Effect of different physicochemical de-tartration methods on red grape juice quality

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Abstract Grape contains a great deal of tartaric acid and minerals such as Potassium and Calcium which during ripening of grape increase in concentrations and result in potassium and calcium tartrate precipitation in grape juice. There have been different methods introduced for de-tartration but none of them have been completely successful. These methods can be categorized into two different groups of physicochemical and instrumental methods. In this study application of cold treatment, cream of tartar (Potassium bitartrate), Carboxymethyl Cellulose (CMC), and Mannoprotein were investigated. Then all these samples were stored at 5 °C to stimulate tartrate crystals precipitation and were investigated for color indices change, the amount of sediments, acidity, pH and taste. Among these methods addition of Mannoprotein and CMC were proved to have ability in stabilizing tartrate crystals in red grape juice.

Keywords Red grape juice · De-tartration · Mannoprotein · Carboxymethyl cellulose

Abbreviations

KHT	Potassium hydrogen tartrate
CD	Color density
CMC	Carboxymethyl cellulose
T or H	Tint or hue
MT	Metric ton
a.u	Degree of redness
MAP	Modified atmosphere packaging
Abs	Absorbance of sample

CaT	Calcium tartrate
$\lambda_{\max}$	Wavelength in which maximum absorbance of special compound observed
A	Treatment with 4 g/L potassium hydrogen tartrate
ANOVA	Analysis of variance
B	Treatment with 0.1 g/L of carboxymethyl cellulose
RMD	Repeated measurement design
C	Treatment with 0.2 g/L of mannoprotein
LSD	Least significant difference
D	Control treatment (cold stabilization)
LSM	Least square means

Introduction

Grape is an excellent source of vitamins and minerals and is known as one of the best anticancer natural agents. In recent years, consumption of grape juice and grape products has continued to increase. Grape is one of the world largest fruit crops and according to FAO statistics, Iran with the gross production of 2,255,670 MT in year 2010 stands as one of the major producer countries of this crop (FAO 2010; Doymaz 2012). According to the Food and Agriculture Organization, 75,866 km² of the world are dedicated to vines and annually about 1,739,503 MT grape produces in the world (FAO 2010).

Sabir et al. (2011) investigated effects of modified atmosphere packing and honey dip treatments on quality maintenance of minimally processed grape cv. Razaki (*V. vinifera* L.) during cold storage. They concluded that MAP, honey solution dip or their combination significantly maintained the general quality of minimally processed grape by delaying quality loss and berry decay.

Augustine et al. (2011) carried out a study to assess the effect of various combined post harvest treatments with chemical preservative, ionizing radiation doses and low

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temperature (LT) storage on the storability and the microbial quality of the grape pomace and retention of anthocyanins. The results showed that combination of sodium benzoate and γ -irradiation (0.1 % SB +2.0 kGy) was effective in improving the storability of grape pomace and in retention anthocyanins during storage. The treatment also reduced the total aerobic bacterial count and the yeast and mould count (1.6 log CFU/g) in the treated grape pomace during 16 days of storage.

Grapes are unique from other fruits because, the tartrate salts (Potassium Hydrogen Tartrate (KHT) and Calcium Tartrate (CaT)) which are naturally present in grape juice, must be precipitated or stabilized after juice extraction. However, these salts will settle upon cooling or even when filtered juice is refrigerated. These crystals, although harmless, are aesthetically unpleasant and can be mistaken for glass fragments (Boadu et al. 2003). Application of fuzzy expert system approach was applied by İnan et al. (2011) to predict some quality characteristics of grape juice concentrate (Pekmez) after different heat treatments. Literature review revealed that little work has been done regarding de-tartration of grape juice and it's mainly achieved via cold stabilization. This traditional approach involves chilling the juice close to its freezing temperature for a period of up to a week to induce precipitation of tartrate salts. Although cold stabilization has been proved to be effective, it includes long processing time, high energy cost and large capital investment to provide tanks and extra refrigeration. Contrariwise, there has been a lot of work done on de-tartration of red wine. The most usual way of stabilization is precipitating the potassium hydrogen tartrate (KHT) which can be carried out in wine through several alternatives: addition of cream of tartar (Potassium Hydrogen tartrate), Carboxymethyl Cellulose (CMC), Mannoprotein and physical and membrane separation methods (Margalit 2004).

