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Evaluation of Grape Pomace in Production of Shalgam Juice: Effects on Some Physicochemical, Microbiological and Sensorial Properties

Mehmet Akbulut[1]*
https://orcid.org/0000-0001-5621-8293

Nedim Tekçe[1]
https://orcid.org/0009-0001-5559-8607

Hacer Çoklar[1]
https://orcid.org/0000-0002-4948-0960

[1]Selcuk University, Agriculture Faculty, Department of Food Engineering, Konya, Türkiye

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*Correspondence: makbulut44@gmail.com; Tel.: +90-332-2232915 (M.A.).

HIGHLIGHTS

- Grape pomace addition affected the microbiological characteristics of shalgam juice.
- The cloudier shalgam juice were obtained with the addition of grape pomace.
- Shalgam juice containing 25% pomace was sensorially similar with traditional shalgam juice.

Abstract: Shalgam juice is a red colored and cloudy traditional fermented beverage produced by lactic acid fermentation of bulgur flour, black carrot, salt, turnip, sourdough, and water. In this study, the usability of grape pomace as a black carrot substitute for the enrichment of shalgam juice with phenolic compounds was investigated. Five different ratios of pomace (0, 25, 50, 75, and 100%) were added to the formulation as a substitute of black carrot and samples were analyzed for pH, acidity, soluble solid content (SSC), color (L*, *a**, *b**, C*, *h*, color density, color tone), microbiologically and sensorially during the fermentation to find out the effects of pomace on the progress of the fermentation. The shalgam juice containing only black carrot was used as control. The cloudiness, pH, and brightness of the shalgam juice increased as the pomace ratio increased. The control contained less yeast and mold counts than the pomace containing the shalgam juices. Grape pomace addition did not have an effect on the total mesophilic aerobic and the lactic acid bacteria counts. The sensorial properties of the shalgam juice containing 25% grape pomace were almost the same as the control sample, and as the pomace ratio increased, the shalgam juice samples could not meet the consumer's taste and expectations. In conclusion, when considering microbiological, sensorial and physicochemical properties, 25% grape pomace can be added to formulation before the fermentation as a carrot substitute.

Keywords: Black carrot; fermented beverage; lactic acid fermentation; waste evaluation; quality parameters.

INTRODUCTION

The negative effects of climate change have increased the sensitivity of human beings to the environment in recent years. Evaluation of industrial wastes to minimize their damage to the environment has become one of the important research and study field. Many agro-industrial wastes are used as animal feed and fertilizer or used as a carbohydrate source in microbial fermentation processes [1,2]. Grape pomace is a waste of grape juice and wine industry and generally evaluated as low value-added products such as animal feed or fertilizer [3]. It has been stated that it causes some negative effects on plants where it is used as fertilizer, and animals especially due to its high polyphenolic content. Therefore, it should be used after purification [1,2]. It is known that grape pomace is rich in various functional components such as tocopherols and phenolic substances, especially resveratrol. In recent years, the positive effects of resveratrol consumption on health have attracted attention and it is recommended to consume resveratrol-containing foods and resveratrol supplements. Resveratrol, a stilbene belonging to the phytoalexin group, has attracted attention in recent years due to its antioxidant, antifungal, anti-inflammatory effects, preventing or delaying cardiovascular diseases and cancer [4,5].

Grapes and wine are recognized as the most important dietary sources of resveratrol. Resveratrol is found in the skin of grapes rather than the flesh. Likewise, the amount of resveratrol in grapes is higher than in its juice. This indicates that resveratrol is removed together with the pomace during the processing. The fact that wine contains more resveratrol than grape juice is attributed to resveratrol transfer from the skin during the fermentation.

Shalgam juice is a traditional fermented beverage with red color, cloudy and sour flavor, produced by the spontaneous lactic acid fermentation of bulgur flour, black carrot, salt, turnip, sourdough and water, and its fermentation takes approximately 2-4 weeks at 10-35 ^{o}C.

Lactic acid produced by lactic acid bacteria throughout the fermentation gives a sour taste to the beverage and prolongs the shelf life of shalgam juice. Shalgam juice contains 2.0-4.0% total dry matter, 0.018-0.09 % protein, 1.1-2.2% salt, 1.46-2.06% ash, 0.106-0.91% total acidity, 0.71-2.06% ethanol. Its pH is ranged between 3.15 and 4.25 [6-8].

Sugar, which is necessary for the growth of lactic acid bacteria, is found in raw materials, especially black carrot. Bulgur flour in the formulation is a source of nutrients for microorganisms in the first stage of fermentation. Microbiota of shalgam juice consists of *Lactobacillus* (89.63%), *Leuconostoc* (9.63%) and *Pediococcus* (0.74%) [6]. It has been noted that the amount of lactic acid bacteria in shalgam juice varies between 1.1×10^{5}-1.5 10^{6} cfu/mL [9]. Black carrot, which is used as the main raw material in the production of shalgam juice, provides the enrichment of shalgam juice with the polyphenolic compounds, while contributing to the taste and aroma as well as the formation of red color.

The aim of this study is to determine the effects of grape pomace, which is used as a black carrot substitute for enrichment with polyphenolic compounds on product quality and fermentation process and to reveal its usability in shalgam juice production.

MATERIAL AND METHODS

Materials

Bulgur flour, salt, water, black carrot, grape pomace, and *Saccharomyces cerevisiae* were used in the production of shalgam juice. Black carrot, *S. cerevisiae* and bulgur flour were obtained from Gunseven Inc. Salt was obtained from a local market. Ekşikara grape was acquired from a vineyard in Hadim-Konya and its pomace was produced in the pilot plant of Selçuk University Faculty of Agriculture.

Fermentation method

Shalgam juice was produced by two-stage fermentation method. Firstly, bulgur (0.91%) was fermented with yeast (0.2%) before the main fermentation in the jar (10 L) at 28 ^{o}C for 24 hours. After the first fermentation, salt (1.16%) and black carrot (16.6%) were added to the jar and filled with the water up to 10 L. The sample prepared according to this formulation was accepted as the control sample. While the amounts of yeast, bulgur flour, salt, and the water remained constant in other formulations, black carrot was substituted with grape pomace at different ratios by weight (25, 50, 75 and 100%). The samples were coded as S1 (100% black carrot), S2 (75% black carrot+ 25% grape pomace), S3 (50% black carrot+ 50% grape pomace), S4 (25% black carrot+ 75% grape pomace), and S5 (100% grape pomace) according to ratios of black carrot and grape pomace. The lids of the jars were tightly closed and left to fermentation. The fermentation was carried out at 20^{o} C. The progress of the fermentation was monitored by measuring the total titratable acidity and terminated when the increase in acidity ended. After the filtration, the production of shalgam juice was completed and lasted an average of 44 days.

