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RESEARCH ARTICLE

Utilization of Black Grape Pomace in the Production of Shalgam Juice: Effect on the Ethyl Alcohol Levels

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Abstract

Ethanol naturally occurs at different levels in some foodstuffs resulting from the plant metabolism or fermentation of carbohydrates. The ethanol content of foods produced by the plant metabolism is highly lower than that of ethanol level arisen from the fermentation. Due to being the end product of anaerobic alcoholic fermentation by yeast and lactic acid fermentation by heterofermentative lactic acid bacteria, the foods subjected to both the alcoholic and lactic acid fermentations contain ethanol. Shalgam juice is a traditional Turkish beverage subjected to lactic acid fermentation. Although the main fermentation (lactic acid fermentation) progress via lactic acid bacteria, *Saccharomyces* and *non-Saccharomyces* yeast also have a role in the production of both ethanol and flavor compounds in shalgam juice.

In this research, enrichment of shalgam juice in terms of polyphenolic compounds via grape pomace addition as a waste of fruit juice industry, evaluation of grape pomace as a value-added product, and determination of the change in ethanol content during the enrichment were aimed. Grape pomace was substituted with black carrot in the formulation and 9 different formulations were obtained according to the grape pomace-black carrot ratios. Fermentation took place at ambient temperature for 44 days, and ethanol content was determined by gas chromatography.

When taking into consideration the grape pomace and black carrot ratios, the lowest ethanol content (0.302 g/100 ml) was determined in the shalgam juice produced from the formulation containing only black carrot. As grape pomace ratio increased in the formulation, higher ethanol contents were found in the shalgam juices. And the highest value (1.048 g/100 ml) was observed in the shalgam juice containing only grape pomace. As a conclusion, the levels of ethanol in the samples containing 50% black grape pomace+50% black carrot and 25% black grape pomace+75% black carrot, which is appreciated and accepted by the consumers, were 0.832% and 0.463%, respectively. Both levels were below 1.2% specified in the relevant legal regulations; Therefore, it was shown in this study that black grape pomace could be used in Shalgam juice production without the need to write the alcohol level on the label.

Keywords: Ethyl alcohol, shalgam juice, black grape pomace, black carrot, fermentation

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Şalgam Suyu Üretiminde Siyah Üzüm Posasının Kullanımı: Etil Alkol Düzeyine Etkisi

Öz

Etanol bitki metabolizması ve karbonhidratların fermantasyonu sonucu bazı gıda maddelerinde farklı düzeylerde doğal olarak oluşmaktadır. Bitki metabolizması yoluyla üretilen gıdaların etanol içeriği fermantasyondan meydana gelen etanol içeriğinden oldukça düşüktür. Maya ile anaerobik alkolik fermantasyonun ve heterofermantatif laktik asit bakteri yoluyla laktik asit fermantasyonun son ürünü olması nedeniyle hem alkolik hem de laktik asit fermantasyonlarına maruz kalan gıdalar etanol ihtiva eder. Şalgam suyu, laktik asit fermantasyonuna tabi tutulan geleneksel bir Türk içeceğidir. Ana fermantasyon (laktik asit fermantasyonu) laktik asit bakterileri yoluyla ilerlemesine rağmen, *Saccharomyces* ve *Saccharomyces* olmayan mayalar da Şalgam suyunda hem etanol hem de aroma bileşiklerinin oluşumunda bir rol oynar.

Bu araştırmada, meyve suyu endüstrisi atığı olarak üzüm posası ilavesi ile Şalgam suyunun polifenolik bileşikler açısından zenginleştirilmesi, üzüm posasının katma değeri yüksek bir ürün olarak değerlendirilmesi ve zenginleştirme sırasında etanol içeriğindeki değişimin belirlenmesi amaçlanmıştır. Kara üzüm posası, formülasyonda siyah havuç ile ikame edilmiş ve üzüm posası-kara havuç oranlarına göre 9 farklı formülasyon elde edilmiştir. Fermantasyon, ortam sıcaklığında 44 gün süreyle gerçekleştirilmiş ve etanol içeriği gaz kromatoğrafisiyle belirlenmiştir.

