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EFFECT OF FERMENTATION PROCESSES ON PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY DURING PRODUCTION OF BLACK CARROT VINEGAR

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ABSTRACT
Black carrots and black carrot-derived products have gained great popularity in recent years due to their significant content of health-promoting bioactive compounds. Therefore, this study focuses on the production of vinegar from black carrot juice (BCJ) derived from black carrot juice concentrate (BCJC), to attain a food product with nutritional added value. In this study, the effect of alcoholic fermentation by *Saccharomyces cerevisiae* and acetic acid fermentation by four different vinegar starters (grape, apple, alcohol vinegar, and the mixture of grape and apple vinegar) on some physicochemical properties (pH, total acidity, total dry matter, reducing sugar, total phenolics, and antioxidant activity) of BCJ was investigated. The results obtained indicated that processing the BCJ into black carrot wine (BCW) led to an overall reduction of only 4% in total phenolic contents, and a further decrease of 21-32% in total phenolic content was observed in black carrot vinegars (BCVs) due to the acetification process, on a weight-to-volume basis (mg/L). A similar decreasing trend was also determined for the antioxidant activity throughout the vinegar production process. Total phenolic content was not affected by inoculation with any of the seed vinegars since the difference between the total phenolic contents with respect to the four different vinegar starters was statistically insignificant ($P > 0.05$). In general, the BCW and BCVs (regardless of the type of inoculum) produced in this study exhibited better bioactive properties compared to their commercial counterparts (C-BCW and C-BCV). In conclusion, vinegar was successfully produced from black carrot by retaining a considerable amount of its nutraceutical components.

Keywords: Black carrot, vinegar, total phenolic content, antioxidant activity

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KARA HAVUÇ SİRKESİ ÜRETİMİ SIRASINDA FERMANTASYON İŞLEMLERİNİN FENOLİK İÇERİĞİ VE ANTİOKSİDAN AKTİVİTESİ ÜZERİNE ETKİSİ

ÖZ

Kara havuç ve kara havuç türevi ürünler, sağlık üzerine olumlu etkili biyoaktif bileşenleri içermeleri nedeniyle, son yıllarda büyük popülerlik kazanmıştır. Bu nedenle, bu çalışmada, besin değeri yüksek bir gıda ürünü elde etmek amacıyla kara havuç suyu konsantresinden (BCJC) elde edilen kara havuç suyunun (BCJ) sirke üretiminde kullanılması üzerinde durulmuştur. Bu çalışmada, *Saccharomyces cerevisiae* ile gerçekleştirilen alkol fermantasyonunun ve dört farklı sirke starteri (üzüm, elma, alkol sirkesi ve üzüm ve elma sirkesi karışımı) ile başlatılan asetik asit fermantasyonunun, kara havuç suyunun pH, toplam asitlik, kuru madde, indirgen şeker, toplam fenolik madde, antioksidan aktivite değeri gibi bazı fizikokimyasal özellikleri üzerine etkisi incelenmiştir. Elde edilen sonuçlar (ağırlık/hacim bazında (mg/L)), kara havuç suyunun (BCJ) kara havuç şarabına (BCW) işlenmesinin toplam fenolik içeriklerinde yalnızca %4'lük bir azalmaya yol açtığını göstermiştir. Ayrıca, kara havuç sirkesinde (BCV), asetifikasyon işlemine bağlı olarak, toplam fenolik içeriğinde %21-32'lik bir azalmanın daha olduğu belirlenmiştir. Sirke üretim süreci boyunca antioksidan aktivite için de benzer bir azalma eğilimi belirlenmiştir. Dört farklı sirke starteri ile üretilen sirke örneklerinin toplam fenolik içerikleri arasındaki fark istatistiksel olarak önemsiz olduğundan ($P > 0.05$), farklı sirke starterleri ile inokülasyonun toplam fenolik madde miktarını etkilemediği sonucuna varılmıştır. Genel olarak, bu çalışmada üretilen kara havuç şarabı (BCW) ve kara havuç sirkesi (BCV), inokulum türünden bağımsız olarak, ticari muadillerine (C-BCW ve C-BCV) kıyasla daha iyi biyoaktif özellikler göstermiştir. Sonuç olarak, nutrasötik bileşenlerinin önemli bir miktarı korunarak kara havuçtan başarılı bir şekilde sirke üretimi gerçekleştirilmiştir.

