1)]	Color Properties				
	"L"	"a"	"b"	Chroma	Hue
A	27.27±1.02^{a}	6.57±2.91^{a}	-0.19±0.69^{a}	6.60±2.90^{a}	5.64±1.99^{a}
B	28.38±1.21^{a}	4.77±1.66^{a}	-0.94±0.48^{a}	4.90±1.52^{a}	13.13±9.61^{a}
C	28.95±1.22^{a}	5.11±1.44^{a}	-1.18±0.23^{a}	5.27±1.34^{a}	14.05±6.79^{a}

Data represent the mean±SD of four replicates.
[abc]Means with similar letters within columns for each samples are not significantly different by Duncan's multiple range test at P=0.05.
[1)]Sample A (without seeds and without stems), Sample B (with seeds) and Sample C (with seeds and with stems).

Color evaluation

The color values of the grape juice samples showed a non-significant increasing trend (Table 1). Sample C is the lightest (28.95) but has the most purple-red color as can be observed in their high "a" (5.11), "b" (-1.18), chroma (5.27), and hue (14.05) values. Analysis also showed that color degrading values were affected by the addition of seeds and stems. Although samples A, B, and C showed no significant differences from each other in terms of color values it can be observed that browning (0.073), color density (11.45) and % polymeric color (52.43) are more noticeable in sample C (Table 2) than in samples A and B.

Table 2. The color degradation indices of the three Campbell grape juice samples

Sample[1)]	Color degradation indices		
	Browning	Color density	polymeric color(%)
A	0.041±0.00^{b}	9.04±0.16^{a}	42.31±1.84^{b}
B	0.051±0.00^{b}	8.81±0.39^{b}	50.91±1.63^{a}
C	0.073±0.00^{a}	11.45±0.12^{c}	52.43±0.51^{a}

[abc]Values with similar letters within columns for each samples are not significantly different.
[1)]Samples A, B and C are the same as in Table 1.

Functional properties

The differences in functional properties were observed in grape juice samples as affected by the presence of seeds and stems and were presented in figures 1-4. The functional properties of the processed juice samples such as total phenolics, % radical scavenging activity, total anthocyanin, and total flavonoid showed an increasing trend which is maybe due to the additions of seeds and stems. The three samples were characterized as significantly different in total phenolics, total monomeric and total flavonoid, however, the samples showed no significant difference in %radical scavenging activity. The analysis showed that sample A has the lowest functional properties while sample C has the highest functional properties. The high phenolic content observed in Sample C is maybe due to the presence and composition of seeds and stems in that sample which is supported by the findings of different researchers(14,16). These authors observed that high phenolic content in grape juice samples is due to the composition and amount of other materials such as petioles, leaves and stems. In line with this, it has been demonstrated that phenolic content of grape depends upon whether the extraction was made on pulp, skin or seed(28,29). In addition, it was also reported that the berry skins and seeds mostly contribute to the flavan 3-ols compounds in juice and wine(30). Only small differences in increase in % radical scavenging activity were observed following the processing of Campbell grape with seeds and with stems, indicating that overall scavenging capacity was not affected by the presence of seeds and stems. Although the increase in % RSA was not significant it can be observed that sample C has the highest total phenolics value and %radical scavenging activity of 1456.67 mg/L and 76.43% , respectively(Fig. 1,2). Vinson and Huntz(31) have also reported the positive correlation of anti oxidant activity and total phenolics in grape and wine. This is in agreement with the previous study demonstrating that antioxidant activity of

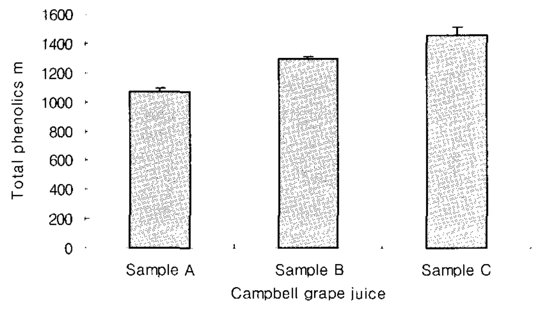

Fig 1. Changes in Total phenolics (mg/L) contents of Campbell grape juice as influenced by the presence of seeds and stems.
Samples A, B and C are the same as in Table 1. Vertical bars represent ± standard deviation of the means (N=3).

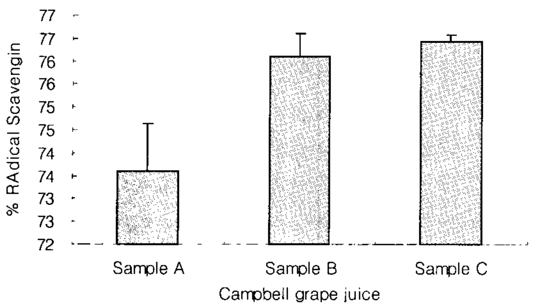

Fig 2. Changes in Antiradical Scavenging activity of Campbell grape juice as influenced by the presence of seeds and stems.

Samples A, B and C are the same as in Table 1. Vertical bars represent ± standard deviation of the means (N=3).