Üzüm posası ve siyah havuç oranları dikkate alındığında, sadece siyah havuç içeren formülasyondan üretilen Şalgam suyunda en düşük etanol içeriği (0.302 g/100 ml) belirlendi. Formülasyonda üzüm posası oranı arttıkça, Şalgam sularında daha yüksek etanol belirlenmiştir. En yüksek değer ise (1.048 g/100 ml) sadece üzüm posası içeren Şalgam suyunda görülmüştür. Sonuç olarak, tüketiciler tarafından beğenilen ve kabul gören %50 siyah üzüm posası + %50 siyah havuç ile %25 siyah üzüm posası+%75 siyah havuç içeren örneklerde etil alkol seviyeleri sırasıyla %0.832 ve %0.463 olarak belirlendi. Her iki seviye de ilgili yasal düzenlemelerde belirtilen %1.2'nin altında olduğu görüldü. Bu nedenle bu çalışmada siyah üzüm posasının etiket üzerine alkol seviyesi yazılmasına gerek kalmadan şalgam üretiminde kullanılabileceği gösterilmiştir.

Anahtar Kelimeler: Etil alkol, şalgam suyu, siyah üzüm posası, siyah havuç, fermantasyon

1. Introduction

Fermentation is a kind of food preservation method that has been applied to perishable foods to produce more stable food products for many centuries. While the main purpose of fermentation is to produce shelf-stable foods, especially in recent years, fermented foods are taken place in the human diet because of their enhanced sensorial and nutritional characteristics. Microbiological activities and chemical reactions simultaneously progress in foods during the fermentation. According to the degree and kind of these activities and reactions various fermentation products such as organic acids, ethanol, CO_2, esters, aldehydes, and ketones occur in fermented foods (Hui et al., 2004). These products lead to produce fermented foods that differ in terms of sensorial and nutritional characteristics. Food fermentations base on three main metabolic pathways which are anaerobic alcoholic fermentation, lactic acid fermentation, and aerobic acetic acid fermentation. Carbohydrates are converted to alcohol and CO_2 in anaerobic alcoholic fermentation and lactic acid in lactic acid fermentation. Different from these two fermentations, alcohol is used as a substrate in acetic acid fermentation and converted to acetic acid. Alcohol and organic acids are the main end products of these fermentations and they have significant effects on both improving the shelf life of food products and contributing flavor (Hui et al., 2004). However, lactic acid fermentation progress according to two different metabolisms as homofermentative and heterofermentative. While the end product is only the lactic in homofermentative fermentation, lactate, acetate, ethanol, and carbon dioxide in addition to lactic acid occur in heterofermentative fermentation (Bamforth & Cook, 2019).

Shalgam juice is a traditional lactic acid fermented beverage and produced from black carrot, turnip, sourdough, salt, and water (Kabak and Dobson, 2011). Yeast, homofermentative, and heterofermentative lactic acid bacteria play a role in the shalgam juice fermentation and shalgam juice contains the metabolic products of these microorganisms such as lactic acid, acetic acid ethanol, carbonyl compounds, volatile acids, higher alcohols, esters, terpenols, norisoprenoids, lactones, and volatile phenols (Altay et al., 2013; Canbaş &Deryaoğlu, 1993; Tangüler & Erten, 2012).

Phenolic compounds are thought to be fingerprints of the plants and each plant has a different polyphenol profile. Black carrots and black grapes contain different phenolic compounds. While black carrot is rich in anthocyanins and phenolic acids, black grape contents higher amounts of flavan-3-ols, flavonol glycosides, and stilbene phytoalexins in addition to anthocyanins and phenolic acids. Each phenolic group possesses different antioxidant activity and health beneficial effects.

From this perspective, this study aimed to determine the ethanol content of shalgam juice whether or not to change, while it was enriched in terms of phenolic compounds by using grape pomace.

2. Material and Method

2.1. Material

The black carrot, bulgur flour, salt, and *Saccharomyces cerevisiae* were obtained from Gunseven Company, Ereğli, Konya, Turkey. Black carrots, washed under running tap water, were cut into slices approximately 4 cm in length. Bulgur flour wetted with pure water was fermented with *Saccharomyces cerevisiae* in a jar at room temperature for 24 h. Ekşikara grape variety used in this study was supplied from a viticulturist in Taşkent, Konya. The pomace was acquired after washing, removing the stalks, and pressing the grapes. The brine was prepared at a concentration of 1.4% and rested overnight.