Determination of titratable acidity, pH, soluble solid content, and turbidity

The pH values of the samples were determined by direct measurement with the pH meter (Inolab 720, WTW, Weilheim, Germany) [10]. To determine the acidity, the samples were titrated with NaOH solution (0.1N) until the pH reached to 8.1 and the results were given as g lactic acid (LA) equivalent/100 mL. The soluble solid content values were determined with the refractometer (HSR-500, Atago, Japan) at 20 °C [10]. The turbidity was measured using the turbidimeter (WTW TURB 430 T) and the results were given as Nephelometric Turbidity Units (NTU) [11].

Color analysis

Spectrophotometric color

Shalgam juice was centrifuged for 10 min at 1048 x g at room temperature and absorbances were measured at 420, 520 and 620 nm on the spectrophotometer (U-1800, Hitachi, Japan) with 1 cm path length cells. The sum of these absorbance values was given as color intensity (CI). The absorbance ratio at 420 and 520 nm was given as color tone. Yellow color, red color and blue color were also calculated from the following equations using absorbance at 420, 520, and 620 nm wavelenghts and color intensity [12].

$$OD420\ (\%) = (ABS_{420}/CI)\ x\ 100 \quad (1)$$

$$OD520\ (\%) = (ABS_{520}/CI)\ x\ 100 \quad (2)$$

$$OD620\ (\%) = (ABS_{620}/CI)\ x\ 100 \quad (3)$$

CIELAB color parameters

The CIELAB color values (*L*, a*, b*,* C* and *h*) of the samples were measured using the spectrophotometer (CM-5, Konica Minolta, Osaka, Japan) equipped with the cell holder (10 mm, CM-A207) [11].

Determination of total mesophilic aerobic (TMA), lactic acid (LA), and coliform bacteria and yeast-mold count

Ten mL of the sample was taken and homogenized with 90 mL of sterile peptone water (0.1%), and a serial dilution was prepared at the levels of 10^{0}-10^{-8}. One mL of dilutions was transferred to separate petri dishes for lactic acid bacteria (LAB), total mesophilic aerobic bacteria (TMAB), coliform bacteria, yeast, and mold counts. Plate count agar, deMan Rogosa Sharpe agar, violet red bile agar, and potato dextrose agar were used for TMAB, LAB, coliform bacteria, and yeast and mold counts, respectively. Petri dishes were incubated under aerobic conditions for 48 hours at 30 °C for TMAB count and 4 days at 28 °C for yeast-mold count. The deMan Rogosa Sharpe agar and the violet red bile agar were incubated under anaerobic conditions for 72 hours at 35 °C and 24 hours at 35 °C, respectively. Tartaric acid (10%) was added to potato dextrose agar just before the samples were poured into the petri dishes to prevent bacterial growth [11].

Sensorial evaluation

The sensorial analysis of shalgam juices was carried out with a group of 20 trained male and female, 22-48 years of age. Ten mL of sample was served to the panelists in the 20 mL glasses at 5 ^{o}C temperature, together with whole grain wheat crackers and water. The panelists were asked to rate how much they liked and disliked each shalgam juice in terms of color, aroma, taste, appearance, and overall acceptability on a 9-category hedonic scale (9 = extremely like; 1 = dislike at all) [13].

Statistical analysis

The sensorial analysis results were given as mean ± standard deviation (SD) and subjected to variance analysis (ANOVA) at 95% confidence interval. Variance analysis was performed in the MINITAB (Released 14, Minitab Inc. USA) package program. Duncan's multiple range test was performed in the MSTAT-C package program to determine the differences between the means.

RESULTS AND DISCUSSION

Titratable acidity, pH, turbidity, and soluble solid content

Shalgam juice is a kind of fermented beverage produced by lactic acid fermentation, and the lactic acid produced by lactic acid bacteria during fermentation makes a significant contribution to prolong the shelf life of the final product. Titration acidity is used to determine the end point of the fermentation and the fermentation of shalgam juice takes about 2-4 weeks. On the other hand, industrially, the fermentation is generally prolonged nearly 3-4 weeks to improve the flavor of shalgam juice, and microbiological activity and chemical interactions also continues during this stage. In this study, the titratable acidity of shalgam juices was determined every 7 days for 52 days (Table 1).

Table 1. The pH, titratable acidity, turbidity and soluble solid content of shalgam juice samples

Sample	Fermentation time (day)	pH	Titratable acidity (g/100 mL)	Turbidity (NTU)	SSC (%)
	9	3.14±0.08^{hi}	0.42±0.07	277.33±104.60	2.07±0.12
	16	3.37±0.07^{b-i}	0.37±0.05	258.67±101.30	2.13±0.12
	24	3.23±0.07^{d-i}	0.41±0.07	49.03±4.92	2.00±0.00
S1	30	3.22±0.03^{e-i}	0.36±0.04	149.80±86.30	2.07±0.12
	37	3.34±0.09^{b-i}	0.37±0.04	178.87±109.60	2.03±0.06
	44	3.30±0.11^{c-i}	0.49±0.02	206.33±112.50	2.07±0.06
	52	3.27±0.16^{c-i}	0.45±0.07	186.67±130.10	2.10±0.00
	9	3.31±0.07^{b-i}	0.35±0.04	305.33±41.50	2.00±0.00
	16	3.50±0.03^{a-h}	0.38±0.04	163.33±20.00	2.00±0.00
	24	3.35±0.03^{b-i}	0.40±0.09	49.27±23.10	2.00±0.00
S2	30	3.32±0.14^{b-i}	0.37±0.04	101.73±57.90	2.03±0.06
	37	3.57±0.09^{a-f}	0.39±0.07	110.47±59.70	2.00±0.00
	44	3.36±0.15^{b-i}	0.51±0.10	179.00±42.10	2.07±0.06
	52	3.41±0.11^{b-i}	0.45±0.08	193.67±78.50	2.03±0.06
	9	3.39±0.14^{b-i}	0.36±0.03	344.30±14.20	1.97±0.06
	16	3.56±0.14^{a-f}	0.34±0.03	248.30±66.30	2.03±0.06
	24	3.28±0.10^{c-i}	0.47±0.09	77.90±24.70	2.00±0.00
S3	30	3.27±0.15^{c-i}	0.47±0.08	181.30±64.70	2.10±0.10
	37	3.41±0.14^{b-i}	0.51±0.07	154.60±76.10	2.07±0.06
	44	3.20±0.03^{f-i}	0.63±0.05	261.30±21.10	2.13±0.12
	52	3.17±0.04^{ghi}	0.59±0.03	258.30±42.40	2.13±0.12
	9	3.22±0.13^{e-i}	0.39±0.09	376.00±66.80	2.13±0.23
	16	3.61±0.19^{a-d}	0.37±0.07	353.30±79.80	2.20±0.35
	24	3.40±0.15^{b-i}	0.41±0.09	74.30±33.00	2.13±0.32
S4	30	3.48±0.13^{a-h}	0.36±0.05	260.00±88.60	2.20±0.36
	37	3.63±0.15^{abc}	0.41±0.06	209.00±117.90	2.20±0.35
	44	3.40±0.09^{b-i}	0.51±0.08	346.30±82.30	2.23±0.32
	52	3.32±0.19^{b-i}	0.48±0.07	340.70±134.50	2.33±0.23
	9	3.06±0.05^{i}	0.30±0.06	441.70±59.50	2.17±0.06
	16	3.52±0.08^{a-g}	0.28±0.02	532.00±33.20	2.20±0.00
	24	3.65±0.09^{abc}	0.33±0.10	323.70±150.10	2.17±0.06
S5	30	3.68±0.15^{ab}	0.30±0.04	383.30±188.00	2.17±0.12
	37	3.85±0.09^{a}	0.31±0.03	256.80±201.00	2.23±0.06
	44	3.60±0.12^{a-e}	0.40±0.07	403.30±168.40	2.27±0.06
	52	3.49±0.20^{a-h}	0.41±0.08	371.00±176.00	2.33±0.12

