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The Effects of Vacuum Heating on the Functional Properties of Grape Juice

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Abstract

The effects of partial vacuum pressure on the functional properties of 'Campbell Early' grape juice were analyzed. It was observed that vacuum pressure beneficially affects these quality parameters in grape juice. Application of vacuum pressure significantly affected ($p<0.05$) the antiradical potential, antioxidant activity, inhibition of polyphenoloxidase and extractive total phenols in the grape juice. There were significant increases in the antiradical activity, antioxidant activity and total phenols content of grape juice whereas polyphenoloxidase activity was significantly inhibited.

Key words: vacuum heating, grape juice, antiradical, antioxidant, polyphenoloxidase, total phenol

Introduction

As consumption of grape juice and grape juice products is increasing (Morris, 1987), researches about grape juice and other grape products are accordingly being conducted by many researchers (Morris, 1989). Various processing methods have been studied for the improvement of quality of grape juice in terms of its functional properties with particular reference to the phenolic contents which contribute not only to the color, flavor and stability of the end product (Netzel et al., 2003) but also towards the antioxidant and antiradical potential of grape juice (Patrineli et al., 1996). During the manufacturing process, grape juice is undergone different treatments which affect the components of juice. Quality defects in grape juice come from collateral reactions during manufacturing process and these reactions are more frequent when the product temperature increases (Teresa et al., 2007). Extraction temperature plays a critical role in preserving the quality and influences juice color by decreasing the activity of degrading enzymes, however some volatile components in grape juice are also sensitive to heat (Threlfall et al., 2005). The main step in enzymatic browning is the oxidation of phenolic compounds to corresponding quinone intermediates that polymerizes to form undesirable pigments and the reactions are mediated by polyphenoloxidase (PPO) in the presence of oxygen. (Rapeanu et al., 2006). Investigations to determine the characteristics of grape PPO and the conditions under which PPO is most active have been widely reported for some grape cultivars (Weemaes et al., 1998). Similarly research in natural antioxidants, especially of plant origin, has greatly increased in recent years (Jayaprakasha & Jaganmohan, 2000).

Processing methods for extraction of juice from grapes may affect functional components in juice. Antioxidants including vitamins, flavonoids, and phenols represent one group of nutraceuticals found in grapes (Threlfall et al., 2005). Natural antioxidants can protect the human body from free radicals that may cause some chronic diseases including cancer, cardiovascular diseases and cataract (Lai et al., 2001). The antioxidant properties of plant extracts have been attributed to their polyphenol contents (Lu & Foo, 2001); therefore plants having high level of phenols have a great importance as a source of natural antioxidants (Baydar et al., 2007). It is of a general belief that the thermal treatments can affect composition and physiological characteristics of juices (Cerdan et al., 2006); therefore it is important to study the affect of heat treatment on the important functional characteristics of grape juice. The goal of this study is to assess the effect of vacuum heating on the quality of grape juice in terms of antioxidant potential, antiradical activity, total phenol contents and activity of polyphenoloxidase.

Materials and Methods

Juice Processing

Grapes 'Campbell Early' grown at a local farm in Kyungbuk province of Korea were used to extract juice. Grapes were de-stemmed and grape berries with sound external appearance

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with optimum ripeness were selected and washed. A small scale machine by H. S. Co. Daegu, Korea specifically designed for heat treatment of grapes under vacuum pressure was used. It consisted of a heating vessel surrounded by water circulation tubes for hot water circulation controlled with a circulator water bath (MCB-3011D; M. E. Co., Daegu, Korea). Vacuum inside the vessel was created by sealing and connecting the heating vessel with a vacuum pump (Model 4001; S. D. Co., Daegu, Korea). Grapes were heated at different temperature and once the desired temperature (59~65°C) was achieved, vacuum pressures of 200, 400 and 600 mmHg were applied for 20 min. In case of control heating was carried out under atmospheric conditions i.e. without the application of vacuum pressure. Afterwards grapes were pressed with the help of cheese cloth to extract the juice followed by over night cold settling of tartartes before analysis of the samples for different quality characteristics.

