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Effect of different clarifying agents on the physico-chemical properties of grape juice concentrate

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ABSTRACT

Grape juice concentrate is a traditional product of grape-harvesting areas of Iran which is generally produced from the year-end harvest of poor quality. In order to examine the effect of different clarifying materials on the quality of grape juice concentrate, a plan was executed using factorial statistical method with completely randomized design. The first factor was type of clarifiers in six levels, comprising grape juice concentrate soil (GJCS), bentonit, silicasol, gelatin-bentonit, gelatin-silicasol, gelatin-bentonit-silicasol; and the second factor was the quantity of clarifiers at three levels with three replicate. Results of statistical analysis showed that an increase in the quantity of GJCS decreased acidity of grape juice concentrate and led to increasing pH, while an increase in other clarifiers concentration (bentonit, silicasol and gelatin) did not influence the acidity and pH significantly. Bentonit and silicasol compared to GJCS, significantly increased transparency and decreased turbidity of grape juice concentrate. Adding gelatin to bentonit and silicasol, compared to the sole application of bentonit and silicasol, decreased the turbidity and increased the transparency. Treatment with gelatin-bentonit-silicasol compound led to a product with highest level of transparency (89.47%) and lowest level of turbidity (8.48%). In grape juice concentrate produced via this treatment, no crucial decrease in important quality criteria; such as pH, acidity, protein, sugar, ash, brix and total dry material; was observed. Finally, treatment of grape juice concentrate using a complex clarifying agent containing 2% gelatin, 4% bentonit and 7% silicasol, is prescribed in order to produce a clear and transparent product.

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KEYWORDS

Grape juice concentrate;
Clarifying agent;
Soil;
Bentonite;
Silicasol;
Gelatin.

INTRODUCTION

Grape juice concentrate, with the local name of Dooshab, is a traditional product of grape-harvesting areas of Iran, produced from boiling and condensation

of grape juice to the brix scale of over 70-80%, in open containers or in vacuum, and without adding sugar or other additives^[1,2]. Grape juice concentrate contains high volumes of natural sugar, minerals, vitamins A, B₁, B₂ and C, organic acids and antioxidants. It, therefore, plays

an important role in the nutrition of various age groups, especially children and athletes^[3-6]. Grape juice concentrate is quickly absorbed by the body as a result of its high volume of digestible monosaccharide. It is, therefore, useful for those weakened as a result of a chronic disease or after undergoing a medical operation^[1-3,7,8]. Grape juice concentrate is a rich source of chemical elements essential to human body, such as copper, zinc and iron. Iron contained in grape juice concentrate may be useful in the treatment of anemia patients^[4,9]. Clarification ways of grape juice concentrate are similar to those of clarification of grape juice. In fruit juice industry, clarification is a unified process that comprises the elimination of undesired color, aroma and flavor; turbidity; bitterness and gassy^[10]. In the process of clarification, clarifiers are utilized which are combined with charged particles of fruit juice such as protein, pectin and phenolic materials and are consequently separated from the environment. Usual clarifiers in fruit juice industry are bentonit, gelatin and silicasol. Bentonit is a kind of clay of montmorillonite group with the characteristic of shallow absorption surficial absorption, and affects proteins, poly-phenolic materials, metal ions and the rest of the toxics^[11]. The soluble protein gelatin is obtained through relative hydrolysis of collagen existing in animal skin, bones and cartilage. In terms of extraction method, gelatin is divided into acid (A) and alkaline (B) variants^[12]. Gelatin characteristics include decreasing the quantity of polyphenols and pectin, making complex with natural proteins of fruit juice and brightening the color of fruit juice. Silicasol is another clarifier which helps to brighten the color of the fruit juice through creating negative charge in fruit juice and flocculating with positively charged compounds^[10,11].

This study also made use of a certain white soil called grape juice concentrate soil as the clarifier material in the production of grape juice concentrate. In addition to depositing suspending material, the soil neutralizes the acidity of the grape juice^[13]. Bodbodak et al. (2009) studied the effect of different clarification treatments on the physicochemical and rheological characteristics of pomegranate juice. Rai et al. (2007) studied the effect of clarifiers on the quality of mosambi orange juice. Gockmen et al. (2001) and OSzmianski and Wojdylo (2007) studied the effect of clarifiers on the quality of apple juice clarification. EhteshamiMoeinabadi et al. (2005) used sodium car-

bonate to reduce acidity and bentonit as clarifier in producing grape juice concentrate. The present study aimed to analyze the effect of different clarifiers on the quality of the grape juice concentrate produced.