S1: 100% black carrot, S2: 75% black carrot+ 25% grape pomace, S3: 50% black carrot+ 50% grape pomace, S4: 25% black carrot+ 75% grape pomace, S5: 100% grape pomace, NTU: Nephelometric Turbidity Unit; SSC: Soluble Solid Content.

Different letters in the same column indicate statistically significant differences between samples ($P<0.05$) The differences between the values in the unlettered column were not statistically significant ($P>0.05$).

The titratable acidity and the pH values of the S1 sample (containing 100% black carrot) on the 9th day of the fermentation found to be 0.42±0.07 g/100 mL and 3.14±0.08, respectively. The titratable acidity of the sample increased to 0.45±0.07 g/100 mL at the last stage. When considering the whole fermentation progress, the grape pomace addition to the formulation before the fermentation caused a decrease in the titratable acidity and increase in the pH value of shalgam juice. Both the use of the grape pomace in different ratios in the formulation ($p<0.01$) and the fermentation time ($p<0.01$) were effective on the pH and titration acidity of shalgam juice. Similar to the results of this study, in a previous study to determine the effects of black carrot ratio on shalgam juice, it was shown that higher black carrot ratios caused an increase in the titratable acidity of the juice [14].

Although fruits are acidic foods, the pH of vegetables is higher than fruits. This is due to the differences in the compounds such as acids, phenolic compounds, sugars, proteins in its structure and their concentrations. These compounds pass from the cell to the liquid fermentation medium via diffusion over time. Therefore, in our study, the pH, which was slightly low at the beginning, increased slightly as time progressed, as the black carrot and grape pomace components passed into the liquid medium by diffusion, and then a decrease was observed again due to the concentrations of fermentation acids formed as a result of the developing fermentation. It is thought that pH and acid content may have shown different tendencies towards increase and decrease due to the difference in the levels of components passing into the liquid medium and the different concentrations of fermentation acids such as lactic acid, acetic acid, propionic acid formed during fermentation [11, 15] and their different acidic powers.

The soluble solid content of the samples varied between 1.97-2.17% and 2.03-2.33% in the first and last stages of the fermentation, respectively. It can be said that the soluble solid content of shalgam juice increased as the grape pomace ratio increased. The S4, and S5 samples, containing 75 and 100% grape pomace ratio in their formulation, had higher soluble solid values than those of the S1, S2, and S3. There was no statistically significant change in the soluble solid content with the progress of the fermentation ($p>0.05$).

Shalgam juice is a cloudy beverage with an intense red color and slightly sour taste. Color and cloudiness are two important quality parameters for consumer acceptance for shalgam juice. The turbidity of the S1 sample containing 100% black carrot was found to be 277.33 NTU in the first stage of the fermentation, and this value decreased to 186.67 NTU towards the end of the fermentation. The addition of the grape pomace caused an increase in the turbidity and was measured as 441.7 NTU in S5 samples containing 100% grape pomace. As in the case of the titratable acidity, both the progress of the fermentation ($p<0.01$) and the grape pomace ratio ($p<0.01$) affected the turbidity of the shalgam juice.

Shalgam juice contains yeast, black carrot, and semolina. These ingredients contain high amounts of protein, hemicellulose, minerals, and polyphenolic compounds. Suspension of the particles consisting of protein, pectin, lipid, hemicellulose, cellulose and other minor components in a fluid food causes cloudiness [16]. The disintegration and fragmentation of macromolecules into small pieces increase the number and surface area of suspended particles and reduce the distance between particles, and thereby the turbidity of a fluid food increases. Disintegration and fragmentation of macromolecules due to microbial and enzymatic activities can lead to increased turbidity. In addition, the protein in yeast can interact with the haze forming-polyphenols in black carrot, or grape pomace to form a protein-polyphenol complex towards to the end of fermentation. The precipitation of this complex towards to the end of fermentation may have caused a clear shalgam juice.

Color results

Color is one of the most important quality characteristics of shalgam juice, and the intense red color of the beverage arises from the anthocyanins of black carrot and also have a contribution in red colored appearance. Table 2 shows the spectrophotometric color values of shalgam juice samples. OD420, OD520 and OD620 indicate the yellow, red, and blue components of the color. The OD420, OD520 and OD620 values of the S1 sample on the 9th day of the fermentation were found to be 60.48±0.69, 38.86±0.69, and 0.66±0.00, respectively and as the fermentation progressed, increases in OD520 and OD620 values and a decrease in OD420 value were observed. Lower OD520, and higher OD420 and OD620 values were observed in the S5 sample at every stage of fermentation compared to the other samples. The lowest color intensity was determined on the 9th day of the fermentation in all samples and increased as the fermentation progressed. The highest value was determined on the 16th day of the fermentation in the S1 sample. It was observed that the color intensity of the S5 sample was lower than the other samples. It was observed that the color intensity of the shalgam juice increased with the increase of the black carrot ratio. According to the variance analysis results, the effects of the fermentation time ($p<0.01$), the grape pomace ratio ($p<0.01$) and their interaction ($p<0.01$) on the color intensity, color tone, OD420, OD520, and OD620 values were found to be statistically significant. As the amount of grape pomace increased, OD420 (yellowness) and OD620 (blueness) values increased, while OD520 (redness) value decreased.

