

## Effects of Cold Stabilization on the Quality of Grape Juice

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### 저온 처리가 포도주스의 품질에 미치는 영향

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#### Abstract

The change in physicochemical and sensory properties and bioactive compounds of Campbell grape juice has been investigated for 30 days at -5°C. The pH and sugar/acid ratio of the cold stabilized juice increased, while titratable acidity and soluble solids decreased as cold stabilization period is prolonged. Juice samples became lighter, yellowish to brownish and lesser red in color as can be observed with the increasing L\* and b\* values, and decreasing a\* value, respectively. The juice cold stabilized for a shorter period of time showed higher levels of bioactive compounds and radical scavenging activity, and was more preferred by the panelists than the juice cold stabilized for a longer period of time. These results suggest that cold stabilization period affected the quality characteristics of grape juice.

Key words : cold stabilization, grape juice, quality, bioactive compounds

### Introduction

Fruit juices like grape juice, nowadays have become very popular products and it was reported that consumption of juice has increased in the developed countries and keeps on increasing in recent years. Moreover, fruit juices are significant sources of nutrients and energy and play an important role in human nutrition (1).

Grapes are unique from other fruits because after juice extraction, the argols (potassium bitartrate) or tartrates must be precipitated. The tartrate crystals will settle out upon cooling or refrigeration and although harmless, are aesthetically unpleasant and maybe considered a quality defect. In addition, the presence of these tartrates may

influence the consumer's acceptability of the product.

Cold stabilization or detartration is a process to remove the tartrate crystals or excess tartaric acid. This step is usually included in the processing of grapes into grape juice, which is more rapid in freezing storage than in cold storage (2). Previous studies also showed the different cold stabilization period used as part of grape juice processing at different temperatures of different grape cultivars (3-5). In addition, Soyer *et al.* (6) reported the effect of cold stabilization on the tartaric acid content and other quality components of white grape juices.

There are many published studies regarding the health benefits that can be derived from grape and grape products such as wine and juice (7-9). However, processing and processing steps of grapes into grape juice may affect some of its quality and nutrient components such as polyphenolic phytochemicals which have antioxidant potency and other

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biological activities (10). Furthermore, Morris *et al.* (11) observed the effects of maturity, extraction temperature, storage temperature and time on color extraction and degradation, quality changes and tartrate formation in grape juice.

Many of the previous studies focused mainly on the removal of tartrates in juice or wine, but the effect of removal of tartrates on the quality of grape juice was not easily available. The aim of this study was to evaluate the effects of different cold stabilization period on the quality and bioactive compounds of Campbell juice.

## Materials and Methods

### Materials

Campbell grapes, grown in Modong-myeon Sangju City, Gyeongbuk, Korea during 2008 season, were used for the processing of grape juice. Folin-Ciocalteu reagent was purchased from Junsei Chemical Co. (Tokyo, Japan), gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and catechin were from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and other chemicals used for analyses were high purity grade.

### Juice processing and cold stabilization

The grapes were washed, destemmed, crushed, added with pectinase, heated at 60°C for 30 min, then heated again at 80°C for 30 min, pressed/filtered using cheesecloth, then the juice was placed in the freezer (-5°C) to cold stabilize for 1, 3, 5, 7, 14 and 30 days. After each cold stabilization period, the juice samples were then filtered using cheesecloth, packed in glass bottles, pasteurized at 70°C for 20 min and analyzed.

### Analyses of soluble solids, pH and titratable acidity

Soluble solids (°Brix) were measured using a hand refractometer (Master-α; ATAGO Co, Ltd, Tokyo, Japan). The pH of grape juice was measured using a pH meter (Delta320; Mettler-Toledo International Inc, Shanghai, China). Titratable acidity as modified by Haight and Gump(12) was measured by adding 10 mL of grape juice sample to 100 mL of distilled water and titrating with 0.1 N NaOH to an endpoint of pH 8.2. The results were expressed as gram tartaric acid per 100 mL.

### Color measurement

Color of the grape juice was determined using a colorimeter

(CR-200; Minolta Co, Ltd, Osaka, Japan). Three reading of L\*, a\*, b\*, chroma (C\*), and hue angle (h°) values were recorded for each sample. The L\* value represents lightness, the +a\* and -a\* values represent redness and greenness, respectively. The +b\* and -b\* values represent yellowness and blueness, respectively. The C\* is a measure of the purity or saturation of the color. The h° expresses the color tone, is defined as red - purple: 0°, yellow: 90°, bluish-green: 180°, and blue: 270°.