2.2. Method

Sliced black carrot and pre-fermented bulgur flour were put into a jar (5 L) and fulfilled to 5 L with the brine. The black carrot was replaced with different amounts of grape pomace to produce other formulations. Nine different formulations were obtained according to the black carrot and pomace ratio and samples were coded as S100, S90, S80, S75, S70, S60, S50, S25, and S0 (Table 1). S100 sample, containing only black carrot, was used as the control. After lidding, all jars were left at ambient temperature to ferment for 40 days. Fermentation was monitored by titratable acidity. The end-point of fermentation was determined according to the titratable acidity remaining constant.

Table 1.
Sample codes as a result of black carrot and pomace ratios

Sample	Code
100% Black carrot + 0% Pomace	S100
90% Black carrot + 10% Pomace	S90
80% Black carrot + 20% Pomace	S80
75% Black carrot + 25% Pomace	S75
70% Black carrot + 30% Pomace	S70
60% Black carrot + 40% Pomace	S60
50% Black carrot + 50% Pomace	S50
25% Black carrot + 75% Pomace	S25
0% Black carrot + 100% Pomace	S0

2.3. Ethanol Analysis

Ethanol content of shalgam juices was determined by a gas chromatography (GC) (Agilent, 7820A). Shalgam juices were filtered through a 0.45 µm pore size syringe filter and 1 µl of it injected to the GC. Separation was achieved in a CP-Wax-57CB column. Hydrogen was used as a carrier gas. FID (Flame Ionization Detector) was used in detection (Anonymous, 2011).

Statistical Analysis

The results were given as mean± standard deviation and subjected to analysis of variance. The differences between the means were determined by Duncan's Multiple Comparison Test. Minitab (Released 14, Minitab Inc. USA) and Mstat C (Mstat C, 1988) programs were used in statistical analysis.

3. Results and Discussion

Ethanol is one of the end products of heterofermentative lactic acid fermentation (Deryaoğlu, 1990; Cogan &Jordan, 1994). However, both yeast and heterofermentative lactic acid bacteria play a significant role in shalgam juice fermentation and yeast also make a contribution to ethanol production during the fermentation (Gardner et al., 2001).

GC chromatogram of ethanol analysis and ethanol contents of shalgam juices are shown in Figure 2 and Table 2 (and Figure 1), respectively. As seen in Table 2, ethanol contents were range between 0.302-1.048 g/100 ml. The lowest value was found to be 0.302 g/100 ml in the sample (S100) produced by only 100% black carrot. As grape pomace amount increased in the formulations, the higher amount of ethanol was obtained in shalgam juices.

