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POLYPHENOLOXIDASE DEACTIVATION IN JUICE FROM "CAMPBELL EARLY" GRAPES BY HEATING UNDER VACUUM PRESSURE

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ABSTRACT

The juice of "Campbell Early" grapes was analyzed to assess the effect of heating under low vacuum pressure on the inhibition of polyphenoloxidase (PPO), the enzyme that catalyzes browning reactions in grape juice. Treatment of grapes by heating under low vacuum pressure of 200–600 mmHg significantly inhibited the activity of PPO. Up to 82.15% reduction in the enzyme activity was observed in the grape juice by heating "Campbell Early" grapes at 65C for 20 min under 600 mmHg vacuum pressure. The vacuum heating of grapes also resulted in a significant increase in total phenols and total sugars in the grape juice, which also had improved sensory properties.

PRACTICAL APPLICATIONS

Vacuum heating enables us to attain the desired quality attributes during processing of fruits and vegetables at reduced temperature. Our study revealed that it not only deactivated polyphenoloxidase in grape juice but also increased other functional and sensory characteristics. Vacuum heating can be effectively applied in the fruits and vegetables processing industry for prevention of enzymatic browning as an alternate to the use of chemical preservatives and more costly enzyme inactivation techniques such as pulse electric fields.

INTRODUCTION

Grapes are one of the important fruit crops in the world. The consumption of grape juice has been associated with various health benefits

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(Ohno *et al.* 2008). Being the largest fruit crop in the world, it has an immense economic importance as it is processed into important food products (Pinelo *et al.* 2006). "Campbell Early" is a popular grape cultivar in Korea with a consistent increase in its cultivation. Grape juice has become a representative grape product with a great deal of research into improving its quality (Ghafoor *et al.* 2008).

Enzymatic browning is a major factor that contributes to the loss of quality in fruits and beverages with associated changes in sensory properties (Kim *et al.* 2005). Enzymatic browning and color degradation during processing of grapes can be prevented by various treatments including thermal treatment and the use of chemical preservatives to preserve the quality of finished products (Icier *et al.* 2008). Higher temperature during the processing of grapes may also trigger chemical reactions that lower the quality of grape products (Teresa *et al.* 2005). During juice processing, grapes are heated before pressing, which not only increases juice yield, but also extracts more tannins, and color components in the grape juice as compared with cold pressing. Heat treatment to the grapes plays a critical role in decreasing the activity of color-degrading enzymes. However, high-temperature heating can also deteriorate other important components (Threlfall *et al.* 2005). Efficiency of a heat treatment process used to deactivate certain enzymes in fruits can be evaluated by indicator enzymes such as polyphenoloxidase (PPO) (Yemenicioglu and Cemeroglu 1998). PPO has been studied in several fruits and plants such as apples, pears, peaches, banana, plums, avocadoes, grapes, litchi fruit, egg plant, herbs, field bean, pepper mint and tea leaves. It is copper-containing oxidoreductase that catalyzes two distinct chemical reactions that involves phenolic compounds and molecular oxygen. It catalyzes first the hydroxylation of monophenols to diphenols, then second, the subsequent oxidation of diphenols to quinines, which are intermediates that polymerize to form undesired pigments (Rapeanu *et al.* 2006a). Investigations to determine the characteristics of grape PPO and the conditions under which PPO is most active has been reported for some grape cultivars (Weemaes *et al.* 1998b). Effects of different processing techniques, processing parameters and their relation with the deactivation of PPO have been studied in various fruits and vegetables (Rapeanu *et al.* 2006b; Icier *et al.* 2008). In case of heat treatments that affect the composition and physiological characteristics of juices (Cerdan *et al.* 2006), intensity and duration of heating, are important with respect to the quality of the final products and the inactivation of PPO (Weemaes *et al.* 1998a). It has been suggested that heat deactivation treatments should be rapid because slow heating processes might result in the activation of PPO in plant tissue instead of deactivating it (Tate *et al.* 1964). Vacuum treatment of foods is a useful technique for altering physical and chemical compositions to improve certain characteristics (Igual *et al.* 2008). Vacuum heating is usually

applied to the heat sensitive products as we can achieve the desired processing objectives and higher product quality at comparatively lower temperature than atmospheric heating (Lewicki 2006). Our principal objective of undertaking the present study was to assess whether heat treatment to grapes under low vacuum pressure could inhibit the activity of PPO and improve the quality of grape juice.

