Physicochemical Characteristics and Antioxidant Capacity of Traditional Yogurt Fortified with Grape (*Vitis vinifera* L.) Seed Extract at Different Levels

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ABSTRACT

The aim of this study is to obtain the grape seed extract with high antioxidant activity and to investigate the quality characteristics of traditional yogurts fortified with seed extracts at different concentrations. For this aim, batch hot water extraction was conducted and extraction conditions were optimized to produce *Vitis vinifera* L. grape seed extract using with the highest antioxidant activity. The fortification of yogurts was conducted at 0.1-0.15 and 0.2 g/100g extract level and antioxidant, physicochemical, microbial and sensory characteristics of the yogurt samples were analyzed. Results showed that antioxidant activity of fortified yogurts were higher and up to 14 % more antioxidant activity was detected compared to the control samples. Yogurt samples preserved their physicochemical characteristics. Although sample with 0.1 g/100 g extract had highest scores in overall acceptance, the sample containing the highest extract concentration of 0.2 g/100 g, exhibited negative perceptions by panelists in sensorial analysis. In conclusion, grape seed may be used in traditional yogurt production by improving the taste and antioxidant potential. The fortification levels of 0.1-0.15 g/100 g grape seed extract might be more feasible in functional yogurt production and might be more recommended for consumer's appreciation in terms of positive health effects.

Keywords: Grape Seed, Extraction, Yogurt, Functional Food, Antioxidant


**Üzüm (*Vitis vinifera* L.) Çekirdeği Ekstraktı ile Zenginleştirilmiş Geleneksel Yoğurdun Fizikokimyasal Özellikleri ve Antioksidan Kapasitesi**

ÖZ

Bu çalışmanın amacı, antioksidan aktivitesi yüksek üzüm çekirdeği ekstraktı elde etmek ve farklı konsantrasyonlardaki çekirdeğin ekstraktı üzerinde tayin edilmiş geleneksel yoğurtların kalite özelliklerini araştırmaktır. Bu amaçla ilk olarak sıcak su ekstraksiyonu uygulanarak en yüksek antioksidan aktiviteye sahip *Vitis vinifera* L. üzüm çekirdeği ekstraktı üretmek için ekstraksiyon koşullarını optimize edilmiştir. Yoğurtların zenginleştirilmiş 0.1 - 0.15 ve 0.2 g/100g ekstrakt düzeyinde yapılmıştır. Yoğurt numunelerinin antioksidan, fizikokimyasal, mikrobiyal ve duyusal özellikleri analiz edilmiştir. Sonuçlara göre, zenginleştirilmiş yoğurt numunelerinin antioksidan aktivitesinin daha yüksek olduğuunu ve kontrol numunelerine kyasla % 14'e kadar daha fazla antioksidandır aktivite gösterdikleri tespit edilmiştir. Yoğurt numunelerinin ise fizikokimyasal özelliklerini korunmuştur. Duyusal analizlerde 0.1 g / 100 g ekstrakt içeren örnekler genel kabul görmüştü en yüksek puana sahip olmasına rağmen, 0.2 g / 100 g içeren örnekler panelistlerce olumsuz algılanmıştır. Sonuç olarak, üzüm çekirdeği ekstraktı geleneksel yoğurt üretiminde tayin ve antioksidan özelliğini arttırmak için kullanabilir. 0.1-0.15 g / 100 g düzeyinde üzüm çekirdeği ekstraktı ile zenginleştirme, fonksiyonel yoğurt üretiminde makul olmakta ve olumlu sağlık etkileri açısından tüketiciye benzeri geleneksel yoğurt üretiminde fazla hitap etmektedir.

Anahtar Kelimeler: Üzüm Çekirdeği, Ekstraksiyon, Yoğurt, Fonksiyonel Gida, Antioksidan
INTRODUCTION

Grape fruit is a rich source of phytochemicals. 60-70% of the extractable polyphenols of grape tissues exist in the grape seed, 28-35% in the fruit peel and 10% in the fruit meat (Shi et al. 2003, Akdeniz 2010). Among these, the grape seeds with high polyphenol content appear as output material during wine, grape juice and grape molasses production. Grape seed contains polyphenols, vitamins, and minerals, (Ranjitha et al. 2014, So-Young et al. 2006). Due to its high antioxidant properties, it was reported to have protective effects on cells, cardiovascular diseases, also act as antiaging and immune-regulatory agent (Shi et al. 2003, Bagchi et al. 2000, Sato et al. 1999, Saito et al. 1998, Jayaprakash et al. 2001, Mandic et al. 2009). Therefore, grape seed extract is a well-known and popular dietary supplement and especially used in foods to provide functionality. Grape seed extract can be obtained from grape seeds using different extraction techniques. However, the bioactivity of the extracts from plants may vary on the extraction conditions such as temperature, solvent composition and time (Kavak and Akdeniz, 2019, Kavak and Kececi 2019, Kavak 2017). Therefore to obtain a bioactive grape seed extract such as having high antioxidant activity, suitable extraction conditions should be set (So-Young et al. 2006, Jayaprakash et al. 2003).