This study is an attempt to compare the efficiency of different chemical methods mainly suggested in order to stabilize tartrate in wine, in stabilizing tartrate in fresh grape juice.

Materials and methods

Sample preparation

Red grapes of *Vitis vinifera* cultivar were chosen for this study. Having squeezed them into juice (Brix °16) by a juicer (Notional, Japan, Model: MJ-176NR), the enzymes of juices inactivated (at 85 °C for 2 min), filtered and divided into 5 parts (the volume of each part is 2 L). The first part (A) was treated with 4 g/L of KHT (based on the dosage recommended by the manufacturer, CLEAN s.r.l., Italy) as seed in order to accelerate precipitation and stored overnight at 5 °C and then filtered through Whatman ashless paper 41. The second (B) and the third (C) parts were treated

with 0.1 g/L of Carboxymethyl Cellulose (CMC) (Fluka, New-Ulm., Switzerland) and 0.2 g/L (based on the tests done by the authors) of Mannoprotein (Ohly, Germany) respectively. The last part (D) was considered as blank. All these 4 treatments were capped in dark bottles then pasteurized (at 90 °C for 20 s) and stored at 5 °C and were analyzed every 2 weeks. The factors analyzed were the amount of sediment, color absorbance, pH, acidity and panel test. All the tests were accomplished in 3 replications.

Methods

Sediment evaluation

Every 2 weeks a bottle of each 4 groups was shaken, opened and 100 ml of it passed through a pre-weighed Whatman paper 41 (dried at 100 °C for 1 h) for separating the sediments. Having dried the wet papers in the oven (at 100 °C for 2 h) and cooled in desiccator to ambient temperature, they were measured for the sediment weight (Scale AND, model GF.3000).

Color evaluation

Color density (CD), Tint (T) or hue (H) and degree of Redness (a.u) were determined using UV/ visible spectrophotometric absorbance (Jenway 6505, Korea) of the grape juice at 420, 520 and 620 nm (Glories 1984), and the following calculations (Eqs. 1, 2 and 3). Distilled water was used as blank sample.

$$CD(a.u) = Abs_{420} + Abs_{520} + Abs_{620} \quad (1)$$

$$T \text{ or } H = Abs_{420}/Abs_{520} \quad (2)$$

$$Redness(a.u) = Abs_{520} \quad (3)$$

Acidity and pH evaluation

A pH-meter (848 pH lab, Metrohm, Switzerland) was used to measure the pH of the grape juices. Total acidity was determined by potentiometric titration with NaOH 0.1 N until pH reached to 8.1.

Sensory evaluation

All the samples was given to group of 10 trained panelists and asked them to evaluate the taste of each juice based on 5 point hedonic scale. Each sample scored in 5(Excellent), 4 (Good), 3(Fair), 2(Poor) and 1 (bad) level.

Statistical analysis

Analysis of variance (ANOVA) was conducted using SAS (9.1, USA) and via repeated measurement design (RMD) ($p \leq 0.05$), in which the effects of treatment, time and double interaction (time*treatment) are evaluated. The significance of differences between mean values was determined by a multiple range test (LSD; Least Significant Difference). For this reason alpha (α) was preferred to be 0.05 which corresponds to a confidence of level of 95 %. In order to investigate the reproducibility of the results, three replicates were performed for all experiments.

Results and discussion

Sediment

The tartrate crystallization was reported as one of the main causes of cloudiness and solid particles in the bottles. Generally, in all treatments the amount of sediment increased during the storage time (Table 1). The amount of sediment

precipitated in 100 ml of each treated group was considered as the method efficiency indicator in stabilizing red grape juice. Additionally, the statistical results showed that time and treatment double interaction (time*treatment) had significant effects ($p < 0.05$) on the amount of precipitation. Disregarding the effect of time on the amount of precipitation, there was also a significant difference ($p < 0.05$) between all the treatments. Among all treatments, as it was expected, the greatest amount was found in blank samples (D), since there was no ingredient applied in order to stabilize tartrate in the juice (Table 1).