MATERIALS AND METHODS

Grapes were purchased from a local farm in Kyungbuk province of Korea, and the grape cultivar was identified as "Campbell Early." All the chemicals used for analysis were purchased from Sigma Chemical Co. (St. Louis, MO).

Juice Processing

The grapes were excised from the plant, and grape berries with good characteristics were selected and washed. A laboratory scale machine (Model 2007GV, H. S. Co., Daegu, Korea) designed in the lab was used for vacuum and heat treatment of the grapes on a set pressure and temperature for a certain period of time. Application of temperature to the grapes was achieved by surrounding the heating vessel with water circulation tubes connected to a circulator water bath (MCB-3011D; M. E. Co., Daegu, Korea). The vacuum was created inside the vessel by sealing and connecting the heating vessel with a vacuum pump (Model 4001; S. D. Co., Daegu, Korea). The grapes were heated at different temperatures, and once the desired temperature (59–65C) was achieved, the vacuum pressure of varying intensities (0–600 mmHg) was applied for different time intervals (10–20 min). Afterward, the grapes were pressed with the help of a cheese cloth to extract juice, followed by overnight cold settling (4C) of tartartes.

Determination of PPO

PPO activity of the grape extract was determined spectrophotometrically following the procedure of Rapeanu *et al.* (2006a) with some modification. A sample of 5 mL of juice was taken, and 10 mL of acetone were added followed by stirring on a magnetic stirrer (Vision Scientific Company, Seoul, Korea). The sample was filtered, and 50 mL of 0.1 M citric acid was added followed by stirring. The samples (2.0 mL each) were taken in test tubes, and 4 mL of 0.1 M catechol solution were added to each tube. The increase in absorbance was measured at 360 nm after incubating the samples at room temperature for 20, 40 and 60 min. The enzyme activity was calculated in triplicates from the

optical density values at 360 nm on a spectrophotometer (TU-1800; Human Corporation, Seoul, Korea). The activity values reported were the mean of the three determinations, and relative standard deviations were less than ±1%.

Analysis for Total Phenolic Compounds

The total phenolic compounds were analyzed using the Folin–Ciocalteu method with some modification (Singleton and Rossi 1965). A 200 µL properly diluted sample or standard solution of varying concentrations was mixed with a 400 µL Folin–Ciocalteu reagent. The deionized water was used for dilution and control. The solution was diluted to a total volume of 4.6 mL using deionized water followed by thorough mixing. After incubation for 10 min at room temperature, 1 mL of 20% Na_2CO_3 solution was added followed by immediate thorough mixing and incubation for two hours. The absorbance was read at A_{765nm} on a spectrophotometer (TU-1800; Human Corporation). Measurements were recorded in triplicates. Gallic acid of 1 mg/mL was used as standard, and total phenolic compounds of the samples were expressed in milligram gallic acid equivalent per 100 mL (mg GAE/100 mL).

Analysis for Total Sugars

Total sugar contents of the grape juice samples were determined by taking 1 mL of appropriately diluted samples or standard solution of varying concentrations and mixed with 0.5 mL of 0.1 N HCl. The mixture was heated in a 100C water bath for 15 min, and then soaked with cooling water. After cooling, 0.5 mL of 0.1 N NaOH was added by mixing. The extracted sample solution was used for the analysis of the total sugar content using the Nelson's reducing sugar test (Hodge and Hofreiter 1962) with a slight modification as reported by Abdullahkasim *et al.* (2007). For the analysis, 1 mL extracted sample or standard solution was mixed with 1 mL alkaric copper reagent and heated in a 100C water bath for 15 min and then soaked in flowing water. After cooling, 0.5 mL arsenomolybdic reagent was added to the mixture followed by immediate mixing to dissolve the sediments. The mixture was diluted to a total volume of 12.5 mL with deionized water. After incubation for 30 min at room temperature, the absorbance at 500 nm was read using a spectrophotometer (TU-1800; Human Corporation). Deionized water was used as control. One percent of D-glucose solution was used for calibration at measuring ranges of 0.01–0.04%. All of the samples were measured in triplicates. The percent relative standard deviation was less than 10%. The total sugar contents of the samples were expressed in grams per serving of D-glucose.

