Food Sci. Biotechnol. Vol. 17, No. 1, pp. 80 ~ 84 (2008)

Food Science and Biotechnology

Effects of Gellan, Xanthan, and λ-Carrageenan on Ellagic Acid Sedimentation, Viscosity, and Turbidity of 'Campbell Early' Grape Juice

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Abstract The effect of gellan (GE), xanthan (XA), and λ-carrageenan (LC) on the viscosity, sedimentation, ellagic acid content, and turbidity of grape 'Campbell Early' juice (CEJ) was investigated. CEJ samples with 0.15% each of GE, XA, and LC were tested for the above variables after 0, 5, 10, and 20 days of storage. The samples containing GE (0.15%) showed the least amount of sediment formation, the lowest ellagic acid content and turbidity, and a rise in viscosity. Sedimentation in CEJ decreased with increased viscosity due to the addition of gums which also limited the ellagic acid content and turbidity. GE was the most effective additive for the stabilization of CEJ.

Keywords: grape juice, viscosity, sedimentation, ellagic acid, turbidity

Introduction

Grapes are one of the important food crops in the South Korea and according to the FAO data for 2005, grape production in Korea was 360,000 metric tons (1). The areas under grape cultivation have been gradually increasing since 1994, and among the grape cultivars grown in Korea 'Campbell Early' maintains a primary position. Grape juice is a representative processed product of grapes, and one of the problems associated with grape juice is the precipitation of acids during transportation and storage. Research is being done to formalize methods for controlling precipitation in grape juice (2). Consumption of grape juice and grape juice products has continued to increase (3) because grape juice is considered to be a healthy food by consumers (4). The popularity of grape juice has motivated a great deal of research into improving its quality. Sediments are considered quality defects in grape juice and their presence may influence consumer acceptability, being a visual deterrent to consumers thus discouraging its purchase.

Gellan gum was allowed as a new food additive by the FDA in 1992, and is famous for its excellent gelling ability at low concentration (5). It is an extracellular polysaccharide produced by *Sphingomonas elodea* fermentation that is a good stabilizer and affects the turbidity and viscosity of reconstituted carrot juice. (6). Xanthan is an extracellular heteropolysaccharide that is widely used in food industries for its thickening and stabilizing properties, and for its unusual stability with regard to temperature, ionic strength, pH, enzymatic and chemical reactivity (7). The viscosity of xanthan solutions is relatively high which partially accounts for its excellent stabilizing properties (8). Carrageenans are biopolymers which are extracted from seaweeds. These are used in the food industry for the gelation and stabilization of food emulsions such as milk with fat, ice cream, and chocolate milk. There are 3 main types of carrageenans, kappa (κ), iota (ι), and lambda (λ), which are linear polysaccharides and differ only in the number of sulfate groups per disaccharide. λ-Carrageenan has 3 sulfate groups and adopts a coiled conformation depending on the ionic and temperature conditions, however it is unable to form gels and is mostly used as a thickener (9).

Sediments formed in grape juice have been analyzed and found to contain ellagic acid (10). Sediments in the juice can result in increased turbidity and require various treatments such as ultrafilteration, treatment with fining agents like polyvinylpolypropyrrolidone (PVPP), egg albumin, gelatin, and casein that have been applied for the quantification and reduction of ellagic acid sediments in grape juice (11). In carrot and apple juice, hydrocolloids have also been used for stabilization by increasing the viscosity and reducing sediments (6,12).

The objectives of the present study were to investigate the effects of gellan, xanthan, and λ-carrageenan on the viscosity, sedimentation, turbidity, and ellagic acid content of grape 'Campbell Early' juice during storage in order to assess the relationship of these quality parameters and their overall effect on the stability of grape juice. We aim to reduce ellagic acid sediments and the turbidity of grape juice by raising its viscosity.

Materials and Methods

Preparation of grape juice Grapes 'Campbell Early' grown in Kyungbuk province of South Korea were used in making grape juice at a local grape juice factory named Kyungpook PhottoMal, Yeongcheon, Korea. The process of making grape 'Campbell Early' juice (CEJ) included the removal of unwanted materials, washing, crushing, heating in a steam jacket (60 to 70°C for 20 min), screw pressing, storage at 0°C (30 days), filtration, bottling, pasteurization (70°C for 20 min), cooling, and storage at 0°C. The processed juice had a °Bx of 15° and an acid value of 0.448 g/L.

