

CHANGES IN TURBIDITY, TOTAL PHENOLIC AND ANTHOCYANIN CONTENTS OF CLEAR RED GRAPE JUICE DURING PROCESSING

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Abstract

This study evaluates the changes in total phenolic content, total anthocyanin content and turbidity with respect to various processes that are used during the clear red grape juice production. Advanced clarification of grape juice was carried out by ultrafiltration using 50 kDa cut-off membrane. Ultrafiltration caused significant lowering in total phenolic content and turbidity value as 30.9 and 99.0 %, respectively. Only 10.5 % total anthocyanin content were lost during the process.

Keywords: Clear red grape juice, ultrafiltration, mash heating, total phenolic content, turbidity

BERRAK SİYAH ÜZÜM SUYUNUN BULANIKLIK DÜZEYİNDE VE TOPLAM FENOLİK MADDE VE ANTOSİYANİN İÇERİĞİNDE İŞLEME SIRASINDA MEYDANA GELEN DEĞİŞİMLER

Özet

Bu çalışma, berrak siyah üzüm suyu üretimi sırasında kullanılan çeşitli prosesler açısından toplam fenolik madde ve toplam antosiyanin içeriği ile bulanıklık düzeyindeki değişiklikleri değerlendirmektedir. Üzüm suyunun ileri düzeyde berraklaştırma işlemi 50 kDa ayırma sınırında membran kullanılarak ultrafiltrasyonla gerçekleştirilmiştir. Ultrafiltrasyon, toplam fenolik madde içeriği ve bulanıklık değerinde sırasıyla % 30.9 ve % 99.0 olmak üzere önemli azalmaya neden olmuştur. Proses sırasında toplam antosiyanin içeriğinin sadece % 10.5' i kaybedilmiştir.

Anahtar kelimeler: Berrak siyah üzüm suyu, ultrafiltrasyon, mayşe ısıtma, toplam fenolik madde miktarı, bulanıklık

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INTRODUCTION

Grape plant (*Vitis vinifera* L.) – the father of alcoholic beverages – is being cultivated for thousands of years (1). The fruit has been widely grown in Northern South America, Central and North America, Asia and Europe (2). Grape seed and skin contains several functional compounds i.e. phenolics and anthocyanins (3-6); though their amount depends on certain factors e.g. grape variety, vintage, fungal infection, cultivation, environmental conditions etc.. Phenolics and anthocyanins are of great interest due to their antioxidant and cardioprotective properties. They are also reported as effective in various cancer research models (7-9). These compounds also influence the sensorial properties of grape juice (bitterness, astringency etc.). Furthermore, their impacts on color and stability (tendency to haze formation and interactions with proteins) are important for fruit juice industry.

Procedural variations e.g. type of extraction, contact time, heat and enzymatic treatments (10, 11) can affect the final composition of anthocyanins and other phenolic contents in grape juice. Along with other variables, colour is the most important indicator of the grape juice quality. It directly depends on the phenolic and anthocyanin contents in the grape (12). Greater juice yields, higher titratable acidity, greater colour extraction, higher anthocyanin and total phenolic concentrations can be resulted if the grapes are heated before pressing (13, 14). Pressed fruit juices have cloudy appearance because of their naturally existing compounds such as polysaccharides (pectin, cellulose, hemicelluloses, lignin and starch), proteins, tannins and metal ions. Aggressive pretreatments before pressing like mash heating and enzymatic fermentation enhance amounts of these components in juice. Consequently, post-bottling haze can be formed during storage even if the juice is clear (15). In fruit juice production, clarification is mostly carried out by fining agents. Recently, microfiltration (MF) and ultrafiltration (UF) systems have replaced conventional fining and filtration methods due to their operational advantages such as mild temperatures, less enzyme consumption, elimination of fining agents and simplicity of operation (16, 17).

Membrane filtration is based on size-exclusion and pressure-driven unit operation in separation technology to concentrate and purify macromolecules from aqueous solutions. The use of membranes with different cut-off ratings allows separation of molecules according to their molecular size (18, 19). Ultrafiltration is widely used to clarify fruit juices by removing large suspended particles and colloids that stabilizes the clarity of juice against haze formation during storage (20, 21).

