Effects of Processing Treatments on the Bioactive Compounds of Campbell Grape Juice

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Abstract – *It is well known that phenolic compounds and flavonoids have a lot of health benefits. Most current heat treatments especially those that are using too high temperature and longer period of processing produce juice with unacceptable analytical and sensory properties. Thus, microwave heating, ultrasonication and blanching before grape juice processing were employed in this study. Each juice sample was subjected to total phenolics, total flavonoid, total anthocyanin and % radical scavenging activity analysis. Analysis of Variance and Duncan's multiple range tests were performed using the SAS program. The concentration of total phenolics, total flavonoids, total anthocyanin and % radical scavenging activity of the grape juices treated with microwave heating and ultrasonication increased significantly with increasing treatment time notably at 5 min treatment. It can be noted that all the bioactive contents and % radical scavenging activity were higher in non-cold stabilized grape juice than in cold stabilized juices. However, blanching whole grapes for longer period of time before processing resulted in the decrease of all bioactive compounds and % radical scavenging activity both in non-cold stabilized and cold stabilized juice especially at 5 min blanching period. It was also showed that 1 min blanching is more effective in increasing the bioactive compounds of the grape juice as compared to other treatment time.*

Keywords: *Campbell grape, Grape juice, Total phenolics, Flavonoid, Anthocyanin, Antioxidant*

INTRODUCTION

Recently, consumers associate the fruit juices such as grape juice as healthy products, thus their commercialization has increased in the last years [1] However, processing grapes into grape juice may affect some of its quality and nutrient component such as polyphenolic pytochemicals which has antioxidant potency and other biological activities by Makris et al.[2]. Campbell grape juice after heating, treatment of fining agents and cold stabilization, although has a positive effect on titratable acidity and sugar/acid ratio decreased its bioactive compounds. It was also believed that the action of polyphenoloxidase enzyme has a detrimental effect on the anthocyanin and other phenolic compounds, thus results to browning and lowered anthocyanin content and other bioactive contents.

Processing grapes into grape juice may affect some of its quality and nutrient component such as polyphenolic pytochemicals [2]. Huckleberry, et al [3] found that less heat or no heat in processing produce more acceptable flavor but the color of the heat processed juice was preferred especially in the red cultivars. It was also reported that the use of enzymes in juice processing can result in greatly improved juice yields as well as quality improvements [4]. However, the use of commercial enzymes does not produce juices with increased phenolic content.

Most current heat treatments especially those that are using too high temperature and longer period of processing produce juice with unacceptable analytical and sensory properties. Thus, microwave heating, ultrasonication and blanching before grape juice processing were employed in this study.

OBJECTIVES OF THE STUDY

This study aimed to determine the effects of microwave heating, ultrasonication and blanching at different time on the bioactive compounds of Campbell grape juice.

MATERIALS AND METHODS

Total phenolics analysis

The total phenolic content was determined by the Folin–Ciocalteu method used by Singleton and Rossi previously modified by Yildirim et al. [5] to reduce the assay volume. To 3.90 mL of dH2O, 0.1 mL of the sample (1 mL grape juice in 9 mL of dH2O) was added 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 Na2CO3 in 100 mL of dH2O) (Yukuri Pure Chemicals Co, Ltd., Kyoto, Japan) was added. After 30 min of vigorous mixing with a vortex mixer the absorbance was measured at 725 nm (2120UV spectrophotometer, Optizen, 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 of grape juice was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH by Brand- Williams et al. [6]. One milliliter of diluted sample 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 sec and then absorbance measurements were taken immediately. The decrease in absorbance at 517 nm was determined with spectrophotometer (Optizen 2120UV, Korea). All evaluations were made in triplicate. The % DPPH radical scavenging activity of the sample was calculated according to the formula of Blois , 1958 (7) .

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 [8]. 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 (pH 4.5) were 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-glucoside was used as a reference compound with a molar absorbance of 28,000 and molecular weight of the pigment (493.2 g) used to calculate the concentration of anthocyanin pigments (mg/L) in the juices.