### Total phenolics analysis

The total phenolic content was determined by the Folin - Ciocalteu method (13) previously modified by Yildirim *et al.* (7) to reduce the assay volume. The results were expressed as gallic acid equivalents (GAE) using a calibration curve with gallic acid as the standard (mg/L).

### Free radical scavenging activity

The antioxidant activity of grape juice was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH (14). The % DPPH radical scavenging activity of the sample was calculated (15).

### Total anthocyanin analysis

The total anthocyanin contents of the grape juice samples were determined using the pH-differential method previously described by Giusti and Wrolstad (16). Malvidin-3-glucoside was used as a reference compound of the pigment used to calculate the concentration of anthocyanin pigment (mg/L) in the juices.

### Total flavonoid analysis

Total flavonoid contents were determined using procedures outlined by Zhishen *et al.* (17). The absorbance of the solution was measured at 510 nm and the flavonoid concentration was determined by using a catechin standard curve.

### Sensory evaluation

The sensory evaluation of the grape juice samples was conducted at the Department of Food Science and Technology at Kyungpook National University, Republic of Korea. About 50 mL of each grape juice were presented in glasses with three digit numbers. Water was also provided for the panelists for rinsing their oral cavity. The 15 trained judges were instructed to evaluate each attribute such as color, aroma, taste and overall acceptability on a nine point hedonic scale of 1-9, in which 1 denotes dislike extremely and 9 denotes

like extremely (18).

### Statistical analysis

Analysis of variance and Duncan's multiple range tests were performed using the SAS program (9.1, SAS Institute, Inc, Cary, NC, USA) to determine the differences between variables of grape juice samples. The level of significance was set at  $p < 0.05$ . The measurements were done in triplicate and presented as mean values  $\pm$  standard deviation (SD).

## Results and Discussion

Previous studies (3-5,19-21) used cold stabilization as a part of grape juice processing, and focused more on the effects of other factors such as cultivar, maturity, juice extraction, storage and other processing methods on the quality of the grape juices. In this study, we focused on the effect of different period of cold stabilization at  $-5^{\circ}\text{C}$  on the quality changes of Campbell grape juice. The results in Table 1 showed that

**Table 1. Soluble solids, pH and titratable acidity of Campbell grape juice as affected by cold stabilization period**

Cold stabilization (days)	Soluble solids ( $^{\circ}$ Brix)	pH	Titratable acidity (g/100 mL)	Sugar/acid ratio
Control	14.50 $\pm$ 0.02 <sup>al</sup>	3.56 $\pm$ 0.02 <sup>g</sup>	0.88 $\pm$ 0.01 <sup>a</sup>	16.42 $\pm$ 0.39 <sup>f</sup>
1	14.40 $\pm$ 0.00 <sup>b</sup>	3.62 $\pm$ 0.01 <sup>e</sup>	0.73 $\pm$ 0.01 <sup>b</sup>	19.73 $\pm$ 0.27 <sup>e</sup>
3	14.20 $\pm$ 0.01 <sup>c</sup>	3.65 $\pm$ 0.00 <sup>d</sup>	0.63 $\pm$ 0.01 <sup>c</sup>	22.66 $\pm$ 0.21 <sup>d</sup>
5	14.20 $\pm$ 0.02 <sup>c</sup>	3.89 $\pm$ 0.01 <sup>a</sup>	0.61 $\pm$ 0.01 <sup>cd</sup>	23.15 $\pm$ 0.22 <sup>cd</sup>
10	14.00 $\pm$ 0.03 <sup>d</sup>	3.66 $\pm$ 0.00 <sup>c</sup>	0.60 $\pm$ 0.01 <sup>de</sup>	23.21 $\pm$ 0.45 <sup>c</sup>
14	14.20 $\pm$ 0.03 <sup>c</sup>	3.61 $\pm$ 0.03 <sup>f</sup>	0.59 $\pm$ 0.01 <sup>e</sup>	24.21 $\pm$ 0.24 <sup>b</sup>
30	12.80 $\pm$ 0.02 <sup>e</sup>	3.84 $\pm$ 0.02 <sup>b</sup>	0.45 $\pm$ 0.01 <sup>f</sup>	28.44 $\pm$ 0.20 <sup>a</sup>

<sup>1)</sup>The results are expressed as the mean  $\pm$  SD (n=3), means with different letters in each column are significantly different at  $p < 0.05$ .