The current study aimed to investigate the effects of main processes i.e. mash heating, clarification, detartarization and ultrafiltration on turbidity, total phenolic and total anthocyanin contents during clear red grape juice production.

MATERIALS AND METHODS

Materials

Fresh grape fruits (Kara Gemre variety), obtained in the fall of 2009, were used for juice production. Gallic acid standard was purchased from Sigma-Aldrich (Steinheim, Germany). Folin-Ciocalteu reagent and sodium carbonate were purchased from Merck (Darmstadt, Germany).

Methods

Grape Juice Production

Grapes were randomly harvested at optimum maturity from the vineyard (Isparta, Turkey) and stored at 0 °C until processing. The schematic diagram of laboratory scale grape juice production is shown in Figure 1. Grapes were washed with tap water, grained by hand and crushed manually. Grape mash was submerged into a jar in water bath shaker (Memmert, Germany) and heated at 65 °C for 60 minutes. Maceration enzyme mix (Pectinex Mash and Pectinex BE Colour, Novoferm, Germany) (50 µL/kg) was added to this mixture, followed by an immediate temperature decrease to 50 °C. Maceration was performed for 30 minutes. After these processes, mash was again manually pressed to obtain juice (yield 83 %). The obtained juice was depectinized by pectolytic enzyme (50 µL/L (Novoferm 61, Novoferm, Germany) at 50 °C for 45 minutes. Clarification treatment was accomplished by adding bentonite (5 %; 7 mL/L), gelatin (5 %; 6 mL/L), and kieselsol

(15 %; 3.3 mL/L); each at 15 minutes interval at 50 °C during 2 hours. Clarified juice was coarse filtrated and detartrated at 0 °C for 12 hours. Potassium bitartrate crystals were formed during this process, which were filtered out.