Determination of antiradical activity

The free radical activity of the grape juice was determined by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Lee et al., 1998). Briefly, 1 mL solution of the juice extract at a concentration of 100 μL/mL methanol was mixed with 2 mL of 10 mg/L methanolic solution of DPPH (Sigma Chemical Co., St. Louis, MO, USA). The mixture was shaken vigorously and allowed to stand at room temperature for 5 min and absorbance was recorded at A517 nm by using a spectrophotometer (TU-1800; Human Corporation, Seoul, Korea). Lower absorbance of the sample indicated the higher free radical scavenging activity. The antiradical activity of the grape juice extract was expressed in percentage.

Determination of antioxidant activity

The antioxidant activity of the grape extracts was evaluated by phosphomolybdenum complex method (Prieto et al., 1999). In brief, 0.4 mL of sample solution (100 μL/mL methanol) was combined with 4 mL of phosphomolybdenum complex containing 0.6 M sulphuric aicd, 2 mM sodium phosphate and 4 mM ammonium molybdate (Sigma Chemical Co.). Test tubes were caped and placed in hot water for 90 min at 95°C. Samples were cooled to room temperature absorbance was measured at A_{695}nm. Antioxidant activity was expressed as mg/mL of grape juice.

Determination of polyphenoloxidase activity

Polyphenolsoxidase activity of grape extract was determined by a method described by Rapeanu et al. (2006) with some modification. A sample of 5 mL juice was taken and 10 mL of acetone (Sigma Chemical Co.) was added followed by stirring. The sample was filtered and to the filtrate 50 mL of 0.1 M citric acid (Sigma Chemical Co.) were added. The sample was stirred and after cooling 2 mL of the sample was taken in the test tube and 4 mL of 0.1 M catechol (Sigma Chemical Co.) solution was added followed by shaking. Absorbance was measured at A_{360}nm after incubating the samples at room temperature for 80 min.

Analysis for total phenolic compounds

The total phenolic compounds in the study samples were analyzed using Folin Ciocalteu method with some modification (Singleton and Rossi, 1965). A 200 μL properly diluted sample or standard solution at various concentrations were mixed with 400 μL Folin Ciocalteu reagent (Sigma Chemical Co.) and 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 (Sigma Chemical Co.) was added followed by immediate thorough mixing and incubation for 2 hr. The absorbance value was recorded at A_{765}nm using spectrophotometer (Human Corporation). Measurements were taken in triplicates. Gallic acid (Sigma Chemical Co.) 1 mg/mL was used as standard and total phenolic compounds of the samples were expressed in milligram gallic acid equivalent per 100 mL (mgGAE/100 mL).

Data analysis

Experimental data were analyzed by using analysis of variance (ANOVA) with significance defined at $p<0.05$. Statistical analysis was done by using Microsoft Excel (MS Office Pofessional Edition 2003 by Microsoft Corporation, Seatle, USA) and Sigma Plot 10 (Systat Software Inc. San Jose, CA, USA).

Results and Discussion

Antiradical activity of grape juice extract

Fig. 1 represents the radical scavenging activity in the juice obtained from grapes after heating for 20 min at 59, 62 and 65°C under different vacuum pressures. Vacuum pressure and mild heating have had significant effects ($p<0.05$) on the radical scavenging capability of grape juice. It was observed from the results that anti radical activity of the samples generally increased with the increase in vacuum pressure. The highest antiradical activity (89.5%) was however observed in the treatment whereby grapes were heated at temperature of 62°C and a 600 mmHg vacuum pressure.