Grape juice is considered as a supersaturated solution of KHT. Under low storage temperature, the dissolved KHT becomes insoluble and small crystals settle to the bottom in the form of sediment. In a supersaturated solution the crystallization process occurs in two stages. In the first stage, crystals are formed. It is important to note that for nucleation in a supersaturated solution the concentration of KHT must reach a critical level. Below this level (such as slightly supersaturated wine) nucleation will be slow. In the second stage the nuclei grow into crystals. The crystal growth occurs when the KHT ions migrate to active sites on the

Table 1 Sediment, acidity, pH values and colorimetric factors of grape juice during storage at 5 °C under deferent treatment conditions

Treatment Factors		A	B	C	D
Sediment (g tartrate /100 ml)	Days				
	0	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
	14	0.74±0.01 ^c	0.98±0.01 ^b	0.15±0.10 ^d	1.44±0.01 ^a
	28	0.76±0.01 ^b	0.74±0.02 ^b	0.80±0.01 ^b	1.48±0.01 ^a
	42	0.76±0.01 ^c	1.30±0.05 ^a	0.88±0.01 ^b	1.48±0.01 ^a
	56	1.26±0.01 ^d	1.54±0.01 ^b	1.38±0.01 ^c	2.28±0.01 ^a
Acidity (g tartaric acid/L)	0	13.83±0.02 ^a	13.83±0.02 ^a	13.83±0.02 ^a	13.83±0.02 ^a
	14	11.07±0.01 ^c	13.09±0.02 ^b	12.55±0.01 ^b	17.80±0.02 ^a
	28	9.52±0.01 ^c	12.22±0.02 ^b	12.50±0.02 ^b	18.55±0.02 ^a
	42	9.51±0.04 ^d	11.50±0.17 ^c	12.30±0.25 ^b	18.53±0.12 ^a
	56	9.50±0.01 ^d	10.25±0.02 ^c	12.13±0.14 ^b	12.70±0.22 ^a
pH	0	3.30±0.03 ^a	3.30±0.03 ^a	3.30±0.03 ^a	3.30±0.03 ^a
	14	3.68±0.04 ^a	3.34±0.01 ^a	3.35±0.03 ^a	3.34±0.02 ^a
	28	3.45±0.01 ^a	3.27±0.01 ^a	3.28±0.01 ^a	3.30±0.01 ^a
	42	3.59±0.00 ^a	3.24±0.01 ^a	3.23±0.01 ^a	3.22±0.01 ^a
	56	3.53±0.01 ^a	3.25±0.02 ^a	3.38±0.01 ^a	3.40±0.01 ^a
Color density (CD) (a.u)	0	4.60±0.00 ^a	4.60±0.00 ^a	4.60±0.00 ^a	4.60±0.00 ^a
	14	4.51±0.01 ^a	4.54±0.03 ^a	4.77±0.02 ^a	4.84±0.20 ^a
	28	4.84±0.04 ^a	4.92±0.06 ^a	4.83±0.02 ^a	3.91±0.61 ^a
	42	4.39±0.56 ^a	4.53±0.64 ^a	4.62±0.60 ^a	3.56±0.02 ^b
	56	5.14±0.02 ^a	5.00±0.01 ^a	4.76±0.00 ^b	4.77±0.25 ^b
Tint (T)	0	0.79±0.03 ^a	0.79±0.03 ^a	0.79±0.03 ^a	0.79±0.03 ^a
	14	0.83±0.04 ^a	0.85±0.01 ^a	0.83±0.03 ^a	0.82±0.02 ^a
	28	0.77±0.01 ^a	0.74±0.01 ^a	0.75±0.01 ^a	0.79±0.01 ^a
	42	0.84±0.00 ^a	0.84±0.01 ^a	0.79±0.01 ^a	0.80±0.01 ^a
	56	0.87±0.01 ^a	0.87±0.02 ^a	0.78±0.01 ^a	0.80±0.01 ^a

Mean values ($n=3$)±S.D. Different letters (a, b, c ...) in the same row are significantly different at the 5 % level. A 4 g/L KHT; B 0.1 g/L of CMC; C 0.2 g/L of Mannoprotein; D control treatment (cold stabilization)

surface of the seed crystals and are incorporated into a crystal matrix (Rhein and Neradt 1979).