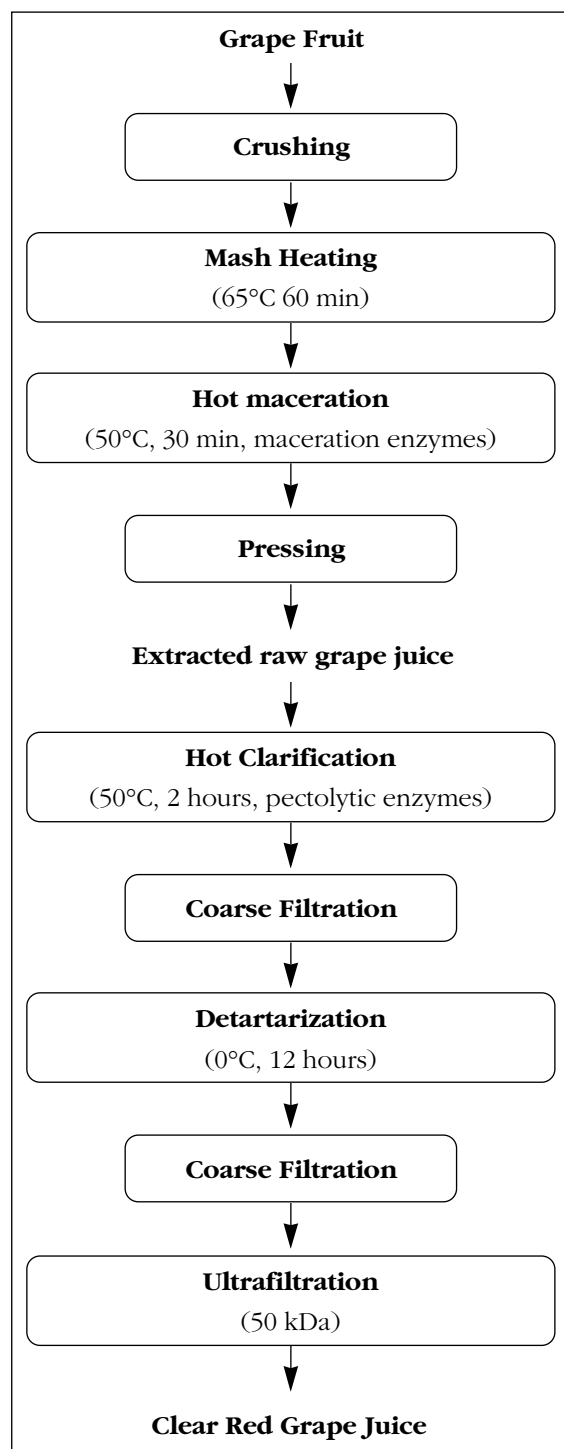


Figure 1. Schematic diagram for clear red grape juice production

Ultrafiltration Process (UF)

UF process was performed by a laboratory scale ultrafiltration system (Sartorius Stedim Biotech, Goettingen, Germany). Detartrated grape juice was feed through polyethersulfone membrane (molecular weight cut-off 50 kDa; kDa = kilodalton) having 200 cm² effective membrane area. Grape juice was ultrafiltered by recirculation of the retentate back to the feed reservoir until the latter was reduced to 10 % of the original volume at 25 °C ± 2. Permeate, obtained from ultrafiltration, was filled into amber-coloured bottles and stored at 4 °C until analysis.

Turbidity

Turbidity values of samples were measured using a turbidimeter (Hach 2100N). Results are given in NTU (Nephelometric Turbidity Unit) (22).

Determination of Total Phenolic Content

Total phenolic content (TPC) of the samples was determined according to the Folin-Ciocalteu's method (23). Gallic acid was used as a standard. Results are expressed as gallic acid equivalents (GAE) (mg GAE/L). The absorbance was measured by using a UV-visible spectrophotometer (Shimadzu UV-160A, Japan) at λ_{max} 765 nm.

Determination of Total Anthocyanin Content

Total anthocyanin content (TAC) of the samples was determined according the pH differential method of AOAC (24). The absorbance was measured by using a UV-vis spectrophotometer (Shimadzu UV-160A, Japan) at λ_{max} 520 nm. Results are expressed as malvidin-3-glucoside equivalents (ME, the predominant anthocyanin in most grape cultivars) (mg ME/L) with a molar extinction coefficient of 28000 L mol⁻¹cm⁻¹ (12).

Statistical Analysis

SAS software, Version 7 (SAS Institute Inc., Cary, NC) was used to analyze the obtained data statistically. Values of all parameters (n = 4) are presented as mean ± standard deviation. Analysis of variance (ANOVA) and Duncan's multiple comparison tests were used to evaluate the means of different treatments at a significance level of 0.05.

RESULTS and DISCUSSION

Post-pressing

Extracted raw grape juice was provided 590.37 mg/L TPC; higher than the value reported by Spanos and Wrolstad (1990). They used Thompson Seedless grape variety to produce grape juice. After pectinase treatment (50 ppm of dosage), they obtained 317 mg/L of TPC (25). Fuleki and Ricardo da Silva (2003) also reported lower TPC value (145.81 mg/L) in Concord grape juice that was extracted by pressing after hot maceration (50 mg/L pectinase at 60 °C for 60 min) (26). dos Santos Lima et al. (2015) investigated the effect of enzyme dosage on TPC while studying *Vitis labrusca* L. They extracted the juice by hot pressing with different enzyme dosages at 60 °C for 60 min. They found the TPC values as 1296 (without enzyme), 1384 (with 1.5 mL/kg of enzyme dosage) and 1203 (with 3.0 mL/kg of enzyme dosage) mg/L in grape juices (27).

In fruit juice industry, hot pressing (after mash heating and hot maceration) is used to extract anthocyanins from fruit skin to the juice that will provide desirable red color as well as higher extraction yield (13). In this study, TAC of extracted raw grape juice was obtained as 48.46 mg/L. Fuleki and Ricardo da Silva (2003) reported a lower TAC value (20.01 mg/L) for the grape juice, which was extracted from Concord grapes (26). However, dos Santos Lima et al. (2015) observed higher TAC values in juice samples of *Vitis labrusca* L. within a range of 103 and 129 mg/L (different enzyme dosages) (27). Considering it and other reported studies, although there are similar procedures on raw grape juice extraction, differences in TPC and TAC values can be attributed to the factors such as variety, maturity, cultivation-extraction-maceration conditions of grapes or processing equipment used.