Sensory Evaluation

The sensory evaluation of each grape juice samples for color, taste, aroma and overall acceptability was conducted by a panel of eight judges selected from the Department of Food Science and Technology at Kyungpook National University, Daegu, Korea. The judges scored each attribute for random samples on a scale of 1–9 in which 1 denotes dislike extremely and 9 stands for like extremely. The measurements were taken in triplicates by each evaluator, and the values were reported as mean scores with standard deviations of ± 1.

Data Analysis

Experimental data were analyzed using analysis of variance with significance defined at $P < 0.05$. Statistical analysis was carried out using Microsoft Excel (MS Office professional Edition 2003 by Microsoft Corporation, Redmond, VA) and Sigma Plot 10 (Systat Software Inc. San Jose, CA).

RESULTS AND DISCUSSION

Inhibition of PPO

Activity of PPO in the grape juice extracts obtained by heating grapes with the application of vacuum pressure of 200, 400 and 600 mmHg for 10, 15 and 20 min at 59C, 62C and 65C is represented in Fig. 1a–c after 20, 30 and 60 min of sample incubation time, respectively. In this study, catechol, a phenolic compound, was used as a substrate. Application of vacuum heating significantly ($P < 0.05$) affected the activity of PPO, and the enzyme activity decreased gradually with an increase in vacuum pressure. Maximum inhibition of the enzyme activity (82.15%) was observed in the grape extract while the grapes were heated at 65C for 20 min under 600 mmHg vacuum pressure, and the sample was analyzed for PPO activity after incubating at room temperature for one hour. However, the least inhibition of PPO (40.45%) was observed at heating grapes without the application of vacuum pressure at 59C for 10 min after 20 min of incubation at room temperature. The effect of heating temperature alone was found nonsignificant on the inhibition of PPO, whereas the effect of heating on the enzyme activity under vacuum was significant ($P < 0.05$).

Researchers have reported different mechanisms to inactivate PPO, which is considered to be the main contributor to browning, discoloration and darkening in fruits and vegetables (Rocha and Morais 2001). Mazzafera and Robinson (2000) found that the activity of PPO was optimum at 25C, and after applying a heating temperature of 76C for 10 min, it was minimal.

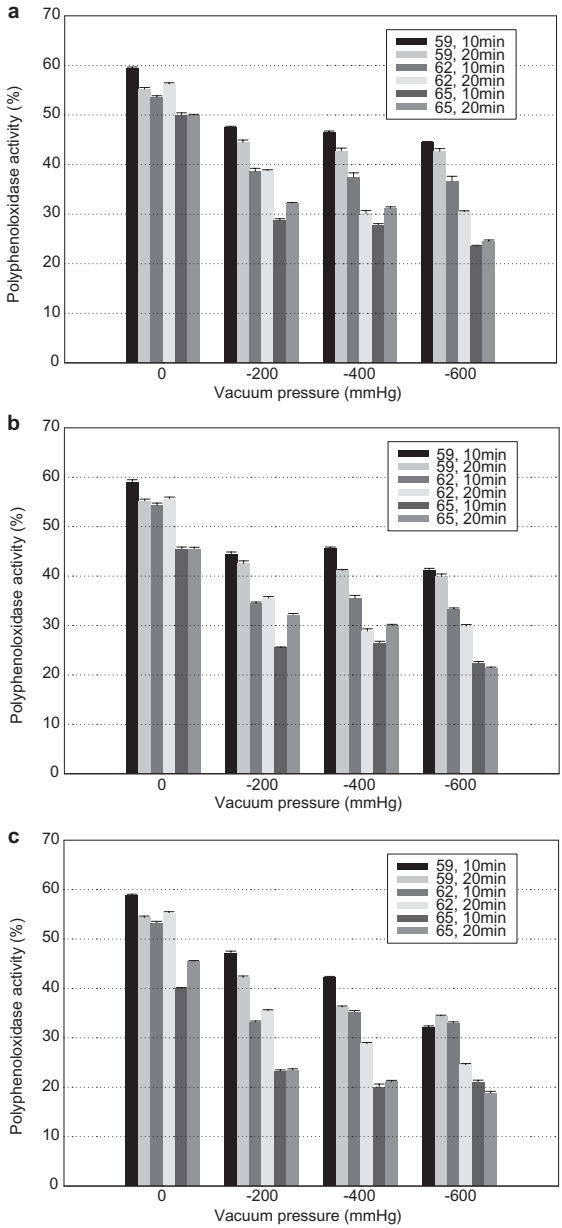

FIG. 1. POLYPHENOLOXIDASE ACTIVITY OF THE GRAPE JUICE AT DIFFERENT PROCESSING CONDITIONS (TIME, TEMPERATURE AND VACUUM PRESSURE) AFTER 20 (a), 40 (b) AND 60 (c) MIN OF INCUBATION OF THE SAMPLE
Bars represent standard error of the mean ($n = 3$).