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Received June 14, 2007; accepted August 1, 2007

Preparation of hydrocolloid solutions Xanthan (XA), gellan (GE), and λ-carrageenan (LC) were purchased from a local chemical supplier (MSC Company, Daegu, Korea). The gums were dispersed in a small quantity of deionized water with a magnetic stirrer (KMC 130SH; Vision Scientific Co., Ltd., Daegu, Korea) and heated for 2 hr in a 70°C water bath (LCB-6D; Daihan Labtech Company Ltd., Namyangju, Gyeonggi, Korea). The concentration of each hydrocolloid in solution was maintained at 1%(w/v).

Mixing of CEJ and hydrocolloids The grape juice was first homogenized at 6,000×g for 15 min by using a homogenizer (Ace-AM; Nihonseiki Kaisha Ltd., Tokyo, Japan). Test samples were made by adding GE, XA, and LC in CEJ to 0.15%(v/v). The juice was stirred at room temperature to completely mix the gums with the juice. Finally, the juice was kept in a sonifier (JAC ultrasonic 2010P; Jinwoo Eng. Co., Ltd., Hwasung, Gyeonggi, Korea) for 3 min and stored at 10°C for analysis of sediments, viscosity, turbidity, and ellagic acid at 0, 5, 10, and 20 days of storage.

Sedimentation Total sediments (g/100 mL) were determined by filtering CEJ samples through previously weighed #5A filter papers. Filter papers with sediments were dried overnight at 50°C and the weight of the sediments was calculated by subtracting the weight of filter paper.

Determination of viscosity The viscosity of CEJ was determined at room temperature by using a viscometer (DV-II; Brookfield Eng Labs. Inc., Stoughton, MA, USA) with spindle No. 1 at a spindle speed of 100 rpm, and expressed as 10^3 Pa·sec.

Quantification of ellagic acid The ellagic acid concentration in CEJ was determined by a simplified quantitative method (10) by diluting a 10 μL sample of CEJ in 1 mL of 10 mM Tris-HCl (Sigma Chemical Co., St. Louis, MO, USA), pH 7 and 1 mM EDTA (Sigma Chemical Co.). The absorbance value at A_{255nm} was determined using a spectrophotometer (TU-1800; Human Corporation, Seoul, Korea). The concentration of ellagic acid in μg/mL of grape juice was calculated by the following formula,

$$\text{Concentraton of ellagic acid } (\mu g/mL) = \frac{(OD - 0.0074)}{0.0050}$$

Turbidity evaluation The turbidity of CEJ samples was analyzed using a spectrophotometer (TU-1800; Human Corporation) by measuring the optical density (OD) of diluted 5 mL samples of CEJ at A_{650nm}.

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 Exel (MS Office professional Edition 2003 by Microsoft Corporation, Redmond, WA, USA) and Sigma Plot 10 (Systat Software Inc., San Jose, CA, USA).

Results and Discussion

The effect of gums and storage time on the viscosity of CEJ The effect of 3 different types of gums and storage time on the viscosity of CEJ is shown in Fig. 1. Gums had a significant effect ($p<0.05$) on the viscosity of CEJ relative to the control sample. GE and XA had a more significant effect on the viscosity of CEJ than LC. With the increase of storage time from 0 to 20 days, CEJ samples with GE and XA showed an increase in viscosity with the maximum viscosity observed on the 20th day of storage. Among all the samples, XA showed the greatest increase in viscosity which may be attributed to its higher molecular weight (13).

The effect of gums and storage time on sedimentation in CEJ The effect of hydrocolloids and storage time on the formation of sediments in 4 samples of CEJ is illustrated in the Fig. 2. The highest amount of sediments was seen in the control sample after 20 days of storage, whereas after the same period less sediment were seen in CEJ with GE or XA, with 0.2885 and 0.2930 g of sediments, respectively. This indicates that the addition of 0.15% GE in CEJ had the maximum effect on preventing sediment formation in CEJ.