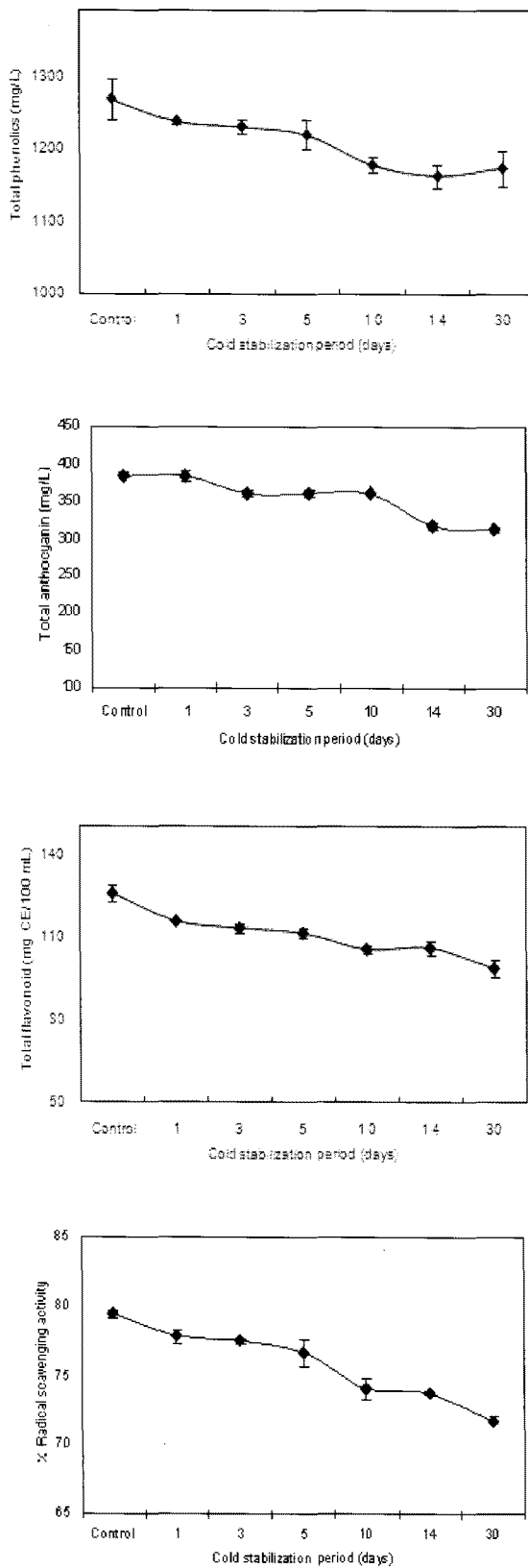
the pH and sugar/acid ratio of the cold stabilized juice significantly increased from 3.56 $\pm$ 0.02 to 3.84 $\pm$ 0.02 and 16.42 $\pm$ 0.39 to 28.44 $\pm$ 0.20 after 30 days of cold stabilization, respectively, while the titratable acidity and soluble solids significantly decreased from 0.88 $\pm$ 0.01 to 0.45 $\pm$ 0.01 and 14.5 $\pm$ 0.02 to 12.80 $\pm$ 0.02, respectively. But it can be noted that between a day to 2 weeks of cold stabilization, not much differences can be observed in physicochemical properties of juice. Grape juice is saturated with potassium bitartarate in which at certain conditions such as low temperature storage, the dissolved potassium tartrates become insoluble and small crystals precipitate to the bottom in the form of sediment (21). This precipitation in grape juice leads to the changes in pH and a decreased in total soluble solids. Furthermore, a significant decrease in titratable acidity can also be observed which is in accordance with the study made by Soyer et al. (6). They reported that acid content of grape juice was not affected by processing steps such as cold stabilization except for tartaric acid. There was also a significant change in color values of the juices (Table 2). The L\*, b\*, and h<sup>o</sup> values increased, while a\* and C\* values decreased. The juice samples became lighter, yellowish to brownish and lesser red in color as can be observed with the increasing L\* and b\* value and decreasing a\* value, respectively. The color changes might be due to the precipitation of pigments together with the tartrates which occurred during cold stabilization. This is in line with the study made by Ingalsbe et al. (22), they observed that color changes in Concord grape juice was due to pigment precipitation through cold stabilization rather than decomposition.