*L**, positive *a**, positive *b**, C*, and *h* values from reflectance color values denotes brightness, redness, yellowness, saturation, and hue angle, respectively. A decrease in *L** value from 100 to 0 indicates a decrease in brightness. The highest *L** value (82.38±5.87) was determined in the S5 sample at the last stage of fermentation (Table 3). With the increase in the grape pomace ratio in the shalgam juice samples, the increases in the *L** and h values, and the decreases in the *C** and *b** values were determined. The highest the *L** and *h* values measured at the shalgam juice containing 100% grape pomace (S5). While all reflectance color parameters affected from the grape pomace rates ($p<0.01$), significant changes were observed just in the *L**, *a** and C* values in the shalgam juices with the progress of fermentation. All reflectance color parameters, except the *L** value of shalgam juice decreased as fermentation progressed. The *a** and C* values of the S1, S2 and S3 samples were almost the same, but higher than the S4 and S5 samples. It is known that higher chroma value indicates higher color intensity [17]. According to the color intensity, the chroma and *a** values, more saturated and red colored beverages were obtained as black carrot ratio increased.

Table 2. The color composition, color intensity and color tone values of shalgam juice samples

Sample	Fermentation time (Day)	Color composition			Color intensity	Color tone
		OD420 (%)	OD520 (%)	OD620 (%)		
S1	9	60.48±0.69^{ab}	38.86±0.69^{k}	0.66±0.01^{j}	4.63±0.05^{cd}	1.56±0.05^{d}
	16	42.25±0.51^{fg}	48.87±0.80^{hi}	8.88±0.33^{ef}	5.44±0.09^{a}	0.87±0.02^{efg}
	24	41.37±0.60^{gh}	49.53±0.75^{ghi}	9.10±0.27^{ef}	5.37±0.08^{a}	0.84±0.02^{e-h}
	30	50.14±0.20^{cde}	48.18±0.37^{hij}	1.68±0.17^{j}	5.27±0.15^{a}	1.04±0.01^{ef}
	37	39.76±1.24^{ghi}	51.59±1.11^{fgh}	8.66±0.14^{ef}	5.15±0.11^{ab}	0.77±0.04^{e-i}
	44	38.69±1.72^{g-j}	52.56±1.63^{fg}	8.75±0.19^{ef}	5.07±0.16^{ab}	0.74±0.06^{f-i}
S2	9	65.59±0.99^{a}	33.14±0.75^{l}	1.27±0.24^{j}	3.99±0.12^{ef}	1.98±0.07^{ab}
	16	35.80±0.35^{h-k}	54.83±0.68^{def}	9.37±0.32^{ef}	4.85±0.06^{bc}	0.65±0.02^{ghi}
	24	35.00±0.47^{ijk}	56.43±1.11^{def}	8.57±0.64^{ef}	4.64±0.09^{cd}	0.62±0.0^{ghi}
	30	49.01±0.72^{de}	48.63±0.60^{hi}	2.37±0.22^{ij}	4.01±0.18^{ef}	1.01±0.03^{ef}
	37	33.56±0.66^{jk}	58.30±0.89^{bcd}	8.14±0.94^{ef}	4.48±0.08^{cd}	0.58±0.02^{ghi}
	44	32.69±0.84^{jk}	59.54±1.01^{abc}	7.78±1.05^{efg}	4.36±0.09^{de}	0.55±0.02^{hi}
S3	9	65.58±1.62^{a}	32.66±1.62^{lm}	1.76±0.02^{j}	2.64±0.17^{h}	2.01±0.15^{ab}
	16	30.73±0.28^{k}	60.44±1.23^{ab}	8.83±1.22^{ef}	3.85±0.11^{fg}	0.51±0.01^{i}
	24	30.78±0.29^{k}	61.38±0.83^{ab}	7.84±0.84^{efg}	3.83±0.09^{fg}	0.50±0.01^{i}
	30	50.12±0.95^{cde}	46.59±0.81^{ij}	3.29±0.15^{g-j}	2.73±0.11^{h}	1.08±0.04^{e}
	37	30.01±0.50^{k}	62.89±0.39^{a}	7.10±0.23^{e-i}	3.65±0.16^{fg}	0.48±0.01^{i}
	44	30.22±0.16^{k}	63.14±0.32^{a}	6.65±0.17^{f-i}	3.58±0.12^{g}	0.48±0.01^{i}
S4	9	66.48±3.16^{a}	30.70±2.63^{lmn}	2.82±0.55^{hij}	1.21±0.12^{kl}	2.18±0.29^{a}
	16	35.47±1.03^{h-k}	53.39±1.94^{ef}	11.15±1.26^{c-f}	2.02±0.21^{i}	0.67±0.04^{ghi}
	24	35.47±1.06^{h-k}	53.47±1.99^{ef}	11.06±1.09^{def}	1.91±0.14^{i}	0.66±0.04^{ghi}
	30	47.95±1.05^{ef}	44.59±0.97^{j}	7.46±0.52^{e-h}	1.30±0.11^{jk}	1.08±0.05^{e}
	37	35.30±1.11^{h-k}	53.14±1.64^{ef}	11.56±0.82^{b-e}	1.73±0.11^{i}	0.67±0.04^{ghi}
	44	35.82±0.91^{h-k}	53.26±0.81^{ef}	10.92±0.17^{def}	1.68±0.09^{ij}	0.67±0.03^{ghi}
S5	9	53.47±8.13^{cde}	31.35±1.09^{lmn}	15.18±7.04^{a-d}	0.36±0.21^{n}	1.71±0.31^{bcd}
	16	54.90±0.53^{bcd}	29.17±0.21^{mn}	15.93±0.33^{abc}	0.76±0.03^{m}	1.88±0.03^{abc}
	24	53.22±1.97^{cde}	29.25±0.81^{mn}	17.53±1.20^{a}	0.82±0.12^{lm}	1.82±0.12^{bcd}
	30	51.33±1.72^{cde}	32.22±0.14^{lmn}	16.45±1.86^{a}	0.49±0.11^{mn}	1.59±0.05^{cd}
	37	55.31±2.07^{bc}	28.69±0.71^{n}	16.00±1.37^{abc}	0.65±0.12^{mn}	1.93±0.12^{ab}
	44	53.75±1.78^{cde}	29.16±0.69^{mn}	17.10±1.09^{a}	0.65±0.12^{mn}	1.85±0.11^{bcd}

S1: 100% black carrot, S2: 75% black carrot+ 25% grape pomace, S3: 50% black carrot+ 50% grape pomace, S4: 25% black carrot+ 75% grape pomace, S5: 100% grape pomace, OD420: Optical Density at 420 nm; OD520: Optical Density at 520 nm; OD620: Optical Density at 620 nm.
Different letters in the same column indicate statistically significant differences between samples ($p<0.05$).