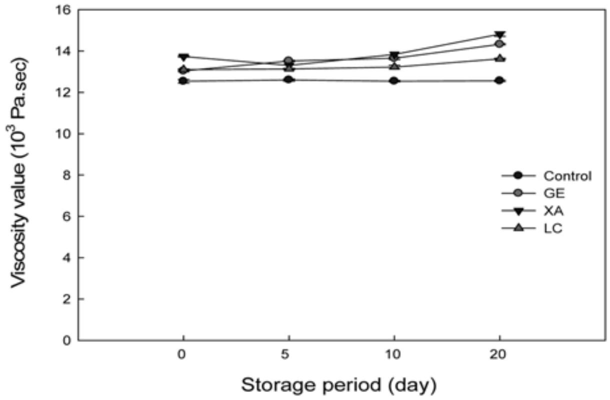

Fig. 1. Effects of gums and storage time on the viscosity of CEJ. Bars represent standard error of the mean (n=3).

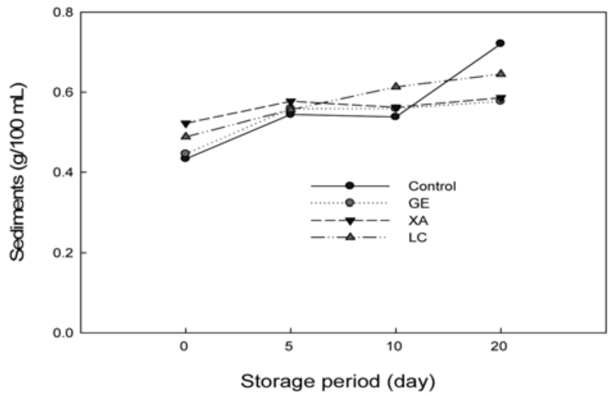

Fig. 2. Effects of gums and storage time on the formation of sediments in CEJ.

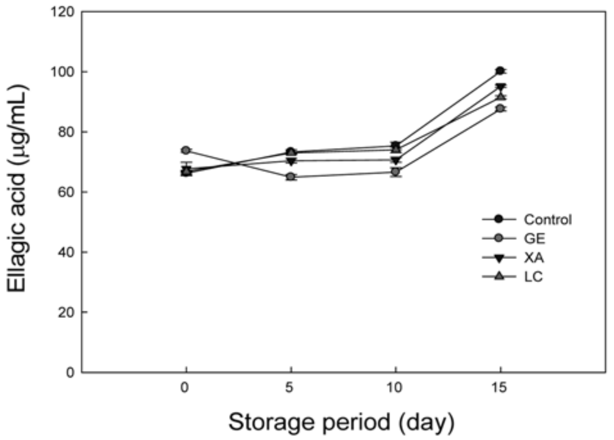

Fig. 3. Effects of gums and storage time on ellagic acid levels in CEJ. Bars represent standard error of the mean (n=3).

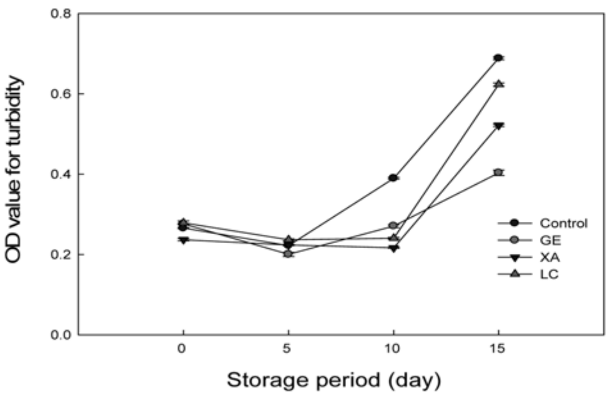

Fig. 4. Effects of gums and storage time on the turbidity of CEJ. Bars represent standard error of the mean (n=3).