Rapeanu *et al.* (2006a) observed a minimal activity of PPO after treatment at 60C and 800 MPa for 15 min. A loss of about 70% PPO activity was reported by Lamikanra *et al.* (1992) for "Welder" and "Noble" grapes at 60C for 30 min of heat treatment. The effect of temperature, holding time and their interaction was found to be significant on the inactivation of PPO in grape juice (Icier *et al.* 2008). However, the application of high temperature heating for a prolonged amount of time is not recommended. Mechanism by which inactivation of PPO may occur is pressure and heat-induced molecular and conformational changes in the secondary and tertiary structures of enzymes (Sun *et al.* 2002). In our studies, 82.15% of PPO in the "Campbell Early" grape juice can be inhibited by heating grapes at 65C for 20 min under lower vacuum pressure of 600 mmHg, and inhibition might have occurred by structural changes in the enzyme induced by vacuum heating of grapes. Besides the heating effect on enzyme activity, vacuum pressure enables to improve the quality of a product at comparatively lower temperature than the other techniques (Lewicki 2006).

Sensory Evaluation

The results of sensory evaluation of grape juice samples for color, aroma, taste and overall acceptability are represented in Table 1. Results reveal that there was significant increase in the sensory quality of grape juice when heated with the application of vacuum pressure. Higher scores for the sensory attributes of color, aroma, taste and overall acceptability were obtained by the grape juice samples prepared by treating grapes under vacuum pressure of 600 mmHg. Highest overall acceptability of grape juice was obtained under 600 mmHg vacuum heating for 20 min at 62C followed by 59C and 65C.

Conventional thermal processing does not contribute significantly toward enzyme inactivation if otherwise aided by the addition of chemical preservatives; however, these processes are associated with sensorial and nutritional losses. Therefore, use of alternative minimal processing techniques is recorded (Matsui *et al.* 2008). It was found that grape juice samples obtained by vacuum heating had higher acceptability for sensory properties of juice possibly because of increased extraction of components responsible for color, taste and aroma of grape juice.

Total Phenol Contents of Grape Juice

Total phenols of the samples at different extraction conditions are presented in Fig. 2. It shows that there was a more extraction of the phenolic compounds into the extract from grapes when they were heated at elevated temperatures and higher vacuum pressures. Both vacuum and heating temperatures significantly affected ($P < 0.05$) the extraction of phenolic

TABLE 1.
SENSORY EVALUATION OF GRAPE JUICE SAMPLES AT DIFFERENT PROCESSING CONDITIONS (VACUUM PRESSURE, TIME AND HEATING TEMPERATURE)

Sensory properties					
Sample number	Vacuum conditions (mmHg, min, C)	Color	Aroma	Taste	Overall acceptability
1	0, 10, 59	4.50	5.25	5.00	4.80
2	0, 10, 62	5.75	3.50	4.75	4.70
3	0, 10, 65	5.25	4.75	4.50	4.90
4	0, 20, 59	5.25	5.50	4.50	5.25
5	0, 20, 62	6.75	5.25	6.00	5.05
6	0, 20, 65	5.50	5.25	6.00	5.10
7	200, 10, 59	5.75	6.75	6.50	6.30
8	200, 10, 62	5.00	5.00	5.50	5.20
9	200, 10, 65	7.25	5.25	5.75	5.95
10	200, 20, 59	6.25	6.50	7.00	6.60
11	200, 20, 62	6.50	6.50	6.00	6.50
12	200, 20, 65	7.50	7.00	5.75	6.75
13	400, 10, 59	6.50	7.25	6.75	6.90
14	400, 10, 62	6.75	7.35	7.65	6.75
15	400, 10, 65	7.75	7.00	6.90	7.10
16	400, 20, 59	6.75	6.25	6.00	6.45
17	400, 20, 62	6.75	7.25	7.55	6.90
18	400, 20, 65	6.50	6.75	6.75	6.75
19	600, 10, 59	7.75	6.55	5.83	6.70
20	600, 10, 62	6.56	6.75	6.75	6.90
21	600, 10, 65	6.55	7.25	7.25	6.90
22	600, 20, 59	7.80	6.55	6.75	7.20
23	600, 20, 62	7.25	6.75	7.50	7.25
24	600, 20, 65	7.00	7.25	7.50	7.10