Levels of bioactive compounds and radical scavenging activity are shown in Fig. 1. A significant decrease in the amount of bioactive compounds and radical scavenging activity as the cold stabilization period is prolonged can be

**Table 2. Color properties of Campbell grape juice as affected by cold stabilization period**

Cold stabilization (days)	Color values				
	L*	a*	b*	C*	h <sup>o</sup>
Control	23.15 $\pm$ 0.66 <sup>e1)</sup>	6.80 $\pm$ 0.36 <sup>a</sup>	-2.51 $\pm$ 0.29 <sup>a</sup>	7.17 $\pm$ 3.15 <sup>a</sup>	339.68 $\pm$ 5.18 <sup>bc</sup>
1	32.18 $\pm$ 0.26 <sup>a</sup>	4.46 $\pm$ 0.72 <sup>bc</sup>	-1.25 $\pm$ 0.41 <sup>ab</sup>	4.64 $\pm$ 6.55 <sup>bc</sup>	344.07 $\pm$ 14.25 <sup>ab</sup>
3	30.76 $\pm$ 0.63 <sup>b</sup>	3.75 $\pm$ 0.18 <sup>bc</sup>	-2.53 $\pm$ 0.21 <sup>c</sup>	4.52 $\pm$ 1.16 <sup>bc</sup>	326.00 $\pm$ 2.49 <sup>d</sup>
5	31.49 $\pm$ 0.38 <sup>ab</sup>	5.80 $\pm$ 2.60 <sup>ab</sup>	-1.15 $\pm$ 0.77 <sup>ab</sup>	6.00 $\pm$ 8.26 <sup>ab</sup>	348.64 $\pm$ 13.65 <sup>ab</sup>
10	31.98 $\pm$ 0.78 <sup>a</sup>	2.62 $\pm$ 0.18 <sup>c</sup>	-2.31 $\pm$ 0.34 <sup>c</sup>	3.50 $\pm$ 2.83 <sup>c</sup>	318.81 $\pm$ 2.70 <sup>d</sup>
14	27.40 $\pm$ 0.06 <sup>d</sup>	2.50 $\pm$ 0.56 <sup>c</sup>	-1.51 $\pm$ 0.36 <sup>b</sup>	2.95 $\pm$ 11.19 <sup>c</sup>	328.31 $\pm$ 3.52 <sup>cd</sup>
30	28.62 $\pm$ 0.45 <sup>c</sup>	4.62 $\pm$ 0.93 <sup>bc</sup>	-0.54 $\pm$ 0.55 <sup>a</sup>	4.68 $\pm$ 7.68 <sup>bc</sup>	353.10 $\pm$ 10.89 <sup>a</sup>

<sup>1)</sup>The results are expressed as the mean  $\pm$  SD (n=3), Means with different letters in each column are significantly different at  $p < 0.05$ .



**Fig. 1.** Changes in bioactive compounds and radical scavenging activity of Campbell grape juice during cold stabilization.

The results are expressed as the mean  $\pm$  SD ( $n=3$ ).

observed in the study. The highest concentration of the analyzed total phenolics, total flavonoid, total anthocyanin and radical scavenging activity of 1638.47 $\pm$ 107.41 mg/L, 125.91 $\pm$ 3.16 mg CE/100 mL, 632.64 $\pm$ 3.14 mg/L and 83.73 $\pm$ 0.32%, respectively, was from the juice sample without cold stabilization. In contrast, the juice sample cold stabilized for one month showed the lowest bioactive compounds and radical scavenging activity of 1220.33 $\pm$ 41.99 mg/L, 98.60 $\pm$ 2.93 mg CE/100 mL, 495.73 $\pm$ 3.14 mg/L and 80.9 $\pm$ 0.25%, respectively. It can be noted that there was a decrease of 25.52%, 21.69%, 21.64% and 3.38% in the total phenolics, total flavonoid, total anthocyanin and radical scavenging activity after 30 days of cold stabilization, respectively. Thus, it can be suggested that bioactive compounds were also removed upon the precipitation of tartrates. The results were similar to the findings of Sistrunk and Morris (19) who reported that there was a significant loss in total anthocyanin during cold stabilization in the Noble grape juice.