Anahtar kelimeler: Kara havuç, sirke, toplam fenolik madde, antioksidan aktivite

INTRODUCTION

The increasing awareness of consumers towards the consumption of healthier products has led to an increasing interest in developing novel products with improved functional characteristics in comparison to traditional products (Kandylis, 2020). The same trend also applies to vinegar production (industry and research), and a number of studies have been conducted with the goal of diversifying vinegar products (Tang et al., 2020). In this sense, various types of alternative vinegars have been produced by using novel raw materials such as fruit and agri-food wastes. Apart from the conventional raw materials like grape, raisin, apple, cereals, different fruit and vegetable sources, such as watermelon, tomato, orange, pomegranate, star fruit berry, mango, onion, have been used as alternative raw materials to produce novel types of vinegars with enhanced nutritional and organoleptic quality (Kandylis, 2020).

The starting material used in the vinegar production is one of the most important factors that determine the quality of the finished vinegar since many of the flavor, aroma and other organoleptic properties of the finished vinegar are derived from the starting material (Hutkins, 2019). Theoretically, any sugar, starch or ethanol-containing material can serve as a substrate for the vinegar fermentation. Accordingly, vinegar can be obtained from almost any fermentable carbohydrate source through a two-step fermentation process. In the first step, fermentable sugars are converted into ethanol by the metabolic activity of yeasts (mainly by strains of *Saccharomyces cerevisiae*) under anaerobic conditions. In the second step, ethanol is oxidized to acetic acid aerobically by bacteria of the genera *Acetobacter* and *Gluconobacter* (Adams and Moss, 2008; Hutkins, 2019; Kandylis, 2020). The final quality of vinegars, as determined by the chemical, nutritional and organoleptic properties, mainly depend on the factors including the selection of the suitable starter cultures, the quality of the starting material, the production method, and the aging process, if applicable (Mas et al., 2014). As a result, this research was carried out in order to use black carrot juice as a raw material for the

production of vinegar, thereby providing the functional benefits of both the substrate and the fermentation process. This raw material was selected mainly in order to increase the content of phenolic compounds and the antioxidant activity of the produced vinegar.