The word "yogurt" is derived from the Turkish word and it is a traditional food in the Balkans and the Middle East (Tamime and Deeth 1980). Yogurt is a fermented milk product which is formed as a result of the culture activities added to milk. As a nutritional source, it has an important biological value for all age groups (Granato et al. 2010). Fermentation occurs in yogurt with lactic acid bacteria and as a result of fermentation, several metabolites with natural biological activity such as antimicrobials against some pathogenic microorganisms are formed (Con et al. 1996). Due to the competitive activity of microorganisms present in the structure of yogurt and the presence of antimicrobial agents, yogurt has a protective effect against a number of infections causing gastrointestinal and urogenital diseases.

Natural products such as grapes, edible berries were successfully explored in different functional foods due to their considerable bioactivities (Jang et al. 1997, Nile and Park 2014). There are different studies using fruit juices, powders, and extracts to investigate their potential as functional ingredients in the dairy sector (Nguyen and Hwang 2016, Wallaca and Guisti, 2008). Families with children are the core consumers of dairy products such as yogurt and it is essential to explore new potent uses of grape seed dairy sector. Therefore, the aim of this study is to obtain the grape (Vitis vinifera L.) seed extract with high antioxidant activity and to investigate the quality characteristics of traditional yogurts fortified with extracts at different concentrations.

MATERIALS and METHODS

Materials

Seeds of grape (Vitis vinifera L.) were obtained from local herbalist (Afyonkarahisar, Turkey). 2,2-Diphenyl-1-picryl-hydrazyl (DPPH), NaOH were purchased from Sigma (Sigma-Aldrich GmbH, Germany). Plate count Agar (PCA) and Violet Red Bile Agar (VRBA) (Merck, Germany) were used for microbiological analysis.

Obtaining Grape Seed Extracts

To produce grape seed extracts, the seeds were grounded and extracted by hot water extraction process (1:10 w/v; mg seed per mL water). Experimental design for the extraction was shown in Table 1. Aqueous extracts were lyophilized (Lyoquest-Telstar, Japan), sealed in tests tubes and stored under refrigerated conditions (+4 ±1 ºC) until usage.

Production of Yogurt Using Grape Seed Extract

Yogurt is produced in traditional method. Cow milk is pasteurized in a household container at ~89±2°C for 5 min. Pasteurized cow milk was cooled to ~44°C. The plain yogurt was mixed in glass container until it was liquid, and the warm milk was added into the mixture where the weight percentage of plain yogurt in milk was 3% (w/w). Samples were stirred for ~1 minute for the yogurt to dissolve well into the milk. Samples were stored at stable temperature of 43±1°C in oven for the incubation of culture for 3.5 hours to a pH value of 4.4-4.6. After curd formation samples were cooled to +4 ±1°C and kept for 1 hour to stabilize the curd structure. Grape seed extracts were added to the samples and completely stirred. Samples were stored at +4°C ±1 for 5 days before the analysis. The codes of the samples were given in Table 2.

Antioxidant Activity Assays

Antioxidant activity of extracts and yogurt samples were determined by DPPH free radical scavenging assay. 50 μL sample was vortexed with DPPH solution. The mixture was incubated at room temperature for 1h at dark. The absorbance of the samples was measured at 517 nm using UV-Vis Spectrophotometer. The antioxidant activity was expressed in terms of percentage of radical scavenging AA (%): 

\[ AA(\%) = \frac{(A_c - A_t)}{A_c} \times 100 \]  

(1)

where \(A_c\) is the absorbance of control (containing all reagents except the test compound ) and \(A_t\) is the absorbance of test sample, respectively.
Pysicochemical Analysis of Yogurt Samples
Samples stored for 5 days at refrigerated conditions were analyzed for their dry matter, by gravimetric method, acidity by titrating with 0.1 M NaOH (until the faint pink color appeared and stable for 2 minutes) in terms of Soxhelet Henkel\(^\circ\)SH, for pH by diluting sample in a ratio of 1/10 with distilled water using pH meter (Ohaus 3100, USA) (Tekinşen et al. 2002).