Mean sensory score (SD ± 1).

compounds in our study. A maximal phenolic content (3.65 mg GAE/100 mL) in the grape juice extract was observed at vacuum pressure of 600 mmHg when the grapes were heated at 65C for 20 min.

From a nutritional point of view, phenolic compounds in grape juice are important because of their health benefits and other functional properties (Pinelo *et al.* 2006). It has also been reported that there is a correlation between antioxidant activity and phenolic and anthocyanin levels of blueberry (Su and Silva 2006). Heating may help in the extraction of more phenolic compounds from the grapes, but higher temperature may degrade the functional properties of the extract (Larrauri *et al.* 1997). However, the application of vacuum heating to grapes significantly increased the extraction of heat-sensitive phenolic compounds, thus improving functional properties of juice.

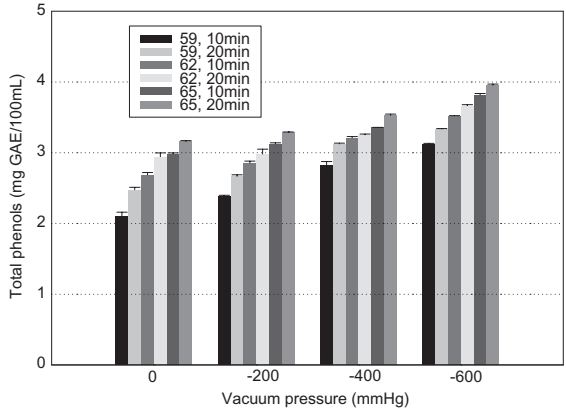

FIG. 2. TOTAL PHENOL CONTENTS OF THE GRAPE JUICE AT DIFFERENT PROCESSING CONDITIONS (TIME, TEMPERATURE AND VACUUM PRESSURE)
Bars represent standard error of the mean ($n = 3$).

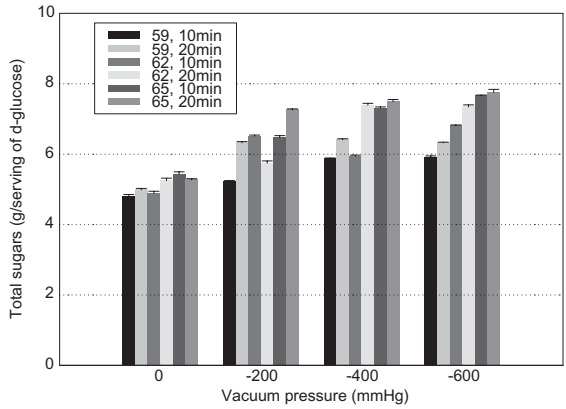

FIG. 3. TOTAL SUGAR CONTENTS OF THE GRAPE JUICE AT DIFFERENT PROCESSING CONDITIONS (TIME, TEMPERATURE AND VACUUM PRESSURE)
Bars represent standard error of the mean ($n = 3$).

Total Sugar Contents of Grape Juice

Total sugar contents of grape juice after heating the grapes under vacuum condition are presented in Fig. 3. A significant increase in sugar contents was observed after heating under vacuum conditions. The highest sugar contents were observed in the grape juice extract when the grapes were heated under 600 mmHg vacuum at 65C for 20 min. Total sugar contents in all the samples

heated under vacuum were higher than those of the samples extracted by the application of same heating temperatures for similar duration of time but without the application of vacuum. Vacuum pressure had a significant effect ($P < 0.05$) on the extraction of total sugars from the grapes.

CONCLUSIONS

Overall, our study shows that the activity of PPO was significantly reduced due to the combined effect of heating and vacuum pressure that also resulted in improved sensory characteristics and increased total phenolic compounds and total sugars in juice. Hence, vacuum heating of grapes can be used for processing of better quality juice at reduced temperature.

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