Black carrot **(***Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef) has received considerable attention in recent years since it constitutes a variety of bioactive phytochemicals such as vitamin C, E and phenolic compounds which are known for their antioxidant activities. The prominent feature of black carrots is their intense purple color, which is caused by the presence of anthocyanins (Algarra et al., 2014). Black carrots are often not consumed as fresh (Kamiloglu et al., 2018) but are rather processed into other products such as juice, concentrate and shalgam (Türkyılmaz et al., 2012). In terms of fermented black carrot juice, shalgam is a highly popular traditional lactic acid fermented beverage in which black carrot, bulgur flour, sourdough, salt, turnip, and water are used for production (Erten et al., 2008). Therefore, most of the studies with regard to fermented beverages in which the main ingredient is black carrot, is based on shalgam produced by lactic acid fermentation. (Turker et al., 2004; Ekinci et al., 2016; Agirman and Erten, 2018). The black carrot vinegar, although not common as traditional types of vinegars, is available on the market. However, there are no published scientific research on this novel type of vinegar. Since black carrots are generally consumed after being processed into various products, it is important to investigate how processing influences the health-associated phytochemicals, such as phenolics, especially anthocyanins (Suzme et al., 2014). In this regard, although several studies have been conducted to examine the effects of food processing, including enzyme-assisted processing (Khandare et al., 2011), juice processing involving clarification and pasteurisation (Türkyılmaz et al., 2012), concentrate processing (Suzme et al., 2014) on black carrot phenolics, there is lack of studies addressing the fermentation-induced changes in bioactive compounds potentially contributing to the health-promoting properties of black carrots. Only in a study conducted by Kocher et al. (2016), the change of total phenolic content and anthocyanins by the alcoholic fermentation process of black carrots was investigated. With regard to the effect of lactic acid fermentation on the beneficial compounds of black carrots, Toktaş et al. (2018) reported increase in total phenolic content, total flavonoid content, anthocyanins content and total antioxidant capacity of shalgam beverages during the progress of fermentation, albeit lower than in the black carrots. Similarly, Alagöz Kabakcı et al. (2022) indicated that fermentation of black carrot juice using kefir culture resulted in increase in the total anthocyanin content, probably due to the pectinolytic activity of lactic acid bacteria. In a similar manner to our study, many studies have been carried out to investigate the effects of alcoholic and acetic acid fermentation on antioxidant activities and phenolic substances of different raw materials for vinegar production, such as grape, apple (Bakir et al., 2016), strawberry (Ubeda et al., 2013; Hornedo-Ortega et al., 2017), cherry (Budak, 2017), pomegranate (Ordoudi et al., 2014; Kharchoufi et al., 2018), orange (Davies et al., 2017), blueberry (Su and Chien, 2007), papaya (Kong et al., 2018) and tomato (Koyama et al., 2017). In terms of the changes in functional compounds during vinegar production, a high diversity of patterns was observed in these studies, because of the differences in the raw material used, the starter strains and the fermentation conditions. For example, during the conversion of papaya juice into vinegar, the total phenolic contents and antioxidant activities increased throughout the process (Kong et al., 2018), while in another case, the process from grape and apple wine to grape and apple vinegar resulted in loss of antioxidant phenolic compounds (Bakir et al., 2016). However, studies addressing alterations in functional compounds of black carrots during acetification process are still lacking.

Vinegar fermentations are traditionally induced by the use of the so-called "seed vinegar" or "mother of vinegar" that is composed of an undefined culture withdrawn from previous fermentations through back-slopping methods. To the best of

our knowledge, there is no single study in the literature that has examined the effect of vinegar starters on the quality of vinegars.

Taking all above into account, the aim of this study was to analyze changes in total phenolics and antioxidant activity during a laboratory-scale production of black carrot vinegar. Furthermore, this study was conducted with the aim of determining the possible effect of different seed vinegars (grape, apple, alcohol vinegar, and the mixture of grape and apple vinegar) on some physicochemical properties of the finished vinegars.

MATERIALS AND METHODS

Materials

Black carrot juice concentrate (BCJC) was kindly obtained from a commercial company located in Konya (ERKON Concentrate Industry and Trade Inc.). A wine yeast strain, *Saccharomyces cerevisiae* Narince 3, which was maintained in the Culture Collection of Ankara University Department of Food Engineering, was used to carry out the alcoholic fermentation of the black carrot juice (BCJ). High acidity grape, apple and alcohol vinegar were used as inoculums in order to initiate acetic acid fermentation of black carrot wines (BCW). These inoculum vinegars as wells as commercial black carrot juice, black carrot wine and black carrot vinegar samples were kindly provided from a commercial company located in Ankara (Yeni Kavaklıdere).

Alcoholic Fermentation of Black Carrot Juice

For the alcoholic fermentation of the BCJ, the indigenous wine yeast strain, *S. cerevisiae* Narince 3 (Bağder Elmaci et al., 2014) was initially activated in 10 mL of YPG Broth (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose; pH 4.5) at 28 °C. Subsequently, the activated yeast cultures were transferred to 50 mL of YPG Broth. After the culture activation, a two-stage adaptation was applied in order to adapt the yeast to the black carrot juice environment. For this purpose, the BCJC at about 65° Brix was diluted to 27-28° Brix. For the first adaptation stage, 50 mL of diluted BCJ was mixed with 50 mL of yeast cultures in YPG Broth and incubated at 28 °C. The yeast growth was observed after the first adaptation step. Then, the second stage of adaptation was performed by adding 250 mL of yeast cultures in YPG Broth to 750 mL of black carrot juice. In order to start alcoholic fermentation, 9 L of BCJ (approxiamately 22° Brix) was inoculated with 1 L of adapted yeast culture. The fermentation was carried out at 28 °C for 3 days.