Extracted raw grape juice showed 14.65 NTU of turbidity (Table 1) due to the suspended solids and colloids; mainly cell wall material. These particles usually pass through the fruit juices at higher extraction temperatures. As these compounds are quite unstable and prone to form aggregates with other haze forming compounds, turbidity of fruit juices increase during storage due to which, undesirable flocculates can be observed.

Effect of Clarification

Clarification occurs in two steps: enzymatic treatment (depectinization) and fining (to remove haze causing compounds). Pectolytic enzyme degrades the pectin that would result pectin-protein complexes to flocculate. Afterwards, fining agents are added to further flocculation and sedimentation depend upon the ionic charges on protein, polyphenols and fining agents. Bentonite and gelatin are mainly used in fining process to remove proteins and polyphenols, respectively, while kieselsol is used to increase the gelatin efficiency when hot clarification is performed. After these processes, 480.00 mg/L of TPC was analysed in the raw grape juice; decreased almost 18.7 % after clarification (Table 1).

The amount of total anthocyanins (48.20 mg/L) in grape juice also reflected 0.5 % decrease after clarification. Post pressing and clarification TAC values of the samples were statistically insignificant ($P>0.05$). Gelatin binds with anthocyanins through supramolecular interactions that can cause a notable decrease in total anthocyanin content. A possible explanation for this result is that the enhancement effect of depectinization due to the release of anthocyanins from the cell. Probably, grape juice had higher TAC after depectinization. When the fining agents were applied to the juice, gelatin agglomerated with anthocyanins and other phenolics. This caused a decrease in the amount of total monomeric anthocyanins. Since post-depectinization was not planned as a sampling point in this research, reason of this inconsiderable decrement in TAC at the end of clarification stage cannot be explained clearly.

Turbidity-causing compounds aggregated and settled down by influence of fining agents that decreased the turbidity value from 14.65 to 6.54 NTU i.e. 55.4 % after whole clarification process (Table 1).

Effect of Detartarization

Grapes, grape juice and wine contains high concentration of tartaric acid and potassium. Detartarization (cold-stabilization) process is generally used to prevent precipitation of potassium bitartrate in bottled juice. As seen in Table 1, detartarization did not influence TPC (479.15 mg/L) but slightly decreased the TAC of the juice (47.63 mg/L).

Table 1. Determination of total phenolic content (TPC), total anthocyanin content (TAC) and turbidity values of grape juice during processing

Processing	TPC* (mg GAE/L)	TAC* (mg/L)	Turbidity* (NTU)
1 st step: Hot-pressing	^a 590.37±1.31	^a 48.46±0.87	^a 14.65±4.15
2 nd step: Clarification	^b 480.00±10.35 (18.7%)	^a 48.20±1.06 (0.5%)	^b 6.54±0.06 (55.4%)
3 rd step: Detartarization	^b 479.15±7.08 (0.2%)	^a 47.63±1.01 (1.2%)	^c 3.15±0.00 (51.9%)
4 th step: Ultrafiltration	^c 408.02±21.06 (14.8%)	^b 43.36±0.35 (9.0%)	^d 0.15±0.01 (95.4%)

*: Different letters in the same column are significantly different from each other ($P < 0.05$), % values in parentheses indicate decrement ratio relative to the previous processing step.

By removing the bitartrate instability, alteration was highly remarkable among the juice samples taken after clarification (6.54 NTU) and detartarization (3.15 NTU). Detartarization was quite effective in respect to decrement in turbidity level (51.9 %).