Many factors such as fermentation time [18], fermentation temperature [19] and salt [20] affect the color of shalgam juice. During the fermentation and storage of shalgam juice, copigmentation with lactic acid, polymerization and/or degradation can occur in anthocyanins, and these can cause color differences [21].

The decrease in pH during fermentation favors the anthocyanin copigmentation, it also increases the color intensity [22].

Black carrot and grape pomace contain different anthocyanins. Each anthocyanin has specific color, and their combination and concentrations determine the color of the food product. Other polyphenols in the fermentation media such as catechin, epicatechin and proanthocyanidins which arise from grape can act as a copigment to make a complex with anthocyanins and this increase the stability of color [23]. *Lactobacillus* strains, especially autochthonous ones, affect the color of fermented products [24,25]. Color losses during fermentation are attributed to decrease in copigmentation, formation of colorless pigment and adsorption of anthocyanins by yeast. Extraction of monomeric anthocyanins from grape skin and/or carrot and polymeric anthocyanin formation during fermentation can affect color [17].

Table 3. The reflectance color parameters of shalgam juice samples

Sample	FT (Day)	*L**	*a**	*b**	*C**	*h*°
S1	9	25.01±1.40	58.30±1.73^{b}	42.85±2.22	72.36±2.70^{abc}	36.30±0.60
	16	24.94±1.32	57.97±1.59^{b}	42.48±1.98	71.87±2.45^{abc}	36.22±0.52
	24	28.02±1.13	61.41±1.17^{ab}	46.64±1.44	77.11±1.79^{a}	37.21±0.36
	30	26.98±1.72	59.71±2.04^{ab}	44.64±2.20	74.56±2.93^{ab}	36.77±0.48
	37	27.12±2.05	59.37±2.52^{ab}	44.11±2.77	73.97±3.62^{ab}	36.59±0.76
	44	28.50±1.12	60.76±1.13^{ab}	45.24±0.81	75.75±0.97^{ab}	36.67±0.75
	52	29.80±0.88	61.89±0.74^{ab}	45.66±1.97	76.92±0.80^{a}	36.41±1.45
S2	9	30.11±0.73	61.38±0.55^{ab}	45.39±0.84	76.34±0.94^{ab}	36.48±0.26
	16	27.51±1.07	57.74±1.21^{b}	39.67±1.68	70.05±1.93^{abc}	34.48±0.62
	24	32.48±2.17	62.87±2.13^{ab}	40.47±1.89	74.77±2.81^{ab}	32.76±0.35
	30	32.72±1.06	62.44±0.72^{ab}	39.45±1.94	73.87±1.42^{ab}	32.27±1.17
	37	33.80±0.73	62.93±0.16^{ab}	38.71±3.29	73.91±1.89^{ab}	31.56±2.08
	44	34.01±0.79	62.66±0.16^{ab}	37.49±4.57	73.09±2.51^{abc}	30.83±2.97
	52	34.83±0.73	62.62±0.54^{ab}	34.47±6.44	71.80±3.38^{abc}	28.62±4.34
S3	9	35.57±1.71	60.90±0.70^{ab}	30.83±4.29	68.34±1.56^{bc}	26.80±3.43
	16	35.19±0.32	59.26±1.28^{ab}	26.35±4.87	64.93±3.07^{c}	23.86±3.57
	24	41.45±1.42	64.36±0.54^{a}	27.80±3.03	70.14±1.61^{abc}	23.33±2.13
	30	40.51±1.83	63.06±1.30^{ab}	30.39±4.02	70.09±0.90^{abc}	25.71±3.38
	37	40.35±2.60	62.21±1.46^{ab}	29.80±4.44	69.08±0.60^{abc}	25.57±3.85
	44	39.69±0.83	61.36±1.23^{ab}	31.33±0.98	68.90±1.36^{abc}	27.05±0.66
	52	41.48±0.96	62.26±0.66^{ab}	29.46±1.30	68.89±0.65^{abc}	25.32±1.09
S4	9	49.59±6.39	49.77±3.76^{c}	17.13±4.36	52.74±4.10^{d}	18.92±4.44
	16	51.47±2.49	45.16±3.46^{cd}	11.47±3.21	46.64±3.95^{de}	14.15±3.12
	24	59.06±3.07	45.31±3.31^{cd}	7.31±2.60	45.93±3.60^{de}	9.07±2.66
	30	55.37±1.47	43.85±3.23^{cd}	11.68±2.64	45.45±2.92^{de}	14.99±3.78
	37	55.98±2.05	42.06±2.84^{d}	10.52±3.96	43.50±2.34^{e}	14.12±5.67
	44	54.44±2.30	41.57±2.13^{d}	13.23±2.55	43.70±1.23^{e}	17.73±4.07
	52	56.88±2.81	41.20±1.32^{d}	12.31±3.58	43.10±1.05^{e}	16.62±4.90
S5	9	79.66±3.30	5.68±0.86^{e}	18.85±2.27	19.69±1.46^{f}	73.30±1.38
	16	76.69±0.58	6.28±0.24^{e}	19.61±0.12	20.59±0.18^{f}	72.27±0.56
	24	81.46±7.24	4.34±2.08^{e}	17.04±2.99	17.61±3.42^{f}	76.23±3.91
	30	78.11±7.79	5.11±2.11^{e}	17.88±3.51	18.62±3.92^{f}	74.61±3.88
	37	79.59±8.82	4.52±2.59^{e}	16.45±4.65	17.10±5.12^{f}	75.73±5.44
	44	77.89±8.05	5.52±2.00^{e}	18.00±2.91	18.84±3.36^{f}	73.36±3.51
	52	82.38±5.87	4.57±1.61^{e}	16.15±2.73	16.80±3.06^{f}	74.52±2.93

S1:100% black carrot, S2: 75% black carrot+ 25% grape pomace, S3: 50% black carrot+ 50% grape pomace,
S4: 25% black carrot+ 75% grape pomace, S5: 100% grape pomace, FT: Fermentation time
Different letters in the same column indicate statistically significant differences between samples ($p<0.05$). The differences between the values in the unlettered column were not statistically significant ($P>0.05$).