Similarly in treatment A, powdered KHT crystals served as seed crystals and crystal growth began immediately. Thus the addition of KHT speeds up the time required to stabilize the juice. Many trials have been conducted to determine the optimum amount of KHT needed to stabilize wine. The research has shown that the addition of 4 g/L of powdered KHT with a particle size of 40 μm is sufficient to render a wine stable (Rhein and Neradt 1979). Adding KHT in amounts less than 4 g/L will require a longer time to stabilize the grape juice. Using higher amounts than 4 g/L is not recommended because it is uneconomical, rather difficult to remove, and the improvement in KHT reduction is limited. In this study also after the first day of storage of samples in refrigerator, a great amount of precipitated tartrate was filtered, capped, pasteurized and stored again. Therefore less sediment is observed.

The results proved that (D) was less effective in tartrate stabilizing. On the other hand, the other treatments didn't have significant differences in amount of sedimentation and showed a better result than the former. Mínguez and Hernández (1998) compared the efficiency of potassium hydrogen tartrate (KHT) crystal seeding and calcium tartrate (CaT) crystal seeding in red wine. The results showed that CaT acts as an inductor of the KHT and CaT crystallization, while KHT seeding only induced KHT crystallization. Therefore Ca levels in wine didn't reduce.

In recent years, researches suggested that by using food additive Carboxymethyl Cellulose (CMC), the better effect of tartar stabilization was obtained (Niu Shengyang et al. 2006). It's proved that due to molecular structure and protective colloid property of CMC, it coats the ions K^+ and HT^- , and prevents the formation of the salt and thus prevents crystal growth. Actually this anionic polymer competes with other ions present in juice to bond on the surface of crystals in order to reduce their size. However, its effect against calcium tartrate is limited and the suggested dose of calcium tartrate is 100 mg/L. In a similar study Bosso et al. (2010) studied the effectiveness of CMC at different doses for the prevention of tartaric precipitations in white wines, they concluded that CMC caused a significant decrease in tartaric precipitations induced by the addition of potassium bitartrate (KHT), by limiting the growth of the added KHT crystals and its effectiveness increased with the dose, following a hyperbolic trend. Another study accomplished by Niu Shengyang et al. (2006) showed that the mechanism of tartaric stabilization by the use of CMC is formation composite with tartar. The composite with potassium bitartrate can stabilize in wine but the composite with calcium tartrate was precipitated mostly. This fact may be because of CMC ability to make physico-chemical balance in wine, thus it can keep stabilization in long time. CMC should be dissolved in cold water and added to the wine before polish filtration and bottling. Experiments have shown that CMC at the dose of 15 to 100 ppm is most effective (Dharmadhikari 2002).

Mannoprotein is the outer layer of the yeast cell (Ballou 1990; Inoue et al. 1995) which allows a better physical and chemical stability. This component behaves as a protector colloid against precipitations, improving the tartaric stability (Moine-Ledoux and Dubourdieu 2000) interacting with phenolic components, improving the color and decreasing the astringency (Fuster and Escot 2002). Also the wines treated with Mannoprotein presents a more stable color. This protective effect can be explained by the strong hydrophilic characteristic of Mannoprotein that makes it's very soluble and stable; preventing precipitation, with the following decreased of color, as has been observed in other methods (Feuillat et al. 2001).

Acidity and pH

Regarding acidity and pH, All variables of time, treatment and double interaction time*treatment showed significant differences ($p < 0.05$). However, comparing means by least square differences method showed no significant difference between the treatments B - C, B - D and C-D in case of pH changes. As it's been reported in Table 1, all the samples followed a decreasing trend in acidity which correlates with combining tartaric acid with potassium and calcium and finally precipitating as tartrate. However, this trend is quite faster in A (Table 1). It may be due to filtration applied in this treatment which separated the sediments containing tartaric acid. This accompanies with an increase in pH value and evidently results in off taste. Mato et al. (2005) claimed that organic acids in grape juice are of great importance as they influence in organoleptic properties (flavor, color and aroma) and in the stability and microbiologic control of this beverage.

Binning (1989) also suggested that tartaric acid concentration in juices not only depend on its initial concentration in grapes, but also related to temperature, minerals (potassium and calcium) concentration and solubility of the salt product. In another study done by Dharmadhikari (2002), increasing the dose of KHT to 12 g/L (3 times) reduced the tartaric acid level by 0.74 g/L or 40.6 %. Thus increasing the KHT addition three times the suggested level (4 g/L) caused tartaric acid reduction.