Acetic Acid Fermentation of Black Carrot Wine

After the alcoholic fermentation of the reconstituted BCJC, the obtained BCW was inoculated with four different vinegar starters at 25% (v/v) inoculum size to conduct acetic acid fermentation by surface culture acetification. The four inoculums included grape, apple, alcohol vinegar, and the mixture of grape and apple vinegar. The black carrot vinegar samples inoculated with grape, apple, alcohol vinegar, and the mixture of grape and apple vinegar were coded as G-BCV, AP-BCV, AL-BCV and M-BCV, respectively. The BCW samples inoculated with different types of vinegars were then incubated at 30 °C in an incubator aerated continuously with an aquarium pump. Acetic acid fermentation was monitored by measuring pH value and total titratable acidity. Acetic acid fermentation was lasted for 35 days. The obtained vinegars were filtered through a roughing filter and then stored at 4 °C for subsequent analysis. Prior to analysis, the samples were filtered through a 0.45 μm PVDF (polyvinylidene fluoride) filter (Millipore, Bedford, MA, USA).

Analytical Measurements

The pH values of the samples were measured potentiometrically at 20 °C by using a pH meter (Mettler Toledo, S-20K, Switzerland). Total titratable acidity was determined by titrating an aliquot of the sample with standard NaOH solution to the end point of pH 8.5 using phenolphthalein as an indicator. Total titratable acidity (g/100 mL) was expressed as citric or acetic acid equivalent (Aktan and Yıldırım, 2011). Total solid contents of the samples were determined gravimetrically in g/L (Aktan and

Yıldırım, 2011). The reducing and total sugar contents were determined according to a modified Miller method using DNS (3,5-dinitrosalicylic acid) (Forouchi and Gunn, 1983). Ethanol content was analyzed using gas chromatography (Shimadzu GC-2010 Plus, Kyoto, Japan) equipped with flame ionization detector (FID). One microliter of filtered samples was automatically injected into the GC system via autosampler (Shimadzu AOC-20S, Kyoto, Japan). TRB-WAX capillary column with length of 30 m and internal diameter of 0.25 mm was used. The column oven, injection and detector temperatures were set at 220, 250 and 230 °C, respectively. Nitrogen, and a mixture of hyrogen and air were used as the carrier and fuel gas, respectively.

The antioxidant activity assay was performed by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging method as described by Molyneux (2004). This method was based on the measurement of the decrease in colour spectrophotometrically at 517 nm as a result of reduction of violet stable radical DPPH after the reaction with test compound. The DPPH free radical lost its intense violet colour and turned to yellow due to the reduction reaction, when mixed with the sample having antioxidant activity. For the DPPH assay, appropriately diluted test sample was mixed with 1 mM DPPH solution. The absorbance was measured at 517 nm after 30 min of reaction at room temperature in the dark. The parameter EC50 ("efficient concentration" value) was used for the interpretation of the results from the DPPH method. The EC50 is defined as the concentration of substrate that causes 50% loss of the DPPH activity (colour). Therefore, the lower EC50 value shows the higher antioxidant activity.