Microbiological Analysis of Yogurt Samples
Samples were evaluated for their total mesophilic aerobic bacteria (TMAB) and total coliform bacteria using PCA at 37\(^\circ\)C incubation for 72 hours, and VRBA at 35\(^\circ\)C for 48 hours incubation under aerobic conditions, respectively (Harrigan and McCance 1976).

Sensorial Analysis of Yogurt Samples
The sensorial characteristics of samples were measured using hedonic test by a total of 12 pre-trained individuals (4 males, 8 females). Yogurt samples of ~40 mL were put in white plastic cups. They served at room temperature with random 3 digit codes in a random order. Water was provided for panelists to rinse their mouth between samples. The evaluation was based on the appearance, taste and color, mouth feel and overall acceptance criteria by scoring on 9 point hedonic scale (Altuğ 1993).

RESULTS and DISCUSSION

Antioxidant Activity of Extracts
To obtain grape seed extract having the highest antioxidant activity, the effect of extraction temperature and time was investigated to optimize the extraction conditions and the results were shown in Figure 1 and 2. Results showed that increase of temperature around from 40 to 55\(^\circ\)C caused an increase in antioxidant activity ~ 8 \% for 60 minutes hot water extraction (Figure 1).

It was reported that temperature was an important factor in extraction since it is highly effective on diffusion of the molecules (Majd et al. 2014, Kavak and Ülkü 2015, Cacace and Mazza, 2003). But, in extraction processes the temperature should not be so high to avoid degradation of the phenolies compounds thus an upper limit to optimize the bioactivity is critical (Kavak and Akdeniz 2019). The antioxidant activity was nearly same for 45 and 55 \(^\circ\)C. Therefore the working temperature for the following extraction process was chosen as 45\(^\circ\)C to obtain the grape extracts with the highest antioxidant activity (Figure 2). In the second step, effect of time on AA\% was the highest for 90 minutes of extraction. Therefore those results revealed that the optimum extraction process was 45\(^\circ\)C for 90 min within the chosen experimental parameter’s range and those extracts with the highest antioxidant activity could be used for further steps to produce functional yogurt.

Antioxidant Activity of Yogurt
To investigate the effect of extract addition on the antioxidant activity, extracts obtained at optimized conditions (having the highest antioxidant activity as previously mentioned) were added to yogurt samples and antioxidant activities of the samples were investigated. Yogurt contains many bioactive components such as casein where it was reported that casein as a major protein constituent of yogurt and had also antioxidant activity (Kavak and Akdeniz 2016, Silva and Malcata 2005). Therefore results in Table 3 indicated a considerable antioxidant activity (around 54\%) even in YK samples. The fortification of traditional yogurt with seed extracts (0.2g/100g) increased AA\% to 68,5 where this increase in AA\% with increase in extract concentration is in good agreement with previous reports (Shyamala et al. 2005). Additionally the antioxidant activity measured at the 5\(^{th}\) day of storage revealed that activity was preserved in refrigerated conditions indicating the stability of the functional product over time (Dabija et al. 2018).

Pysicochemical Analysis Results
The results of physicochemical analysis were shown in Table 4. The total acidity represents the amount of lactic acid produced during fermentation in the yogurt and varied between 36.4 and 38.1 which were in good agreement with the previous reports for the yogurt stored for 5 days at +4 \(^\circ\)C (Tomovska et al. 2016). Dry matter contents were almost same for the control and extract added samples which was consistent with the previous reports about the traditional yogurts where 13.3\% dry matter was reported. The pH change for the fortification of yogurt is reported to depend on the nature of the added component as well as it was related to the development of the growth of the bacteria in samples (Pelaes et al. 2015). In literature, the addition of hawthorn extract (in 0.5 \% concentration) affected the pH and resulted a decrease in yogurt acidity but the addition Pleuranus ostratus aqueous extract did not affect the pH where the pH of the control sample was approximately the same as pH of other samples (Dabija et al. 2018, Pelaes et al. 2015). However, results in Table 3 indicated that the pH values of samples slightly decreased during storage at +4 \(^\circ\)C and with the addition of grape seed extracts. The decrease in acidity of yogurt after the production was clear with the extracts added samples compared to the control sample.

Microbiological Analysis Results
The total coliform bacteria group can be regarded as a large collection of different kinds of bacteria where there is a strong relationship between yogurt safety and quality. Results in Table 5 showed that total
coliorm bacteria were under the detection limit of 1 log cfu/g. These results indicated the raw materials quality, adequate heat treatment applications or hygienic production. The total mesophilic aerobic bacteria counts of the samples ranged between 7.29-8.24 log cfu/g. In literature, it was reported that flavor additives had no effect on the mesophilic aerobic count in yogurt (Con et al. 1996). But this study showed that there was a slight decrease in TMAB with fortification of yogurt with grape seed extract.