MATERIALS AND METHODS

Materials

Grape (*Razeghi* variety) was harvested from the gardens of Nazloochoy district in Urmia. Material used for clarification including bentonit (SIHA, Paranit Na-Cabentonit), gelatin (mesh 35, type A, bloom 80, DGF Stoess), commercial silicasol 15% (Baykisol 15%) and calcium carbonate (Charleaux brand, EU0) was provided by Saroone Co. Urmia. Also, GJCS was obtained from the grape juice concentrate producers' bazaar in Urmia.

Methods

(a) Production of grape juice concentrate

Fifty four samples of grape juice concentrate (including six treatments in three levels and three repetitions) produced around early October 2011 in the research center of ministry of agriculture in Urmia (TABLE 1). For each sample, about 5 liters of grape juice squeezed from 10 kg of grape by a juicer (Toshiba, Japan), and the pH, acidity and brix of the juices were measured.

GJCS reduces acidity and eliminates materials blurring the grape juice. The soil was first dissolved into part of grape juice and then added to the samples and was thoroughly mixed. After 2-3 hours, grape juice concentrate cracks on the surface. At this time the existing foam should be removed from the surface and sieved through a piece of percale. In all other treatments, the acidity of grape juice was set off by calcium carbonate (42.5 g/5 liters of grape juice) to the final pH=8.5. Then the clarifying agents were added and the juice was sieved by a piece of percale after 30 minutes. All samples were finally transferred to the cooking section and concentrated to brix=70±2.

(b) Physicochemical and microbiological analysis of grape juice concentrate

The solid material in the solution (brix) was measured by a digital refractometer (Ceti, Belgium) equipped with temperature modifier^[16]. Total solid matter (dry

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extract) was measured at 70°C, under pressure less than 250 mm/Hg, in vacuum oven (Memmert, Germany), and total ash was measured, using an electrical furnace (Barnstead Thermolyne F6000, Germany) in 550°C. pH was measured by pH-meter (WTW 720, Germany) and total acidity was measured by titration method. Also, total sugar, reducing sugar and sucrose were determined, using Lane-Eynon method with Fehling's solution; fat, raw fiber and protein content of the samples were determined by digital Soxhlet extractor (Buchi, Swiss), Fibertec (Foss, Swede) and digital macro-Kjeldahl (Buchi, Swiss) respectively^[19]. To determine the color, transparency and turbidity, grape juice concentrate diluted up to brix=12 degrees with distilled water, and then the transparency and color of the samples were respectively determined by measuring the transmission of light in 625 and 440 nm using spectrophotometer (Uv-Visible Varian, Australia) and turbidity measured with turbidimeter (Wagtech, England)^[20]. Total bacterial, mold & yeast, coliforms and acidophilus bacteria counts were determined using plate count agar (PCA), YGC agar, Crystal Violet Neutral Red Bile Glucose Agar (VRBA) and orange extract agar respectively^[21].

TABLE 1 : Clarifying agents used in grape juice concentration.

Treatments ^a	Clarifying agent	Concentration
T _{1-A}	Soil	3(g/100ml)
T _{1-B}	Soil	4(g/100ml)
T _{1-C}	Soil	5(g/100ml)
T _{2-A}	Bentonit	4(g/lit)
T _{2-B}	Bentonit	5(g/lit)
T _{2-C}	Bentonit	6(g/lit)
T _{3-A}	Silicasol	5(ml/lit)
T _{3-B}	Silicasol	6(ml/lit)
T _{3-C}	Silicasol	7(ml/lit)
T _{4-A}	Gelatin + Bentonit	2(g/lit) + 4(g/lit)
T _{4-B}	Gelatin + Bentonit	2(g/lit) + 5(g/lit)
T _{4-C}	Gelatin + Bentonit	2(g/lit) + 6(g/lit)
T _{5-A}	Gelatin + Silicasol	2(g/lit) + 5(ml/lit)
T _{5-B}	Gelatin + Silicasol	2(g/lit) + 6(ml/lit)
T _{5-C}	Gelatin + Silicasol	2(g/lit) + 7(ml/lit)
T _{6-A}	Gelatin + Bentonit + Silicasol	2(g/lit) + 4(g/lit) + 7(ml/lit)
T _{6-B}	Gelatin + Bentonit + Silicasol	2(g/lit) + 5(g/lit) + 6(ml/lit)
T _{6-C}	Gelatin + Bentonit + Silicasol	2(g/lit) + 6(g/lit) + 5(ml/lit)

^aall treatments done in three replicate.