Color

Color is the most important quality index influences consumers' acceptability of grape juice. This characteristic is directly dependent on the phenolic composition of the juice and the anthocyanins present in the grape juice. The color of grape skins is determined by the accumulation of red anthocyanins pigments (Boss et al. 1996). These pigments participate in many reactions that promote changes in the color of grape products, mainly through copigmentation and formation of polymeric pigments (Wrolstad et al. 2005). During the juice storage it turned gradually brown. This event can be due to

decrease in anthocyanin content irreversibly formation of more stable polymeric pigments which are responsible for changing the grape juice aroma, color and flavor (Francia-Aricha et al. 1997) (Fig. 1).

Statistical results showed that treatment, time and double interaction (time*treatment) also caused significant differences in color changes ($p<0.05$), however LSM showed no differences between all the treatments ($p<0.05$). During the storage time Abs₅₂₀ (which is an indicator of red pigment), didn't show any changes in all the samples (Table 1). Hendry and Houghton (1996) reported that the temperature used during the processing and storage of juices can cause changes in the color of the product. This is due to formation of chalcones (colorless) causing loss of color due to anthocyanin degradation. Therefore it's conclude that as all the samples were bottled in dark bottles and stored in a stable temperature, no significant color change is observed.

Color density (CD) of grape juice indicates the amounts of color present in juice. As it is depicted, CD values ranged from 4.60 to 5.14 on average. Although CD and H can be measured quickly, their usefulness is limited. CD and H are assumed to represent the color of all the pigments in the juice. CD values are maximum at $\lambda_{\max}$ of anthocyanins (around 520 nm). But CD measurement does not include the color provided by anthocyanin-derived pigments. However, total color red pigment can be measured at 520 nm (Hülya Orak 2007). Many researchers have also presented $\lambda_{\max}$ values (Bakker and Timberlake 1997) and associated absorbance profiles of anthocyanins and anthocyanin-derived pigments. Regarding Table 1, it's deduced that Mannoprotein and CMC and cream of tartar could retain the colorants quite well. Vivas and Saint-Cricq de Gaulejac (2001) also proved that Mannoprotein has a positive effect in protecting the anthocyanins present in grape juice. The absorbance maxima and extinction coefficient values of anthocyanins will differ by variation of pH values. At low pH, anthocyanins have a high molar absorptivity. Thus, at low pH, anthocyanins will appear to be intensely colored and possess purple-red hues. On the other hand there is a reduction in absorbance and quantification of anthocyanins when the pH value increased (Wrolstad et al. 2005).

This finding is in accordance with the results of this study. The comparison of the hue value means, showed no significant difference ($p>0.05$).

Taste

All 4 samples were given to group of 10 person panelists and asked to score them according to 5 point hedonic analysis at the last day of storage. As it's illustrated in Fig. 2, sample A and samples B and C were ranked as the worst and the best samples respectively. There was no significant differences ($p>0.05$) between the acceptance of groups B and C by the panelists. The other 2 samples (A and D) were also preferred in the same

Fig. 1 Changes in red pigment in grape juices during storage at 5 °C under deferent treatment conditions. Mean values ($n=3$) $\pm$ S.D. a.u degree of Redness; **a** 4 g/L KHT; **b** 0.1 g/L of CMC; **c** 0.2 g/L of Mannoprotein; **d** control treatment (cold stabilization)

rank, suggesting there's also no difference between these two groups. Unacceptability of sample of group A could be justified by adsorption of the substances influencing the taste to the surface of the seed applied in this method. According to the panelists comments rejection of group D was due to the abundance of sediments which made the juice quite astringent and unpleasant.

Conclusions

Comparing all the results, it's concluded that all the methods are effective in reducing the amount of sediment in the final product. However, taking other factors into consideration such as color, acidity and organoleptic properties oust A. On the other hand, the factors measured both in B and C didn't show noticeable differences and they both revealed an outstanding effect in retaining the grape juice properties.

Fig. 2 The overall sensory acceptability score of grape juices by the panelists under deferent treatment conditions on the last day (56th day) of storage at 5 °C. Mean values ($n=15$) $\pm$ S.D. Different letters (**a**, **b**, **c**...) in the columns are significantly different at the 5 % level. **a** 4 g/L KHT; **b** 0.1 g/L of CMC; **c** 0.2 g/L of Mannoprotein; **d** control treatment (cold stabilization)

Therefore it's concluded that in order to stabilize the tartrate salts, treating grape juice either with CMC or Mannoprotein is preferred to the other methods introduced in this study.

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