The effect of gums and storage time on the ellagic acid content of CEJ Ellagic acid levels in grape juice are closely related to the occurrence of sediments, therefore the effect of gums and storage time on ellagic acid in CEJ was investigated. Figure 3 shows that the addition of GE, XA, and LC to CEJ significantly decreased the amount of ellagic acid compared to the control ($p<0.05$). An increase in the ellagic acid contents of all CEJ samples was observed on the 20th day of storage, however the samples containing hydrocolloids had a lower ellagic acid content than the control, with a minimum of 87.58 μg/mL due to the addition of GE. These results reveal a significant effect ($p<0.05$) of gums and storage time on the ellagic acid content of CEJ.

The effect of gums and storage time on the turbidity of CEJ Figure 4 illustrates the effect of hydrocolloids and storage time on the turbidity of CEJ. The type of gum significantly affected the turbidity of CEJ ($p<0.05$). CEJ samples with GE showed minimal turbidity with OD values of 0.200 and 0.403 on the 5th and 20th days of storage, respectively. All CEJ samples after 20 days of storage showed an increase in turbidity, however samples with added GE, XA, and LC had OD values of 0.403, 0.521, and 0.623, respectively, which were lower than the control which had an OD value of 0.688. The loss of turbidity may be attributed to the formation of sediments in CEJ during storage. Ellagic acid is formed in grape juice as a result of the hydrolysis of ellagic tannins (14). The sediments (yellowish to red crystals) formed in grape juice were found to be composed of ellagic acid which is a phenolic compound found in several fruits such as grapes and strawberries (10). Precipitates were formed during the storage of clarified and pasteurized 'Mangolia', 'Carlos', and 'Muscandine' juices, and analysis of the juice sediments revealed the presence of ellagic acid (15). Similarly, evergreen black berry juice sediments consist of ellagic acid, protein, and unidentified compounds (11). The phenolic composition of grape juice can be significantly affected by processing and storage conditions, and several treatments are known to reduce phenolic levels in grape juice (12). Gelatin and ployvinylpolypyrrolidone were found to have a lowering effect on ellagic acid levels in 'Muscandine' grape juice (13). In another study (16) it was found that the treatment of grape juice with ployvinyl-polypyrrolidone (0.1-0.2 g/L juice), egg albumen (6-10 mg/L juice), and gelatin (0.05-0.4 g/L juice) resulted in a significant reduction of juice phenolics and sediment formation. In our experiments the amount of sediments gradually increased in the control samples, and the quantity of ellagic acid was also found to be higher with increased storage time. However, juice samples with gellan gum showed the least amounts of both sediments and ellagic acid as shown in Fig. 2 and 3, respectively.

Another factor which accounts for the stability of the dispersion system is higher viscosity (17). In our experiments the addition of GE, XA, and LC to CEJ resulted in an increase in the viscosity of juice compared to the control sample, and according to Stokes law the sedimentation rate of particles in the juice is inversely related to its viscosity. Therefore we can assume that higher juice viscosity may increase the stability of juice. This hypothesis seems to be true with regard to the viscosity of the control CEJ which was lowest after 20 days of storage, corresponding with the highest sediment levels (0.360 g/100 mL) among all samples. In this study we observed that the viscosity of samples containing XA was higher (0.0148×10^3 Pa·sec) and the sediment levels were lower (0.293 g/100 mL). In the case of juice containing GE (0.15%), the sediment levels even lower (0.288 g/100 mL) and the observed viscosity on the 20th day of storage was 0.0143×10^3 Pa·sec.

Cloudiness in juices is largely due to the presence of suspended particles (18), and the higher the quantity of sediments, the higher the turbidity of the juice (19). We observed that the addition of hydrocolloids to CEJ changed the turbidity of grape juice samples. Grape juice samples containing less sediment showed lower values of turbidity whereas higher sediment levels increased the turbidity value of CEJ. Juice samples containing GE had the least amount of sediments while the turbidity (0.404) was also the lowest after storage for 20 days, whereas the control sample contained the most sediments was the most turbid (0.688). These results are in agreement with an earlier study (6) which found that GE at a concentration of 0.02% in carrot juice resulted in the least amount of pulp, white sediments, and lower juice turbidity.