Microbiological properties

The changes in total mesophilic aerobic bacteria, yeast and mould, and lactic acid bacteria during the fermentation in the shalgam juice samples are shown in Figure 1. Coliform bacteria were not detected in any of the samples during the fermentation. Yeast-mould counts were lower than LAB and TMAB counts in all formulations throughout the fermentation. There were fluctuations in TMAB, yeast-mould, and LAB counts in all samples during fermentation. A decrease in the TMAB count was observed towards the 30th day of fermentation, and then an increase was observed. The lowest values in the S1, S2, S3, S4, and S5 samples were found as 7.34, 7.80, 8.12, 7.80, and 7.90 log CFU/mL on the 30th day of fermentation. The samples containing grape pomace possessed higher total bacteria in all periods, except the 9th, 24th, and 44th day of fermentation, than the control sample (S1). On the other hand, both the progress of fermentation ($p>0.05$), the grape pomace ratio ($p>0.05$) and also their interaction ($p>0.05$) had no statistically significant effects on the TMAB count of shalgam juice. The lowest LAB counts in all samples were observed on the 30th day of fermentation and ranged between 8.44 and 9.06 log CFU/mL. The highest values were determined in the S1 (7.41 log CFU/mL), and the S3 (7.79 log CFU/mL) on the 37th day, in the S2 (7.67 log CFU/mL), and the S4 (7.40 log CFU/mL) on the 44th day, and in the S5 (7.65 log CFU/mL) on the 16th day. While the LAB count of S5 was lower than the control (S1) in all stages, the S2, S3, and S4 samples contained higher LAB than the S1.

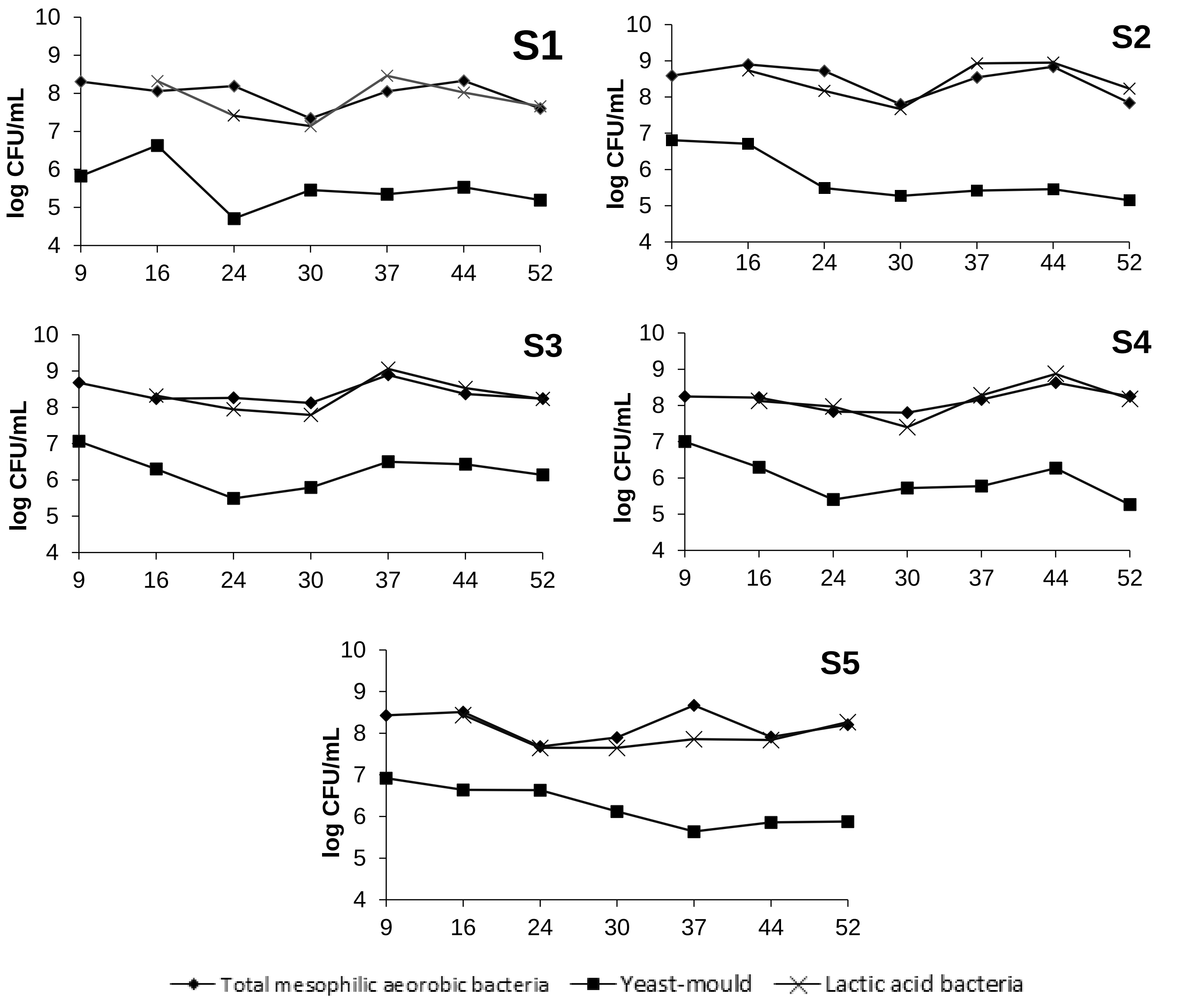

Figure 1. The total mesophilic aerobic bacteria, lactic acid bacteria and yeast-mould counts of shalgam juice samples (S1: 100% black carrot, S2: 75% black carrot+ 25% grape pomace, S3: 50% black carrot+ 50% grape pomace, S4: 25% black carrot+ 75% grape pomace, S5: 100% grape pomace)

However, among the parameters used in this study, only the fermentation time had a statistically significant effect on the LAB count (p<0.01) of shalgam juice. The highest yeast-mold count was observed on the 9th day of fermentation in all samples except the S1 sample. In the S1 sample, the highest yeast-mount count was observed on the 16th day and the lowest on the 24th day. In general, as the fermentation progressed, lower yeast-mould counts were determined in the samples, and it was found statistically significant (p<0.01). The highest count was found to be 7.06 log CFU/mL in the S3 on the 9th day and followed by the S4 (7.00 log CFU/mL), S5 (6.92 log CFU/mL), S2 (6.81 log CFU/mL), and S1 (6.63 log CFU/mL). Yeast-mould counts of the control sample (S1) in all stage from beginning to the end of fermentation were lower than the other samples. Grape pomace addition to the fermentation media at different rates caused increments in the yeast-mould count of shalgam juice, and these increments were found to be statistically significant (p<0.01).

Similar tendency in TMAB, LAB and yeast-mould counts during the fermentation of prunus mahaleb fruit [26], curly kale juice [27], radish roots [28], sauerkraut [29], solid residue of olive mill wastewater [30], shalgam juice [19, 14], mango juice [31], dongchimi [32] have been reported.