Total flavonoid analysis

Total flavonoid contents were determined using procedures outlined by Zhishen *et al.* [9]. One milliliter of diluted grape was placed in a flask. Four milliliters of distilled water was added followed by 0.3 mL of NaNO2 (Sigma Chemical Co., USA). After 5 min, 0.3 mL of $AlCl_3$ (Junsei Chemical Co. Ltd., Japan) was added. After another 6 min, 2 mL of 1 N NaOH (Duksan Pure Chemical Co. Ltd., Korea) was added and then the solution was diluted to a total volume of 10 mL with distilled water. The absorbance of the solution was measured at 510 nm and the flavonoid concentration was determined by using a catechin standard curve.

Statistical analysis

Analysis of Variance and Duncan's multiple range tests were performed using the SAS program ($P < 0.05$).

RESULTS AND DISCUSSION

The concentration of total phenolics, total flavonoids, total anthocyanin and % radical scavenging activity of the grape juices treated with microwave heating and ultrasonication increased significantly with increasing treatment time notably after 5 min of treatment (Fig. 1 and 2). It can be noted that all the bioactive contents and % radical scavenging activity were higher in non-cold stabilized grape juice than in cold stabilized juices. These differences between cold stabilized and non-cold stabilized juice might be attributed to the precipitation of pigments together with the tartrates during cold stabilization period. However, it can be observed that the content of the cold stabilized juice which was microwave heated for 5 min showed higher total phenolics, total flavonoids, total anthocyanin and % radical scavenging activity of 1697.54± 73.59 mg/L, 239.67±1.94 mg CE/ 100 mL, 1079.91±12.43 mg/L, and 75.10%±0.31, respectively, than non-cold stabilized control juice, 1414.24±18.37 mg/L, 185.91±3.16 mg CAE/ 100 mL, 627.84±12.77 mg/L and 69.77%±0.36, respectively. The increase for total phenolics, total flavonoid, total anthocyanin and radical scavenging activity were 20%, 29%, 72% and 7.64 %, respectively. These results are in line with the study on apple mash by Gerard and Robert[10]. They reported that microwave heat treatment of the mash increased extraction of phenolics and flavonoids from apple mash and resulted in juice with increased concentrations of total phenolics and flavonoids.

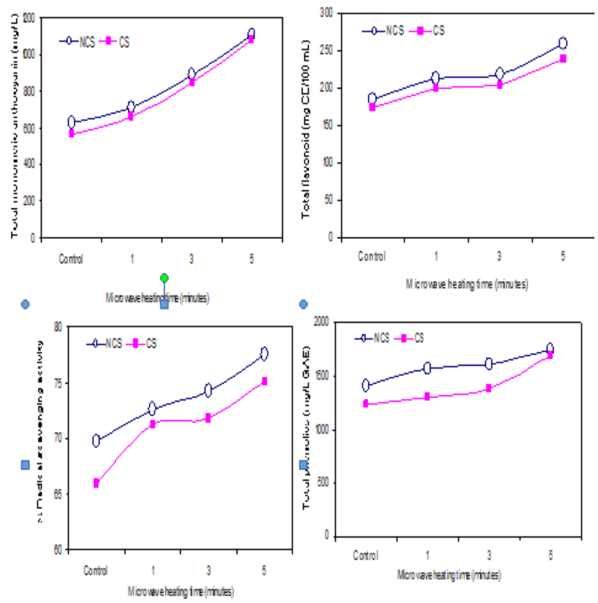

Fig1. Bioactive compounds of Campbell grape juice at different microwave heating time. NCS: Not cold stabilized ; CS: Cold stabilized stabilization

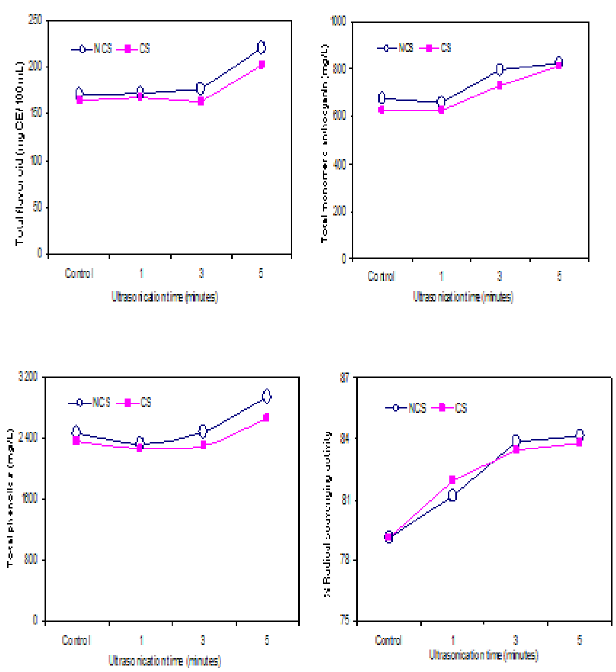

Fig2. Bioactive compounds of Campbell grape juice at different ultrasonication time NCS: Not Cold Stabilized; CS: Cold stabilized