XA, GE, and LC are negatively charged ionic polysaccharides (20), which accounts for the decrease of sediments after the addition of negatively charged polysaccharides. In a previous study (18), it was found that the electronegative carboxymethylcellulose (CMC) had a greater stabilization effect on cloudy apple juice. Electronegative Na-alginate was found to have a preventive effect on sedimentation in combined vegetable juice (21). Due to negative charges on juice particles, the electronegative hydrocolloids when added to the juice will increase electrostatic repulsive forces between juice particles to obtain stable juice (17).

We can assume that the prevention of sediments in CEJ by GE (0.15%) was caused by the combined effect of electrostatic repulsion and higher viscosity, and this in turn resulted in decreased ellagic acid and turbidity values in CEJ. When dissolved in water at higher temperature and cooled, GE formed double helices which aggregated to form junction zones and finally formed a 3-dimensional network structure with mono or divalent cations (22). It was found (23) that certain cations, such as K^+, Cs^+, and Ca^{++}, form networked structures with double helices, and that Ca^{++} had the maximum capacity to induce these structures followed by Cs^+. Recently, confocal laser scanning microscopy (CLSM) has also confirmed such network formation in GE (0.005%) systems containing Ca^{++} (24). They (25) also observed that increasing the concentration of GE as high as 0.5% will result in a massive and intricate network structure. Grape juice could donate Ca^{++} cations (25) for the formation of 3-dimensional networks with GE double helices. These stabilizing properties of GE are similar to other gelling agents e.g., alginate (26). Various gums have been reported to improve rheological properties and increase viscosity in other food dispersions resulting in better stability of food products (27,28). In this study we used 0.15% GE in CEJ which can potentially form a strong enough network for the suspension of particles and prevent their precipitation. This leads us to infer that GE has a good stabilizing effect on CEJ by reducing the precipitation of sediments hence lowering the ellagic acid content and turbidity of the grape juice. The addition of 0.15% each of GE, XA, and LC to CEJ could affect the physical and chemical variables of juice including viscosity, ellagic acid content, sedimentation, and turbidity. However despite the increase in viscosity of CEJ due to XA, minimal sedimentation was observed with the addition of GE (0.15%) to samples which also resulted in lesser turbid juice with lower ellagic acid content. The improved stability of CEJ due to GE may be a result of the increase in viscosity, control of sedimentation by the formation of network structures with cations, and electro-static repulsion between molecules of GE and ellagic acid in CEJ. GE (0.15%) proved to be most effective for the stabilization of CEJ and can be recommended as a suitable stabilizer for grape juice.

Acknowledgments

This study was supported through Grape Research Projects' Group by Agriculture R&D Promotion Center, Ministry of Agriculture and Forestry, Korea.