Sensorial Analysis Results

Figure 3 shows the sensory analysis results of the yogurt samples fortified with grape seed extract in different concentrations. Results showed that fortifying yogurt with grape seed affected the sensory scores of quality attributes. The impact of fortification on color was distinct where Y2 had the highest (8.9) and YK took the lowest score of 7.8.

The grape seed extract has orange-light brown color due to its pigments and this might have positively contributed to yogurt color. Similar results were previously reported in literature (Nguyen and Hwang 2016). Overall acceptance scores indicated that Y1 got the highest score (8.1) where it was 7.6 for YK. Although the addition of extract was more acceptable for all characteristic for Y1 and Y2 compared to control samples, the values of Y3 were the lowest except color. Those results revealed that higher amounts of extract addition were not appreciated. Since the grape seed extract itself usually has astringent taste, higher concentrations (0.2g/100g) might possibly showed negative effects on other sensorial attributes. Therefore addition of 0.1-0.15 g/100 g grape seed extract might be recommended for consumer’s appreciation in terms of positive health effects.

![Figure 1. Effect of extraction temperature on antioxidant activity in terms of radical inhibition (1 g/10 mL extract; extraction time 60 min.)](image1)

![Figure 2. Effect of extraction time on antioxidant activity of extracts (1 g/10 mL extract; extraction temperature 45 °C.)](image2)
Table 1. The parameters of experimental design used for extraction

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>60</td>
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<tr>
<td>45</td>
<td>75</td>
</tr>
<tr>
<td>55</td>
<td>90</td>
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Table 2. Codes and Definitions of the Samples

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Definition</th>
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<tbody>
<tr>
<td>YK</td>
<td>Control sample (with no extract)</td>
</tr>
<tr>
<td>Y1</td>
<td>Sample with 0.1g extract/100g yogurt sample</td>
</tr>
<tr>
<td>Y2</td>
<td>Sample with 0.15 g extract/100g yogurt sample</td>
</tr>
<tr>
<td>Y3</td>
<td>Sample with 0.2 g extract/100g yogurt sample</td>
</tr>
</tbody>
</table>

Table 3. Antioxidant activity of yogurt samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>AA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YK</td>
<td>54.2±2.3</td>
</tr>
<tr>
<td>Y1</td>
<td>58.3±2.2</td>
</tr>
<tr>
<td>Y2</td>
<td>63.6±1.8</td>
</tr>
<tr>
<td>Y3</td>
<td>68.5±2.1</td>
</tr>
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</table>

Table 4. Pysicochemical analysis results of yogurt samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Dry Matter (%)</th>
<th>pH</th>
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</thead>
<tbody>
<tr>
<td>YK</td>
<td>12.1±0.3</td>
<td>4.24±0.04</td>
</tr>
<tr>
<td>Y1</td>
<td>12.2±0.7</td>
<td>4.16±0.05</td>
</tr>
<tr>
<td>Y2</td>
<td>12.1±0.8</td>
<td>4.11±0.06</td>
</tr>
<tr>
<td>Y3</td>
<td>12.3±0.2</td>
<td>4.08±0.03</td>
</tr>
</tbody>
</table>

Table 5. Microbial analysis results of yogurt samples (log cfu/g)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total coliforms</th>
<th>TMAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>YK</td>
<td>&lt; 1.0</td>
<td>8.24</td>
</tr>
<tr>
<td>Y1</td>
<td>&lt; 1.0</td>
<td>7.61</td>
</tr>
<tr>
<td>Y2</td>
<td>&lt; 1.0</td>
<td>7.33</td>
</tr>
<tr>
<td>Y3</td>
<td>&lt; 1.0</td>
<td>7.29</td>
</tr>
</tbody>
</table>
CONCLUSION

In this research, it was demonstrated that grape seed extract can be successfully used for the fortification of traditional yogurt. The addition of grape seed extract did not change the physicochemical quality characteristics of yogurt samples. There was an increase in antioxidant activities of traditional yogurt samples and sensory analyzes showed that the addition moderate (0.1-0.15 g / 100 g) concentrations of extract had a desirable effect on sensorial characteristics. Therefore, the fortification of traditional yogurt with of low concentrations of grape seed extract may result favorable product in terms of sensorial characteristics and the yogurt gains functionality which makes this healthier dairy product more desirable in terms of consumer perception.

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