Statistical analysis

The design of experiment used was random complete blocks (factorial) with two factors (type and quantity of clarifiers) and three repetitions. Results were statistically analyzed, using the MSTAT-C software and ANOVA test. The medians were compared through LSD test at $p < 0.05$.

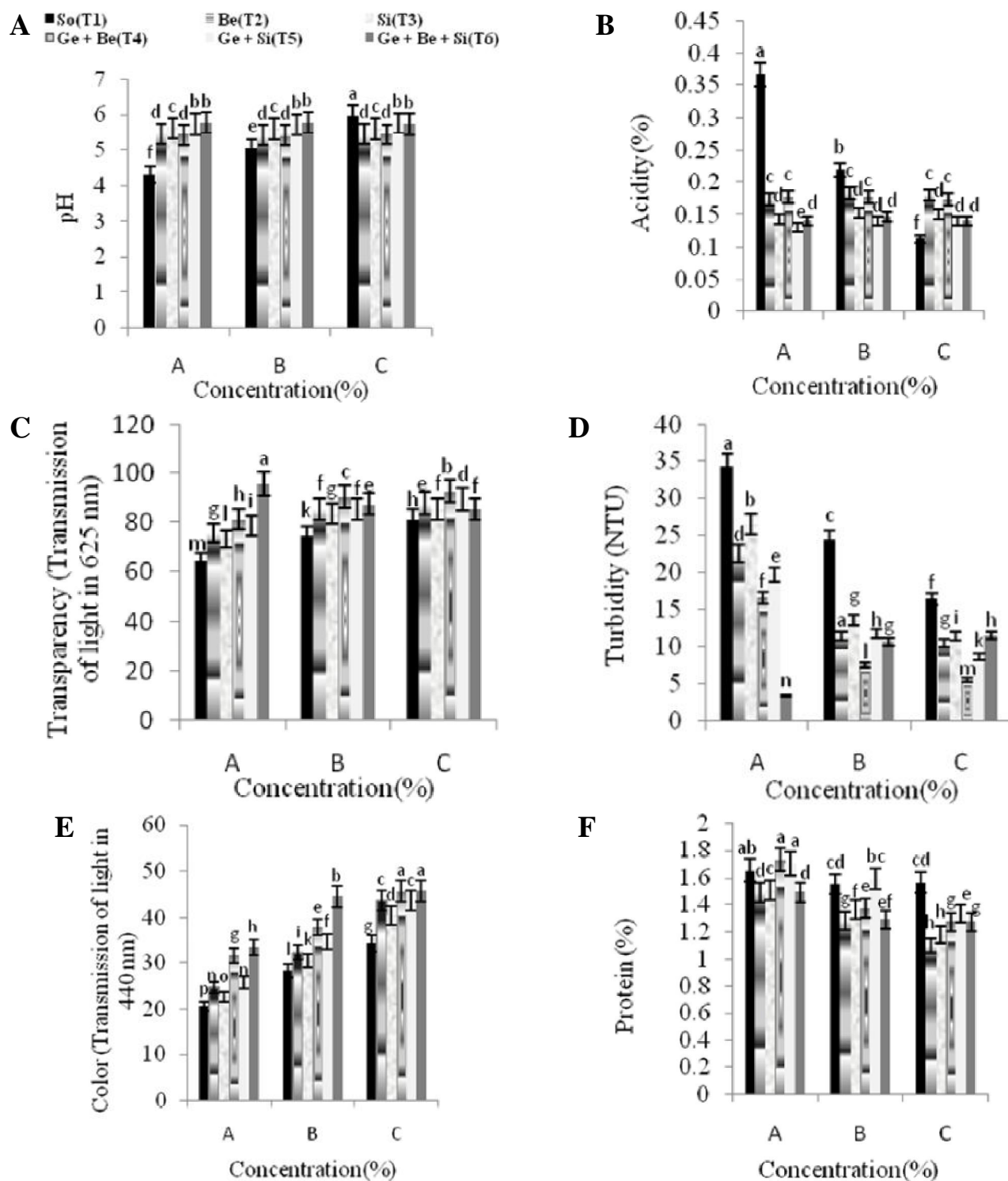
RESULTS AND DISCUSSION

Effect of clarifying agent on physico-chemical properties

(a) pH and acidity

Analysis of grape juice samples for pH and acidity showed these parameters respectively equal to 3.56 ± 0.01 and 0.59 ± 0.01 . Concentration of grape juice led to significant changes in these properties. Figure 1a and 1b show pH and acidity of grape juice concentrate samples produced by different clarifying agents. According to these figures there is no significant difference between pH and total acidity of different treatments ($p < 0.05$). The analysis of medians shows an increase in pH and a decrease in acidity of the samples as a result of increasing the GJCS content. But the increase in other materials did not have a significant effect in pH and acidity. GJCS is an alkali agent (pH=8.5) which reduces the acidity of the grape juice significantly, while other filter-aid materials are chemically neutral (bentonit pH=7.3, gelatin pH=7.1 and silicasol pH=6.8) and therefore have no effect on the acidity and pH.

In the acidity neutralization stage of grape juice, tartaric acid changes into insoluble calcium tartrate and removed from the juice after being deposited and sieving stage. Overall, among different treatments, the lowest pH belongs to GJCS treatment because of its alkaline nature. Our findings are in conformity with the results of Zomorodi et al. (2002) and Basiri (2007). Also, Rai et al. (2007) analyzed the effect of filter-aid material on mosambi orange juice. Bodbodak et al. (2009) analyzed various clarification agents on physicochemical and rheological characteristics of pomegranate juice. Both Rai et al. and Bodbodak et al. observed no significant changes in pH. Oszmianski and Wojdylo (2007) also did not report a significant change in the acidity of apple juice samples while studying the traditional method of juice clarification.



So: Soil, Be: Bentonit, Si: Silicasol, Ge: Gelatin; Different letters on bars differ significantly ($P < 0.05$, $n = 3$). Different treatments refer to TABLE 1.