A spontaneous fermentation takes place in shalgam juice, which starts with alcoholic fermentation and then continues with lactic acid fermentation. Sourdough, carrot, and pomace used in fermentation contain hetero- and homo-fermentative lactic acid bacteria and yeast [20,33] and these microorganism work together during fermentation. Yeast and gram-negative bacteria are inhibited at the early stage of lactic acid fermentation due to the metabolites of LAB such as organic acids, carbon dioxide, ethanol, antifungal compounds, and bacteriocins [34,35]. Heterofermentative microorganisms predominate in the initial steps of vegetable fermentation and are replaced by more homofermentative microorganisms [36]. Homofermentative lactic acid bacteria produce more lactic acid and other microorganism in the media are inhibited due to their lower resistant to higher lactic acid concentration [37]. The fluctuation in LAB and yeast-mold populations may be due to the metabolites produced by microbial flora, organic acids, and pH of the media.

Sensorial properties

The shalgam juices were evaluated in terms of sensory at the end of fermentation. The radar/spider diagram of the shalgam juice samples created according to the sensory scores given by the panelists is shown in Figure 2. Statistically significant differences (p<0.01) in the color, flavor, aroma, taste, appearance, and overall acceptability have been found between the samples. The S1 (containing 100% black carrot) and S2 (containing 75% black carrot) samples were nearly same in terms of color, aroma, taste, appearance, and overall acceptability, while the S1 sample had the highest flavor. The S3 and S4 samples had nearly same, but lower than the S1 and S2 samples, hedonic scores in color, aroma, and appearance. Depending on the increase in the grape pomace ratio in the formulation, decreases were observed in hedonic scores and consumer preference. Consumer acceptance sharply decreased as grape pomace rate in the formulation increased. The S5 sample had the lowest color, flavor, and appearance scores, was the least preferred sample among all shalgam juices.

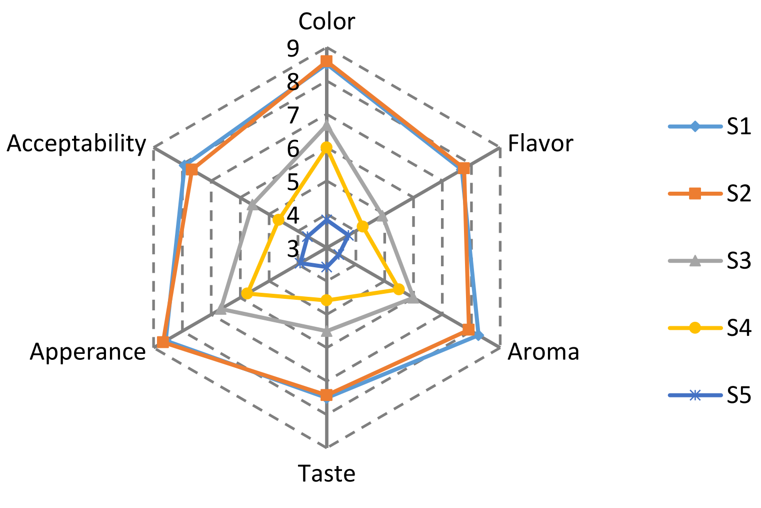

Figure 2. The radar plot of sensory evaluation of samples (S1: 100% black carrot, S2: 75% black carrot+ 25% grape pomace, S3: 50% black carrot+ 50% grape pomace, S4: 25% black carrot+ 75% grape pomace, S5: 100% grape pomace)

Color is one of the most important quality characteristics of shalgam juice, and consumers generally prefer specific dark red colored ones. As mentioned above, it was determined that both redness and color saturation decreased as the carrot ratio decreased, which negatively affected the consumer preference. The shalgam juices with higher pomace rates received lower scores because the desired taste, aroma and flavor in the shalgam juice could not develop due to lack of black carrot and also the aroma compounds originated from grape were added a different and unexpectable flavor to the shalgam juices. The panelists have stated that the vinegar-like taste and aroma were formed in the shalgam juices containing grape pomace, and became more distinct as pomace ratio increased, and the shalgam juice produced with 25% pomace is not different from traditional shalgam juice in terms of sensory characteristics.

CONCLUSION

In this study, the grape pomace was added to the shalgam juice formulation before the fermentation as a carrot substitute to improve the polyphenolic profile. The changes in some quality parameters such as color, acidity, pH, turbidity, and microbiological properties were investigated during the fermentation. At the end of the fermentation, all samples were sensorially evaluated to find out the consumer perspective. It was determined that the grape pomace addition at the levels of 75 and 100% negatively affected the color of shalgam juice. The use of higher levels of grape pomace caused cloudier shalgam juice. The shalgam juice without grape pomace contained higher yeast and mould count than other samples, while there was no difference between the samples in terms of their total mesophilic aerobic and lactic acid bacteria counts. No difference was observed in the samples containing 100% carrot and 25% pulp in terms of sensory properties. Consumer acceptance decreased sharply as the proportion of grape pomace in the formulation increased.

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REFERENCES

1. Das D, Panesar PS, Panesar G, Timilsena Y. Sources, Composition, and Characterization of Agro-Industrial Byproducts. P. S. P. E. Anil Kumar Anal, *Valorization of Agro-Industrial Byproducts*. Boca Raton, CRC Press. 11-30. ISBN: 9781003125679, 2022.
2. Mohamed H, Shah AM, Song Y. Conversion of Agro-industrial Wastes into Value-Added Products. M. I. Sarker, L. Liu, M. P. Yadav, H. O. Yosief and S. A. H. (Eds.), Conversion of Renewable Biomass into Bioproducts. Washington, DC, ACS Publications. 197-217. ISBN13: 9780841298149, 2021.
3. Vaccarino C, Curto RL, Tripodo M, Patané R, Ragno A. Grape marc as a source of feedstuff after chemical treatments and fermentation with fungi. Bio. Technol. 1992;40(1):35-41.
4. Mongioì LM, Perelli S, Condorelli RA, Barbagallo F, Crafa A, Cannarella R, et al. The role of resveratrol in human male fertility. Molecules 2021 26(9): 2495.
5. Vestergaard M, Ingmer H. Antibacterial and antifungal properties of resveratrol. Int. J. Antimicrob. Agents 2019 53(6):716-23.
6. Altay F, Karbancıoglu-Güler F, Daskaya-Dikmen C, Heperkan D. A review on traditional Turkish fermented non-alcoholic beverages: Microbiota, fermentation process and quality characteristics. Int. J. Food Microbiol. 2013;167(1):44-56.
7. Canbaş A, Deryaoğlu A. Shalgam suyunun uretim teknigi ve bilesimi uzerinde bir araştirma. Doga-Turk. J. Agric. For. 1993;17:119–29.
8. Özdestan O, Uren A. Biogenic amine content of shalgam (salgam): a traditional lactic acid fermented Turkish beverage. J. Agric. Food Chem. 2010;58(4):2602-8.
9. Baysal AHD, Çam M, Harsa HŞ. Functional properties of “Şalgam Juice”, a traditional fermented Turkish beverage. International Symposium on Functional Foods in Europe International Developments in Science and Health Claims, St. Julian’s, Malta, 9-11, 2007.
10. Cemeroglu B. Food analysis, Food Technology Society Publication. ISBN: 9789759857868, 2007.
11. Ulucan E, Çoklar H, Akbulut M. Application of ultrasound to extend the shelf life of shalgam juice: Changes in various physicochemical, nutritional, and microbiological properties. J. Food Process. Preser. 2022;46(5): e16501.
12. Ribéreau-Gayon P, Glories Y, Maujean A, Dubourdieu D. Handbook of Enology, Vole 2: The chemistry of wine stabilization and treatments, John Wiley & Sons. ISBN: 978-0-470-01038-9, 2021.
13. Magalhães KT, Dias DR, de Melo Pereira GV, Oliveira JM, Domingues L, Teixeira JA, et al. Chemical composition and sensory analysis of cheese whey-based beverages using kefir grains as starter culture. International J. Food Sci. Technol. 2011;46(4):871-8.