Antioxidant activity of grape juice

Antioxidant value of grape juice extract was represented in

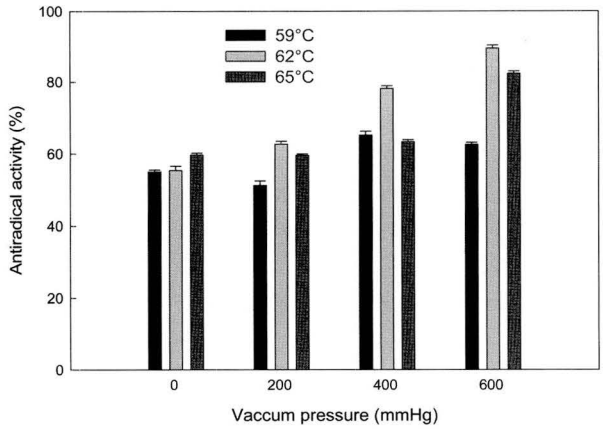

Fig. 1. Antiradical activity percentage of the grape extracts at different pre-extraction treatments. Bars represent standard error of the mean (n=3).

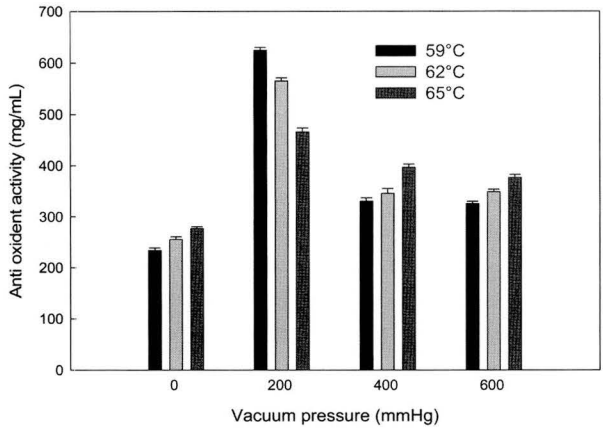

Fig. 2. Antioxidant activity of the grape extracts at different pre-extraction treatments. Bars represent standard error of the mean (n=3).

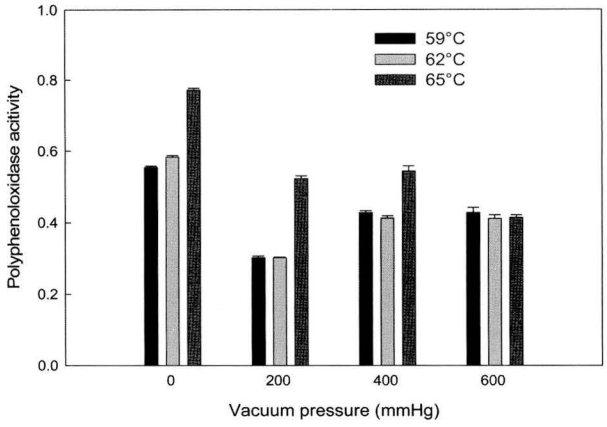

Fig. 3. Polyphenoloxidase activity of the grape extracts at different pre-extraction treatments after 80 minutes of incubation of the sample. Bars represent standard error of the mean (n=3).

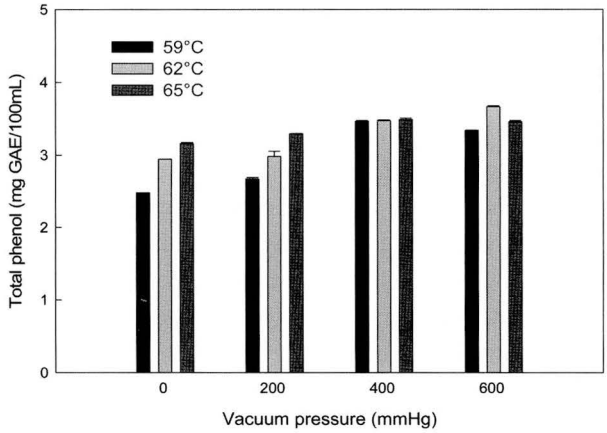

Fig. 4. Total phenol contents (mg GAE/100 mL) of the grape extracts at different pre-extraction treatments. Bars represent standard error of the mean (n=3).

the Fig. 2. Optimal antioxidant value was observed at heating temperature of 59°C under 200 mmHg vacuum pressure. Vacuum pressure and mild heating have had significant effect ($p<0.05$) on the antioxidant potential of grape juice. Generally higher antioxidant values were observed by heating grape juices under 200 mmHg vacuum pressure.