Ultrasonication of whole grapes before the juice processing also showed an increase in bioactive compounds and % radical scavenging activity both in non- cold stabilized and cold stabilized juice especially at 5 min ultrasonication. It can also be observed that the content of the cold stabilized juice which was treated with ultrasonication for 5 min showed higher total phenolics, total flavonoids, total anthocyanin and % radical scavenging activity of 2662.72± 84.18 mg/L, 201.72±2.93 mg CE/100 mL, 810.80±7.85 mg/L, and 83.80%±0.24, respectively, than non-cold stabilized control juice, 2460.46±14.61 mg/L, 170.21± 5.77 mg CE/ 100 mL, 676.05±1.34 mg/L and 79.13%±0.12, respectively. The increase was almost 8.22% in total phenolics, 19% for total flavonoid, 19.84% for total anthocyanin and 5.90 % for radical scavenging activity. It is worth noting that even the juice was cold stabilized, the bioactive contents are still higher in treated juice as compared to the control.

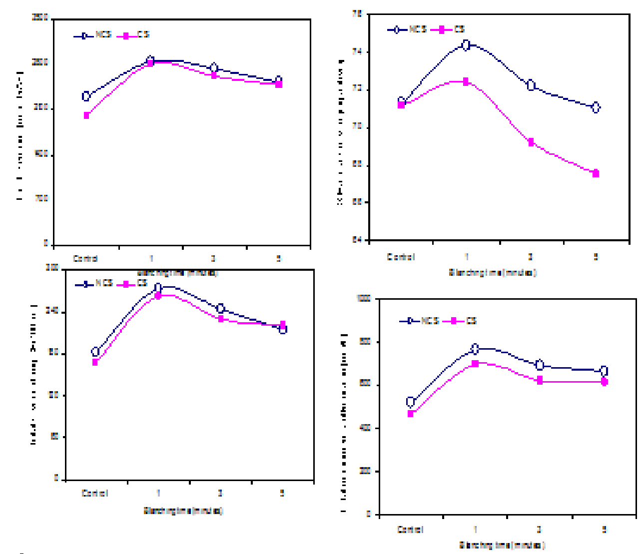

Fig3. Bioactive compounds of Campbell grape juice at different blanching time. NCS: Not cold stabilized ; CS: Cold stabilized stabilization

Fig. 3 presents the effects of blanching on the bioactive compounds of Campbell grape juice. Blanching whole grapes for longer period of time before processing resulted in the decrease of all bioactive compounds and % radical scavenging activity both in non-cold stabilized and cold stabilized juice especially at 5 min blanching period. It was observed that during blanching the color pigment of the grape is extracted and mixed with the water, thus lowering the amount of the desired bioactive compounds. However, the results also showed that 1 min blanching is the most effective way of increasing the bioactive compounds of the grape juice.

This effect may be the result of polyphenoloxidase being inactivated during the microwave heat treatment, blanching and ultrasonication treatment of whole grapes before processing, thus increased bioactive compounds and % radical scavenging activity was observed in the grape juice.

CONCLUSION AND RECOMMENDATION

The concentration of total phenolics, total flavonoids, total anthocyanin and % radical scavenging activity of the grape juices treated with microwave heating and ultrasonication increased significantly with increasing treatment time notably at 5 min treatment. It can be noted that all the bioactive contents and % radical scavenging activity were higher in non-cold stabilized grape juice than in cold stabilized juices. Even the juice was cold stabilized, the bioactive contents are still higher in treated juice as compared to non-cold stabilized control grape juice. The study is limited to Campbell grape variety, however this process can also be use to other tropical fruits with high antioxidant and bioactive compounds.

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