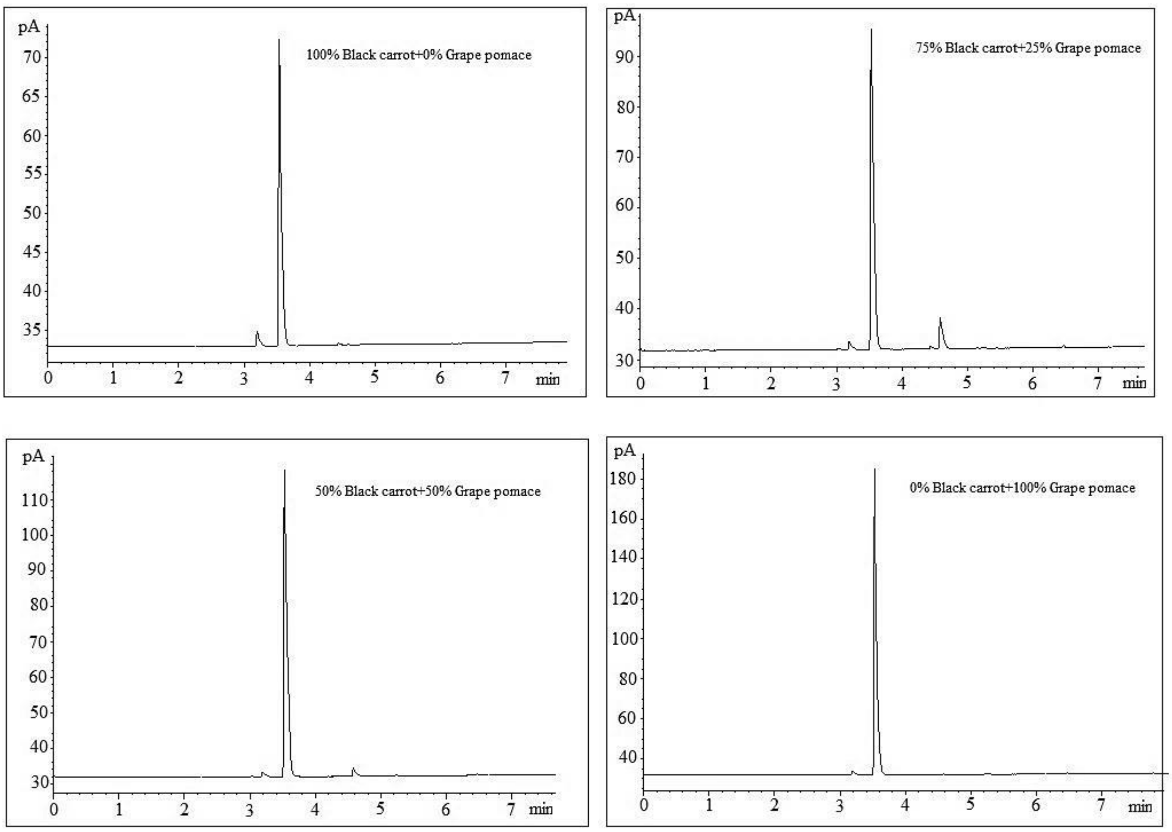

Figure 2: GC chromatogram of ethanol in shalgam juices

In a previous study, ethanol values in 20 (industrial) shalgam juices acquired from local markets produced by different suppliers reported between 0.19-4.76 g/L (Öztürk, 2009). According to previous studies, there is a significant difference in the ethanol concentration of shalgam juices. These can arise from variations in the formulation, materials used in the production, and fermentation conditions.

Table 2.
Ethanol contents of shalgam juices

Black carrot-grape pomace ratio	**Ethanol content (g/100mL)**
S100	0.302±0.030f
S90	0.360±0.023f
S80	0.441±0.012e
S75	0.463±0.009e
S70	0.512±0.015de
S60	0.590±0.030cd
S50	0.655±0.010c
S25	0.831±0.049b
S0	1.048±0.048a