The sensory evaluation of the juice samples showed that the sample cold stabilized for 5 days was the most preferred in terms of color, while the samples cold stabilized for 3 days was the most preferred in terms of aroma (Table 3), but the sample cold stabilized for 1 day was the most preferred samples in terms of taste and over all acceptability. This means that the panelists mostly preferred the juice cold stabilized for a shorter period of time than the juice sample cold stabilized for a longer period of time. It was also observed that the juices cold stabilized for longer period of time almost lost its sweet-sour taste. However, cold stabilization period based on the panelists' scores did not significantly affect the changes in color of the grape juice.

In conclusion, cold stabilization period significantly affect the physicochemical properties and bioactive compounds of grape juice. It is also suggested to cold stabilize the Campbell grape juice for 1 day at  $-5^{\circ}\text{C}$ , although it still has high titratable acidity but lower than control, it was the most preferred in terms of taste and overall acceptability. This means that the consumer likes the sweet-sour taste in grape juice not just a sweet taste. The sample also showed a higher amount of bioactive compounds and % radical scavenging activity as compared to other juice samples which were cold stabilized for a longer period of time. Further studies to maintain the bioactive compounds of the grape juice after cold stabilization is needed.

Table 3. Sensory scores of Campbell grape juice as affected by cold stabilization period

Cold stabilization (days)	Color	Aroma	Taste	Overall acceptability
Control	7.42±0.67 <sup>ab1)</sup>	7.25±0.99 <sup>abc</sup>	6.5±1.45 <sup>ab</sup>	7.17±0.94 <sup>ab</sup>
1	7.25±0.87 <sup>a</sup>	7.58±0.67 <sup>ab</sup>	7.33±0.98 <sup>a</sup>	7.58±0.79 <sup>a</sup>
3	7.42±0.51 <sup>a</sup>	7.67±0.49 <sup>a</sup>	7.25±0.75 <sup>a</sup>	7.42±0.51 <sup>a</sup>
5	7.58±0.52 <sup>a</sup>	7.25±0.62 <sup>abc</sup>	6.92±0.79 <sup>ab</sup>	7.17±0.72 <sup>ab</sup>
10	7.50±0.52 <sup>a</sup>	6.92±0.79 <sup>bc</sup>	6.92±0.67 <sup>ab</sup>	7.00±0.60 <sup>bc</sup>
14	7.17±1.27 <sup>a</sup>	6.83±1.19 <sup>c</sup>	5.41±1.31 <sup>c</sup>	5.50±1.09 <sup>c</sup>
30	7.08±1.00 <sup>a</sup>	7.41±0.67 <sup>abc</sup>	6.25±1.06 <sup>bc</sup>	6.75±0.75 <sup>ab</sup>

<sup>1)</sup>The results are expressed as the mean ± SD (n=3), Means with different letters in each column are significantly different at p<0.05.

## 요 약

‘캠벨’ 포도주스의 이화학적 및 관능적 품질특성에 저온 안정화 처리기간이 미치는 영향을 조사하기 위하여, 포도 과실로 압착 주스를 제조하여 -5℃에서 30일간 보관하면서 주스의 각종 품질특성 변화를 조사하였다. 주스의 당/산비와 pH는 증가하였으나 적정산도와 가용성 고형분 함량은 경시적으로 감소함을 보였다. 주스의 명도(L\*값)와 황색도(b\*값)는 증가하는 경향을 보였으며 적색도(a\*값)는 감소하는 경향을 각각 나타내었다. 주스의 총페놀, 안토시아닌, 플라보노이드 함량과 유리기 소거능 및 관능적 기호도는 안정화처리 기간이 길어질수록 점차적으로 감소하였다. 이로써 저온 안정화 처리기간은 포도주스의 품질 변화에 영향을 미치며 장기간 처리는 품질을 오히려 저하시키는 것으로 확인되었다.

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