Figure 1: Effect of type and quantity of clarifiers on quality of grape juice concentrate: A: effect on pH; B: effect on total acidity; C: effect on transparency; D: effect on turbidity; E: effect on color, F: effect on protein content.

(b) Brix, dry material and sugar content

According to ANOVA analysis there was no significant difference between brix and dry material of GJC produced using different clarifiers (data not shown). The

mean value for brix of grape juice samples was 23.1 ± 0.37 which increased to $71.1 - 72.8$ afted concentration using different clarifiers. The sugar content of GJC samples differed statistically (data not shown);

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with the highest concentration (72.6%) in the treatments T_{11} and T_{17} and the lowest concentration in T_3 (70.4%). Bodbodak et al. (2009) studied the effect of different clarifying agents on physiochemical and rheological properties of pomegranate juice and saw no significant difference in solid material in different treatments. Gockmen et al. (2001) reported similar results while clarifying apple juice using different clarifiers.

(c) Total ash

ANOVA test showed no significant difference between ash content of different treatments (data not shown) ($p > 0.05$). The ash content of GJC samples was between 0.91-0.98%.

(b) Fat and raw fiber

The quantity of fat and raw fiber was reported nil in all treatments, which indicates that the basic material (grape juice) and all clarifiers do not contain fat and raw fiber (data not shown).

(e) Transparency

ANOVA test showed a significant difference between different treatments in terms of transparency ($P < 0.05$). Treatment with gelatin-bentonit-silicasol resulted to the clearest product ($T_{\lambda 625\text{nm}} = 89.47\%$) while the transparency of the samples treated with GJCS was at least ($T_{\lambda 625\text{nm}} = 73.47\%$). By increasing the soil concentration, the product transparency increased (Figure 1c) due to colloids and impurities separation.

As both bentonit and silicasol have negative electric charge, they can flocculate positively charged compounds (such as proteins). Colloidal materials and agents that make the grape juice opaque are mostly positively charged particles. In addition, these two compounds bind phenolic materials via surface absorption^[10,23]. Also, Triberti and Castinor (1992) showed that bentonit decreased flavonoid compounds such as tannin.