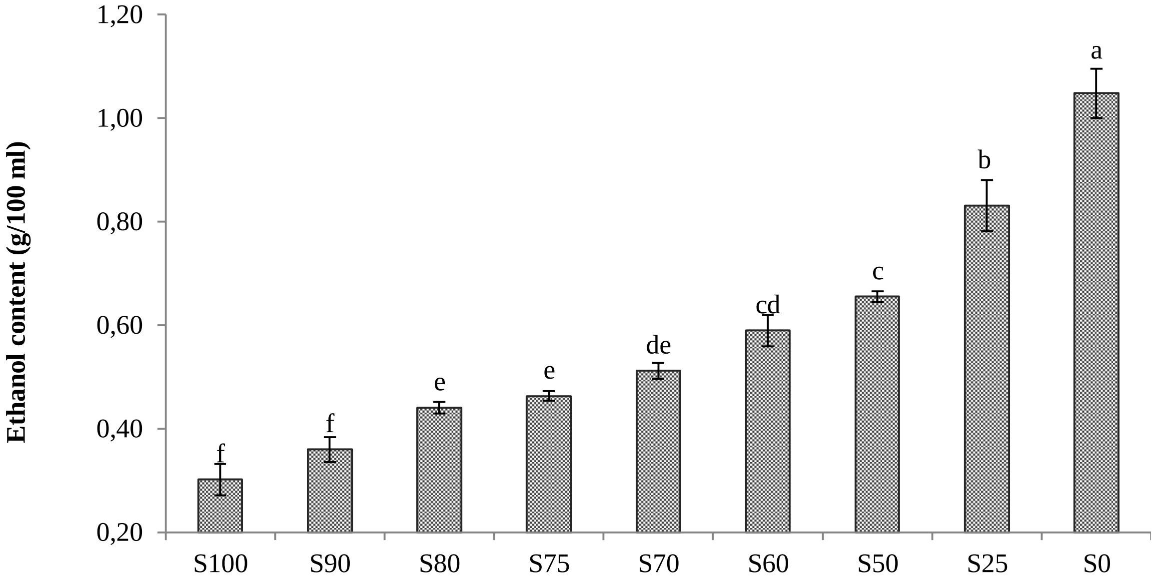

Figure 1. Ethanol contents of shalgam juices

Fruits and vegetables naturally contain very little amounts of alcohol, and higher ethanol values indicate that heterofermentative lactic acid and alcoholic fermentation have occurred. In a study, ethanol contents of fresh pear, orange, lemon, apple, and pineapple fruits have been detected as 0.019, 0.021, 0.002, 0.0073, 0.048%, respectively (Gündüz et al, 2013). An increase in ethanol content from 0.048 to 0.99% of the pineapple fruit after the storage at 4 ^{o}C for ten days was determined by Gündüz et al, 2013. Similarly, in the same study, it was reported that the ethanol content of unprocessed grape juice (2.11%) increased to 5.60% at the end of the 10 days (Gündüz et al, 2013).

Ekşikara grape has sweet, dark-skinned, and aromatic berries and is generally consumed as fresh or after processing to the raisin and molasses (Coklar, 2017). Its fructose, glucose, and saccharose contents were reported as 10.35, 9.48, and 0.35 g/100 g, respectively (Coklar&Akbulut, 2016).

The main fermentable sugar source is the black carrot in shalgam juice fermentation (Altay et al., 2013). Nagraj et al. (2020) have reported sucrose, glucose, and fructose contents of carrots as 3.59, 0.59, and 0.55%, respectively. Grape pomace contains higher amounts of fructose and glucose than that of black carrot. Corbin et al. (2015) have reported the glucose and the fructose concentrations for the pomaces of two different grape varieties (Sauvignon Blanc and Cabernet Sauvignon) as 18.6-19.0% and 2.1-2.5%, respectively.

Glucose, fructose, and sucrose are the most common fermentable sugars found in vegetables. Glucose is the major and fructose is the second major substrate for lactic acid fermentation. Sucrose can be fermented after hydrolyzed to glucose and fructose by invertase released by the yeast. A limited number of lactic acid bacteria can ferment the sucrose. In most cases, sucrose remains without hydrolyzing and fermented at the end of the fermentation of vegetables (Hui et, al., 2004). In a previous study by Fleming et al. (1983) 46.43% of the sucrose found in carrot fermented using *Lactobacillus plantarum* for 35 days remained at the last stage of fermentation without metabolized. Increases in ethanol content of shalgam juices with increasing the grape pomace ratio can arise from passing of the higher amounts of fermentable sugars from grape pomace to the fermentation media than that of black carrot.

While the ethanol formation indicates the spoilage of fresh fruit and vegetable juices, ethanol formed during lactic acid fermentation contributes to the specific taste and flavor of shalgam juice (Erten et al., 2008). On the other hand, the ethanol content of non-alcoholic beverages has been limited by legal regulations, and also according to ethanol level beverage producers have to declare the ethanol content on product labels. In the unfermented non-alcoholic beverages such as fruit juice, nectar, fruit-flavored soft drink that are within the scope of Non-alcoholic Beverages Notification of Turkish Food Codex (Number: 2007/26), the highest permissible ethanol content, naturally formed, is 3.0 g / L (Anonymous, 2007). According to the Turkish Food Codex Regulation on labeling and provision of food information to consumers (January 2017, 29960), producers have to state the alcohol contents of beverages on product labels, if the actual alcohol content of beverage by volume is more than 1.2% (v/v) (Anonymous, 2017).

4. Conclusion

In this study, the effect of adding black grape pomace to the fermentation medium on alcohol formation was investigated in order to improve the physical, chemical and nutritional properties of Shalgam juice. With the proportional increase of black grape pomace, the alcohol level formed by fermentation increased. The levels of ethyl alcohol in the samples containing 50% black grape pomace + 50% black carrot and 25% black grape pomace + 75% black carrot, which is appreciated and accepted by the consumers, were 0.832% and 0.463%, respectively. Both levels were below 1.2% specified in the relevant legal regulations; therefore, it is not necessary to write it on the label. As a conclusion, when taking into consider the legal regulations on ethanol content of beverages 50% black carrot+50% black grape pomace ratio can be used in shalgam juice fermentation.

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