References

1. Food and Agriculture Organization of the United Nations. FAOSTAT Crop Production Data. Available from: http://faostat.fao.org. Accessed June 1, 2007.
2. Kim JS, Yook C. Color and flavor changes of grape juices stored in a cool and dark place for long-term preservation. Food Sci. Biotechnol. 12: 439-444 (2003)
3. Morris JR. Grape Juice: Influences of pre harvest, post harvest practices on quality. pp. 129-175. In: Quality Evaluation of Fruits and Vegetables. Pattee H (ed). AVI Publishing Company, Westport, CN, USA (1987)
4. Teresa GC, Margaluz AG, Robert MF, Carmen AA, Olga MB. Effects of thermal and non-thermal processing treatments on fatty acids and free amino acids of grape juice. Food Control 18: 473-479 (2005)
5. Pszczola DE. Gellan gum wins IFT's food technology industrial achievement award. J. Food Technol. 47: 94-96 (1993)
6. Chulin L, Xianosong H, Yuanying Ni, Jihong W, Fang C, Xiaojun L. Effect of hydrocolloids on pulp sediment, white sediment, turbidity, and viscosity of reconstituted carrot juice. Food Hydrocolloid 20: 1190-1197 (2006)
7. Speers RA, Tung MA. Concentration and temperature dependence of flow behavior of xanthan gum dispersions. J. Food Sci. 51: 96-98 (1986)
8. Zhan XB. Production, Properties, and Application of Food Hydrocolloids (in Chinese). China Light Industry Press, Beijing, China. pp. 8-9 (2003)
9. Rodd AB, Davis CR, Dunstan DE, Forrest BA, Boger DV. Rheological characterisation of 'weak gel' carrageenan stabilized milks. Food Hydrocolloid 14: 445-454 (2000)
10. Boyle JA, Hsu L. Identification and quantitation of ellagic acid in 'Muscandine' grape juice. Am. J. Enol. Viticult. 41: 43-47 (1990)
11. Siriwoharn T, Wolstad RE, Durst RW. Identification of ellagic acid in blackberry juice sediment. J. Food Sci. 70: C189-C197 (2005)
12. Matcheix JJ, Sapis J, Fleuriet A. Phenolic compounds and polyphenoloxidase in relation to browning in grape and wines. Crit. Rev. Food Sci. 30: 441-486 (1991)
13. Bagley EB. Mechanistic basis of rheological behavior of foods. pp. 573-595. In: Physical Chemistry of Foods. Schwartzberg HG, Richard WH (eds). Marcel Dekker Inc., New York, NY, USA (1992)
14. Lee JH, Talcott ST. Ellagic acid and ellagitannins affect on sedimentation in 'Muscandine' juice and wine. J. Agr. Food Chem. 50: 3971-3976 (2002)
15. Lin TY, Vine RP. Identification and reduction of ellagic acid in 'Muscandine' grape juice. J. Food Sci. 55: 1607-1613 (1990)
16. Garrido VM, Sims CA, Marshall MR, Bates RP. Factors influencing ellagic acid precipitation in 'Muscandine' grape juice during storage. J. Food Sci. 58: 193-196 (1993)
17. Genovese DB, Lozano JE. The effect of hydrocolloids on the stability and viscosity of cloudy apple juices. Food Hydrocolloid 15: 1-7 (2001)
18. Okoth MW, Kaahwa AR, Imungi JK. The effect of homogenization, stabilizer, and amylase on cloudiness of passion fruit juice. Food Control 11: 305-311 (2000)
19. Singleton VL, Marsh GL, Coven M. Identification of ellagic acid as a precipitate from loganberry wine. J. Agr. Food Chem. 14: 5-8 (1966)
20. Wang Z, Xu SY, Tang J. Food Chemistry (in Chinese). China Light Industry Press, Beijing, China. pp. 81-82 (1999)
21. Zhang M, Li C, Cao P. Effects of processing conditions of the green-leafy vegetable juice enriched with selenium on its quality stability. J. Food Eng. 62: 393-398 (2004)
22. Chandrasekaran R, Radha A. Molecular architectures and functional properties of gellan gum and related polysaccharides. Trends Food Sci. Tech. 6: 143-148 (1995)
23. Ikeda S, Nitta Y, Temsiripong T, Pongsawatmanit R, Nishinari K. Atomic force microscopy studies on cation-induced network formation of gellan. Food Hydrocolloid 18: 727-735 (2004)

24. Rodríguez-Hernández AI, Durand S, Garnier C, Tecante A, Doublier JL. Rheology-structure properties of gellan systems: Evidence of network formation at low gellan concentrations. Food Hydrocolloid 17: 621-628 (2003)
25. Olalla M, Gonzalez MC, Cabrera C, Gimenez R, Lopez MC. Optimized determination of calcium in grape juice, wines, and other alcoholic beverages by atomic absorption spectrometry. J. Assoc. Off. Ana. Chem. Int. 85: 960-966 (2002)
26. Zuo L, Lee JH. Application of statistical experimental design to improve the quality of fresh-cut apple cubes by edible coating with alginate. Food Sci. Biotechnol. 15: 825-832 (2006)
27. Chun SY, Kim HI, Yoo BS. Effect of gum addition on the rheological properties of rice flour dispersions. Food Sci. Biotechnol. 15: 589-594 (2006)
28. Yoo BS, Shon KJ, Chang YS. Effect of guar gum on rheological properties of acorn flour dispersions. Food Sci. Biotechnol. 14: 233-237 (2005)