14. Tanguler H, Gunes G, Erten H. Influence of addition of different amounts of black carrot (*Daucus carota*) on shalgam quality. J. Food Agric. Environ. 2014;12(2):60-5.
15. Akbulut HF, Akbulut M. Mineral composition, the profile of phenolic compounds, organic acids, sugar and *in vitro* antioxidant capacity, and antimicrobial activity of organic extracts of *Juniperus drupacea* fruits. Food Sci. Nutr. 2023;11(10): 6435-46.
16. Tiwari B, Muthukumarappan K, O'donnell C, Cullen P. Effects of sonication on the kinetics of orange juice quality parameters. J. Agric. Food Chem. 2008;56(7):2423-8.
17. Garrido M, Bote ME, Moreno D, Adamez JD. Developme *1*(10), 6435-6446. nt of a Jerte Valley cherry-based beverage by fermentation of lactic acid bacteria and characterization of its potential functional value. Emir. J. Food Agric. 2020;32(10):711-22.
18. Tanguler H, Cankaya A, Agcam E, Uslu H. Effect of temperature and production method on some quality parameters of fermented carrot juice (Shalgam). Food Bio. 2021;41:100973.
19. Cirak MA, Agirman B, Erten H. The chemical, microbiological and sensory characteristics of şalgam during fermentation process. J. Food Process. Preserv. 2022;46(6): e15440.
20. Agirman B, Settanni L, Erten H. Effect of different mineral salt mixtures and dough extraction procedure on the physical, chemical and microbiological composition of Şalgam: A black carrot fermented beverage. Food Chem. 2021;344:128618.
21. Kabakcı SA, Türkyılmaz M, Özkan M. Changes in the quality of kefir fortified with anthocyanin-rich juices during storage. Food Chem. 2020;326:126977.
22. Kabakcı SA, Türkyılmaz M, Özkan M. Effects of fermentation time and pH on quality of black carrot juice fermented by kefir culture during storage. J. Sci. Food Agric. 2022;102(6):2563-74.
23. Hernández T, Estrella I, Pérez-Gordo M, Alegria EG, Tenorio C, Ruiz-Larrrea F, et al. Contribution of malolactic fermentation by *Oenococcus oeni* and *Lactobacillus plantarum* to the changes in the nonanthocyanin polyphenolic composition of red wine. J. Agric. Food Chem. 2007;55(13):5260-6.
24. Di Cagno R, Coda R, De Angelis M, Gobbetti M. Exploitation of vegetables and fruits through lactic acid fermentation. Food Microbiol. 2013;33(1):1-10.
25. Kwaw E, Ma Y, Tchabo W, Apaliya MT, Wu M, Sackey AS, et al. Effect of *lactobacillus* strains on phenolic profile, color attributes and antioxidant activities of lactic-acid-fermented mulberry juice. Food Chem. 2018 250: 148-54.
26. Gerardi C, Tristezza M, Giordano L, Rampino P, Perrotta C, Baruzzi F, et al. Exploitation of Prunus mahaleb fruit by fermentation with selected strains of *Lactobacillus plantarum* and *Saccharomyces cerevisiae*. Food Microbiol. 2019;84:103262.
27. Szutowska J, Rybicka I, Pawlak-Lemańska K, Gwiazdowska D. Spontaneously fermented curly kale juice: Microbiological quality, nutritional composition, antioxidant, and antimicrobial properties. J. Food Sci. 2020;85(4):1248-55.
28. Pardali E, Paramithiotis S, Papadelli M, Mataragas M, Drosinos EH. Lactic acid bacteria population dynamics during spontaneous fermentation of radish (*Raphanus sativus* L.) roots in brine. World J. Microbiol. Biotechnol. 2017;33:1-9.
29. Zhou Q, Zang S, Zhao Z, Li X. Dynamic changes of bacterial communities and nitrite character during northeastern Chinese sauerkraut fermentation. Food Sci. Biotechnol. 2018;27:79-85.
30. Nanis I, Hatzikamari M, Katharopoulos E, Boukouvala E, Ekateriniadou L, Litopoulou-Tzanetaki E, et al. Microbiological and physicochemical changes during fermentation of solid residue of olive mill wastewaters: Exploitation towards the production of an olive paste–type product. LWT. 2020;117:108671.
31. Cele NP, Akinola SA, Manhivi VE, Shoko T, Remize F, Sivakumar D. Influence of lactic acid bacterium strains on changes in quality, functional compounds and volatile compounds of mango juice from different cultivars during fermentation. Foods 2022;11(5):682.
32. Jeong SH, Jung JY, Lee SH, Jin HM, Jeon CO. Microbial succession and metabolite changes during fermentation of dongchimi, traditional Korean watery kimchi. International J. Food Microbiol. 2013;164(1):46-53.
33. Torres S, Verón H, Contreras L, Isla MI. An overview of plant-autochthonous microorganisms and fermented vegetable foods. Food Sci. Human Well. 2020;9(2):112-23.
34. Alan Y, Yıldız N. Effects of *Lactobacillus* used as the starter culture on naturally fermented pickled cabbage. Food Sci. Technol. 2021 42(e45020):1-9.
35. Olsen A, Halm M, Jakobsen M. The antimicrobial activity of lactic acid bacteria from fermented maize (kenkey) and their interactions during fermentation. J. Appl. Bacteriol. 1995;79(5):506-12.
36. Bautista-Gallego J, Medina E, Sánchez B, Benítez-Cabello A, Arroyo-López FN. Role of lactic acid bacteria in fermented vegetables. Grasas y Aceites, 2020 71(2): e358-e358.
37. Chen AJ, Luo W, Peng YT, Niu KL, Liu XY, Shen GH, et al. Quality and microbial flora changes of radish paocai during multiple fermentation rounds. Food Control, 2019 106: 106733.