Effect of Ultrafiltration

UF significantly affected TPC of permeate when compared with detartarated feed sample (Table 1). Amount of TPC in clear red grape juice was found as 408.02 mg/L when UF was performed through a membrane of 50 kDa molecular weight cut-off (MWCO). Removal of phenolics and proteins during grape juice production is essential, otherwise they cause hazing in the juice bottles (20). The amount of total phenolic compounds varied from 590.37 to 408.02 mg/L during pressing to ultrafiltration. The UF membrane showed 14.8 % rejection towards total phenolics. While the reduction in TPC was 18.7 % after clarification, totally 30.9 % was achieved by using ultrafiltration (Table 1). Cassano et al. (2008) also reported 13.5 % of decrease in TPC of kiwi fruit juice with a 30 kDa cellulose acetate UF membrane (28).

TAC in red grape juice was influenced significantly by the UF. In detartarated juice, TAC was 47.63 mg/L that decreased to 43.36 mg/L after UF. Total anthocyanins reduction in filtered juice was 10.5 % as compared to the raw juice. The rejection of examined UF membrane towards total anthocyanins was determined as 9.0 % (Table 1). Cassano et al. (2007) found that the rejection of 15 kDa tubular PVDF membrane towards total anthocyanins was of 9.4 % in clear blood orange juice (29). Acosta and co-workers (2014) also observed the retention of total anthocyanins as 60 % in blackberry juice with 150 kDa MWCO at 0.5 MPa transmembrane pressure while 99 % with 5 kDa MWCO at 3 MPa (30).

UF influenced the turbidity of the grape juice significantly (before 3.15 NTU, after 0.15 NTU). The variation during processing indicated that, ultrafiltration was the most effective one among the processes to decrease the turbidity level (95.4 %). UF treatment caused almost completely reduction in turbidity and total reduction at the end of processing reached to 99.0 % when compared to pressed juice (Table 1). It can clearly be seen that UF process provided high-quality juice in terms of clarity.

CONCLUSION

TPC, TAC and turbidity values (590.37 mg/L, 48.46 mg/L and 14.65 NTU, respectively) of clear red grape juice are highly affected by the processes applied in this study. It means the values reduced to 30.9 %, 10.5 % and 99.0 %, respectively. Among the methods applied in juice production, ultrafiltration (UF) had the most significant influence on TAC and turbidity level. However, the most significant decrement in TPC values was monitored after clarification process. After UF, turbidity value became negligible (0.15 NTU). Therefore, UF can be an alternative method to eliminate the potential sources (e.g. phenolic contents) of post-bottling haze formation in clear red grape juice. Clarification prior to UF process can prevent membrane from extreme fouling.