Addition of gelatin to clarifying agent complex had a synergistic effect in clarification. Gelatin with positive charge absorbs negative-charge materials like phenolic compounds, tannins and pectin while silicasol and bentonit absorb materials with positive charges^[25]. Bravo et al. (1991) showed that the combination of gelatin-bentonit reduced phenolic materials, which confirms the findings of the present study. The treatment containing 2% gelatin-4% bentonit-5% silicasol had the

most transparency ($T_{\lambda 625\text{nm}} = 95.57\%$). This result is in conformity with those of Zomorodi et al. (2002), Bodbodak et al. (2009), Rai et al. (2007) and Gockmen et al. (2001). Figure 1c shows the effect of type and quantity of clarifiers on the transparency of grape juice concentrate.

(f) Turbidity

ANOVA test showed a significant difference between different treatments in terms of turbidity ($P < 0.05$) in a way that gelatin-bentonit-silicasol treatment had the least turbidity (8.48. NTU), and the GJCS treatment had the most turbidity (25.062 NTU). The most opaque GJC belonged to treatment containing 3% GJCS (34.293 NTU) while the clearest sample produced using 2% gelatin-4% bentonit-7% silicasol (3.327 NTU). Figure 1d shows the effect of type and quantity of clarifiers on the turbidity of grape juice concentrate.

(g) Color

ANOVA test showed a significant difference between color intensity of different treatments ($p < 0.05$). The darkest GJC produced in treatment containing 3% GJCS, and the brightest one produced by clarifying complex containing 2% gelatin-6% bentonit-5% silicasol. According to Figure 1e, an increase in GJCS, bentonit and silicasol significantly decreases color intensity and brightens GJC. GJCS, bentonit and silicasol have a high absorption potential and reduce pigments with surficial absorption method^[10]. Molina et al. (1995) showed that bentonit reduces color intensity and the quantity of pigments. Also, Triberti and Castinor (1992) showed that bentonit reduced the color intensity of wine, which confirms the findings of the present study. In another hand, positively charged gelatin flocculates phenolic compounds. Bravo et al. (1991) showed that the combination of gelatin-bentonit reduced free anthocyanins (reduction of pigments). Moreover, Gockmen et al. (2001) studied on clarification of apple juice and found that traditional clarification using gelatin-bentonit complex reduced phenolic compounds, which confirms the findings of the present study. The highest reduction rate in color intensity was gained in treatment containing 2% gelatin-6% bentonit-7% silicasol which is due to a synergistic effect in the elimination of phenolic compounds and pigments. Bodbodak et al. (2009) studied different clarification treatments on physiochemical and rheological properties of pomegranate juice and indi-

cated that gelatin-bentonit-silicasol complex reduced 23% of the total anthocyanins.

(h) Protein

ANOVA test showed significant differences ($p < 0.05$) between protein content of different treatments. The highest protein content gained in grape juice concentrate treated with soil (1.59%) and the lowest protein content belonged to grape juice concentrate treated with bentonit (1.29%). However increasing the soil concentration reduced the protein content of the samples. This may be due to the soil ability to absorb protein compounds. In treatments containing bentonit and silicasol, gelatin-bentonit and gelatin-silicasol, as the quantity of bentonit and silicasol increase, the protein content of the samples decreased. Bentonit and silicasol have negative electric charges and the spectacular ability to combine with positively charged proteins^[28,29]. Molina et al. (1995) used bentonit suspension to clarify red wine and showed that bentonit reduced the protein. According to Figure 1f, treating the grape juice by sole bentonit or silicasol compared to gelatin-bentonit and gelatin-silicasol complexes led to greater decrease in protein content of the final product. The reason may be the competition between positively charged gelatin and protein to combine with negatively charged bentonit and silicasol. The treatment containing 2% gelatin-4% bentonit-7% silicasol led to highest protein content in comparison to other triple complexes. Treatment with gelatin-bentonit-silicasol complex reduces protein content of final product effectively more than treatment with gelatin-bentonit or gelatin-silicasol. The reason is the increase in density of negative charge due to simultaneous use of bentonit and silicasol, and increase in the speed of reaction between bentonit-silicasol and protein^[10].

CONCLUSION

According to the findings, treatment of GJC with gelatin-bentonit-silicasol complex resulted to clearest (89.47%), least opaque (8.48%) and brightest sample compared to other treatments. This treatment did not affect significantly the qualitative characteristics of GJC such as pH, acidity, protein, carbohydrates, ash, brix and total dry materials. Finally, production of GJC using clarifying complex including gelatin 2%-bentonit 4%, silicasol 7% is recommended.

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