Inhibition of polyphenoloxidase activity

Inactivation of polyphenoloxidase in grape juice by heating grapes at various extraction conditions is represented in Fig. 3. Application of vacuum pressure and heating significantly affected the activity of polyphenoloxidase and it was found to decrease gradually with the increase of vacuum pressure. Maximum inhibition of the enzyme was observed in the grape juice heated at 59°C and 62°C under 200 mmHg of vacuum pressure whereas least inhibition of polyphenoloxidase activity was observed while heating grapes without the application of vacuum pressure i.e. under atmospheric conditions. The effect of heating temperature alone was found non significant on the inhibition of polyphenoloxidase however that of heating under vacuum was significant.

Total phenol contents of grape juice

Total phenol contents of the samples from different pre-extraction treatments are presented in the Fig. 4. It shows a higher extraction of the phenolic compounds into the extract from grapes while they are heated at elevated temperatures and vacuum pressures. Both degrees of vacuum and temperature of heating significantly effected ($p<0.05$) the extraction of phenolic compounds in our study. Maximum phenolic contents (3.66 mgGAE/100 mL) in the grape juice extract were observed at vacuum pressure of 600 mmHg while grapes were heated at

62°C for 20 min.

Generally the antiradical and antioxidant properties of the grape extracts are ascribed to the phenolic contents (Cabrera & Moon, 2007; Lu & Foo, 2001; Revilla & Ryan, 2000). Free radical scavenging activity depends on the structural conformation and amount of phenolic compounds, thus phenolic composition greatly influence the antiradical activity (Larrauri et al., 1997). It has also been reported that there is a correlation between antioxidant activity and phenolic levels of the samples (Duh, 1999; Park et al., 2005). Processing may effect the phenolic compounds as reported by Hamam & Nawar, (1991) that higher temperatures may help in the extraction of more phenolic compounds from the grapes however it can also degrade heat sensitive functional components. In our results we have observed that heating grapes at low temperature under vacuum conditions beneficially affects these functional characteristics in the grape juice. Vacuum heating also allows us to achieve the desired objectives of grape juice quality at reduced temperature thereby helping in preservation of heat sensitive volatile components.

Heating treatment is often used to deactivate certain enzymes in fruits and vegetables, as these enzymes aid in enzymatic browning reactions during different processing operations and storage (Yemenicioglu & Cemeroglu, 1998). Polyphenoloxidase catalyze the oxidation reaction of o-phenolic substrates into o-quinones which subsequently polymerizes into dark pigments. This enzyme is widely distributed in plants and it is considered to be the main contributor to browning, discoloration and darkening in fruits and vegetables (Billaud et al., 2004). Enzymatic browning can be inhibited by using chemicals such as ascorbic acid, sulphites, sodium diethyl dithiocarbamate, and heat treatment (Kim et al., 2005). It is suggested that heat deactivation treatments should be rapid since slow heating processes might result in activation of the PPO in the plant tissue rather than deactivation (Tate et al., 1964). Therefore heating grapes under vacuum and reduced temperature enables us to preserve the quality of grape juice and also aids in extraction of more functional components in grape juice. As we have already discussed, the antioxidant and free radical scavenging activities of grape juice are related to the phenolic contents of grape juice and thus any change in phenolic composition will also affect the functional properties of juice (Lu & Foo, 2001) such as antioxidant and antiradical potential. We have observed in our study that the extraction of more phenolic compounds resulted in increase in the antiradical and antioxidant activities of grape juice. We observed that the activity of polyphenoloxidase was significantly reduced and antioxidant and antiradical potential of juice extract significantly increased by heating grapes with the application of vacuum pressures; therefore this process can have a potential application for the prevention of enzymatic browning, enhancement of functional characteristics and processing of higher quality grape juice and grape products. By heating under partial vacuum or reduced pressure we can increase the extraction of functional components from grapes into juice which are otherwise heat sensitive.

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