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REFERENCES

1. Gashkova IV. *Vitis vinifera* L. - Grape-vine. http://www.agroatlas.ru/en/content/cultural/Vitis_K/ (Accessed 16 January 2015).
2. Zecca G, Abbott JR, Sun WB, Spada A, Sala F, Grassi F. 2012. The timing and the mode of evolution of wild grapes (*Vitis*). *Mol Phylogenet Evol*, 62: 736-747.
3. Iacopini P, Baldi M, Storchi P, Sebastiani L. 2008. Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: Content, in vitro antioxidant activity and interactions. *J Food Compos Anal*, 21: 589-598.
4. Revilla E, Ryan JM. 2000. Analysis of several phenolic compounds with potential antioxidant properties in grape extracts and wines by high-performance liquid chromatography–photodiode array detection without sample preparation. *J Chromatogr A*, 881: 461-469.
5. Ghafoor K, Al-Juhaimi F, Choi YH. 2011. Effects of grape (*Vitis labrusca* B.) peel and seed extracts on phenolics, antioxidants and anthocyanins in grape juice. *Pak J Bot*, 43: 1581-1586.
6. Bhat KPL, Kosmeder JW, Pezzuto JM. 2001. Biological effects of resveratrol. *Antioxid Redox Signal*, 3: 1041-64.
7. Pervaiz S. 2003. Resveratrol: from grapevines to mammalian biology. *FASEB J*, 17: 1975-1985.
8. Zhou K, Raffoul JJ. 2012. Potential anticancer properties of grape antioxidants. *J Oncol*, 2012: 803294.
9. Frankel EN, Bosanek CA, Meyer AS, Silliman K, Kirk LL. 1998. Commercial grape juices inhibit the in vitro oxidation of human low-density lipoproteins. *J Agric Food Chem*, 46: 834-838.
10. Creasy LL, Creasy MT. 1998. Grape chemistry and the significance of resveratrol: An overview. *Pharm Biol*, 36: 8-13.
11. Pinelo M, Arnous A, Meyer AS. 2006. Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. *Trends Food Sci Technol*, 17: 579-590.
12. Mazza G, Miniati E. 1993. *Anthocyanins in fruits, vegetables, and grains*. CRC Press, London, UK, 362 p.
13. Leblanc M, Johnson C, Wilson P. 2008. Influence of pressing method on juice stilbene content in Muscadine and Bunch grapes. *J Food Sci*, 73: H58-H62.
14. Sistrunk W, Morris J. 1982. Influence of cultivar, extraction and storage temperature, and time on quality of muscadine grape juice. *J Am Soc Hortic Sci*, 107(6): 1110-1113.
15. Vaillant F, Millan A, Dornier M, Decloux M, Reynes M. 2001. Strategy for economical optimisation of the clarification of pulpy fruit juices using crossflow microfiltration. *J Food Eng*, 48: 83-90.
16. Laorko A, Li Z, Tongchitpakdee S, Chantachum S, Youravong W. 2010. Effect of membrane property and operating conditions on phytochemical properties and permeate flux during clarification of pineapple juice. *J Food Eng*, 100: 514-521.
17. Mondal S, Cassano A, Tasselli F, De SA. 2011. Generalized model for clarification of fruit juice during ultrafiltration under total recycle and batch mode. *J Membr Sci*, 366: 295-303.
18. Yeh H, Dong J, Shi M. 2004. Momentum balance analysis of flux and pressure declines in membrane ultrafiltration along tubular modules. *J Membr Sci*, 241: 335-345.
19. Ennigrou DJ, Gzara L, Ben Romdhane MR, Dhahbi M. 2009. Cadmium removal from aqueous solutions by polyelectrolyte enhanced ultrafiltration. *Desalination*, 246: 363-369.
20. Siebert KJ. 1999. Effects of protein-polyphenol interactions on beverage haze, stabilization, and analysis. *J Agric Food Chem*, 47: 353-362.
21. Girard B, Fukumoto LR. 2000. Membrane processing of fruit juices and beverages: A review. *Crit Rev Food Sci*, 40: 91-157.
22. Tajchakavit S, Boye J, Couture R. 2001. Effect of processing on post-bottling haze formation in apple juice. *Food Res Int*, 34: 415-424.
23. Spanos GA, Wrolstad RE. 1992. Phenolics of apple, pear, and white grape juices and their changes with processing and storage. A review. *J Agric Food Chem*, 40: 1478-1487.

24. Lee J, Durst RW, Wrolstad RE. 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. *J AOAC Int*, 88: 1269-1278.
25. Spanos GA, Wrolstad RE. 1990. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. *J Agric Food Chem*, 38: 1565-1571.
26. Fuleki T, Ricardo-da-Silva JM. 2003. Effects of cultivar and processing method on the contents of catechins and procyanidins in grape juice. *J Agric Food Chem*, 51: 640-646.
27. dos Santos Lima M, Dutra MDCP, Toaldo IM, Corrêa LC, Pereira GE, de Oliveira D, Bordignon-Luiz MT, Ninow JL. 2015. Phenolic compounds, organic acids and antioxidant activity of grape juices produced in industrial scale by different processes of maceration. *Food Chem*, 188: 384-392.
28. Cassano A, Donato L, Conidi C, Drioli E. 2008. Recovery of bioactive compounds in kiwifruit juice by ultrafiltration. *Innov Food Sci Emerg*, 9: 556-562.
29. Cassano A, Marchio M, Drioli E. 2007. Clarification of blood orange juice by ultrafiltration: analyses of operating parameters, membrane fouling and juice quality. *Desalination*, 212: 15-27.
30. Acosta O, Valliant F, Perez AM, Dornier M. 2014. Potential of ultrafiltration for separation and purification of ellagitannins in blackberry (*Rubus adenotrichus* Schlttdl.) juice. *Sep Purif Technol*, 125: 120-125.

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