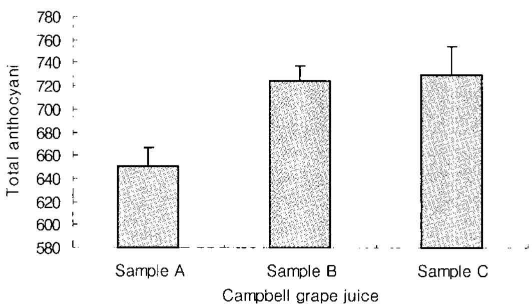

Fig 3. Changes in total anthocyanin (mg/L) of Campbell grape juice as influenced by the presence of seeds and stems.

Samples A, B and C are the same as in Table 1. Vertical bars represent ± standard deviation of the means (N=3).

grapes is positively correlated with the concentration of phenolic compounds(32).

As can be seen from figures 3 and 4, Sample C has the highest total anthocyanin and total flavonoid values with 730.03mg/L and 2112.80 mg/L, respectively. The result showed that the sample without seeds and without stems has the lowest total anthocyanin and total flavonoid values. Total anthocyanin was significantly altered by the addition of seeds and stems despite the non significant difference in color values of the samples. Among the samples total anthocyanin most likely contributed to higher color density, browning and other color values. It has been reported that color densities, degree of redness, hue and chroma are highly correlated with the amount of anthocyanin(33). Thus, the increase in total anthocyanin corresponds to an increase in color density(34). Talcott et al.(35) and Garcia and Bridle(36), reported that differences among individual anthocyanins present in the juice and their ability to form copigment complexes can influence the stability of the anthocyanin in the samples. The total flavonoid has also been significantly increased with the addition of seeds and stems. It has been reported that in grapes, seeds make up a small percentage of the weight but it contains about two thirds of the extractable phenols which are essentially all flavonoids(37,38). In addition, Huang et al.(16) also observed that berry stems showed a substantial amount of total flavonoids. Thus, the increase in functionality highlights the importance of the presence of seeds and stems in the processing of Campbell grape into juice.

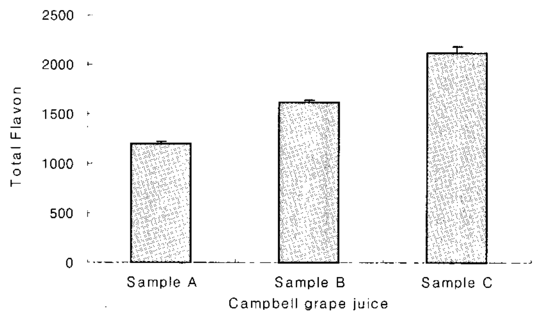

Fig 4. Changes in total flavonoid (mg/L) of Campbell grape juice as influenced by the presence of seeds and stems.

Samples A, B and C are the same as in Table 1. Vertical bars represent ±standard deviation of the means (N=3).

Sensory evaluation

Table 3 represents the mean sensory scores of Campbell grape juice processed without seeds and stems, with seeds only, and with seeds and stem. Interesting results were obtained from the sensory evaluation of the three processed Campbell grape juice samples. It can be noted that there is no significant difference in the panelist's preferences among samples A, B, and C in terms of color, aroma, taste and overall acceptability. The preference scores of the panelists to Sample C are almost similar to Sample B. Most of the sensory attributes were rated between 2 to 3 which means that all the samples were liked moderately to like very much by the panelists. Thus, the results demonstrated that Campbell

Table 3. mean sensory scores of Campbell grape juice processed without seeds and stems, with seeds only, and with stem and seeds

Sample[1)]	Sensory Attributes			
	Color	Aroma	Taste	Overall acceptability
A	2.8^a	2.6^a	2.2^a	2.4^a
B	2.8^a	2.6^a	2.4^a	2.6^a
C	2.4^a	2.6^a	2.4^a	2.2^a

[abc]Values with similar letters within columns for each samples are not significantly different.

[1)]Samples A, B and C are the same as in Table 1.

grape can be process into juice with added stems and seeds without altering its compositional and sensory quality and indeed increase the juice total phenolics, % radical scavenging activity, anthocyanin content, and total flavonoid content of the of the juice.

요 약

포도주스는 건강에 유익한 phenolic compounds의 함량이 풍부하여 최근 많은 주목을 받고 있다. 본 연구에 사용된 Campbell 포도주스는 Sample A(씨와 줄기를 첨가하지 않은 경우), Sample B(씨만 첨가한 경우), Sample C(씨와 줄기를 첨가한 경우)의 세 가지로 제조하여 포도 씨와 줄기의 첨가가 포도주스의 성분, 관능적 특성, 기능적 특성에 미치는 영향을 시험하였다. 포도주스 제조 시 포도 씨와 줄기의 첨가는 pH와 가용성 고형분(°Brix)에는 유의적인 영향을 주었지만 다른 물리화학적 특성이나 색에는 유의적인 영향을 주지 않았다. 또한 total phenol, radical scavenging activity, total anthocyanin, total flavonoid는 증가하였다. 관능적 특성시험의 결과에서도 sample들 사이에서 유의적인 차이를 볼 수 없었다. 이번 연구로 Campbell 포도주스 제조 시 포도 씨와 줄기의 첨가가 포도주스의 물리화학적 특성이나 관능적 특성에 영향을 주지 않으면서 기능적 특성을 향상시킬 수 있는 것으로 여겨진다.

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