The total phenolic contents of the samples were determined in accordance with the Folin-Ciocalteu method of Singleton and Rossi (1965). Briefly, 75 mL of distilled water was put into 100 mL volumetric flask and 1 mL of appropriately diluted sample was added. Subsequently, 5 mL of Folin-Ciocalteu reagent was added to the sample solution and the mixture was allowed to react for 3 min. Then, 10 mL of saturated Na_2CO_3 solvent was added and made up to the flask line with distilled water. The thoroughly mixed sample was allowed to incubate for 60 min and then the absorbance was determined against a blank at 720 nm using a spectrophotometer (Shimadzu UV-1208). The concentration of total phenolics was determined by using the standard curve of gallic acid and expressed as "mg gallic acid equivalent/L (mg GAE/L)".

The colour measurements were performed by using a light reflectance spectrophotometer (Minolta CM-3600d, Osaka, Japan). Measurements were recorded in L^* (lightness), a^* (redness), b^* (yellowness) colour coordinates as defined by CIE (Commission Internationale de I'Eclairage). The spectrophotometer was calibrated with a white ceramic plate using an illuminant C source. 1 cm path length quartz cuvettes with a total volume of 10 ml were used for colour measurements. C^* (chroma) and h° (hue) values were calculated on the basis of the following equations:

$$C^* = (a^{*2} + b^{*2})^{1/2}$$
$$h° = \arctan (b^*/ a^*)$$

Statistical analysis

All vinegar experiments were performed in three independent replicates. The experimental data were expressed as mean±standard deviation (SD) and statistically analyzed by one-way ANOVA using the Minitab Software Version 17 (Minitab Inc., State College, PA, USA) followed by the Duncan's multiple comparison test at the 5% level.

RESULTS AND DISCUSSION

Physicochemical Properties of Black Carrot Juice

Table 1 shows the pH, total titratable acidity, total solid content and reducing sugar concentration of BCJ (raw material), BCW (interim product) and BCV (final product) samples. pH values of BCJ and BCW samples were found as 3.72 and 3.85, respectively. pH values of vinegar samples inoculated with different seed vinegars ranged from 3.70 to 3.75 with an average of 3.72, in accordance with the commercial vinegar sample (C-BCV) with pH of 3.77. In accordance with our results, Baysal et al. (2013) reported that the pH

value of the black carrot juice with Brix of 9.10 was 3.85. In other previous studies, depending on the Brix value, different pH values were reported for black carrot juice. Kırca et al. (2007) found the pH of black carrot juice with a Brix of 10.8 to be 6.0; Dereli et al. (2010) found the pH of black carrot juice to be 4.35, with a °Brix of 11.02. As can be seen from Table 1, the acidity of BCJ was found to be 2.4 g/100 mL, which was also same for C-BCJ.

The total solid content (g/L) of the samples were determined to calculate the results on a dry weight basis. Since one of the aims of this study was to evaluate the effects of vinegar processing on total phenolic content, it was important to give the total phenolic content results on a dry weight basis in order to make comparisons more reliable.

Alasalvar et al. (2001) reported that the main soluble fermentable sugars of black carrot are sucrose (1.96-4.11 g/100 g), followed by glucose (0.69-1.77 g/100 g) and fructose (0.58-1.47 g/100 g). The total sugar concentration in BCJ samples was considerably higher (49.73±0.06 g/L) since BCJ diluted from concentrate (BCJC) was used in this study.

Table 1. Physicochemical analysis of black carrot juice, wine and vinegar samples

Samples	pH	Total titratable acidity (g/100 mL)[a]	Total solid content (g/L)	Reducing sugar (g/L)	L*	a*	b*	C*	h°
BCJ	3.72	2.40	217.57±1.82	49.73±0.06	0.47	1.51	0.82	1.71	28.45
BCW	3.85	2.43	106.22±1.71	13.66±0.18	0.40	2.23	0.42	2.32	18.99
G-BCV	3.70	6.78	59.43±0.71	17.27±0.45	0.11	0.47	0.12	0.59	85.62
AP-BCV	3.75	6.28	65.09±3.84	21.74±1.03	0.16	0.83	0.17	0.90	64.70
AL-BCV	3.70	6.91	67.31±2.33	17.78±0.43	0.25	1.37	0.26	1.43	79.13
M-BCV	3.72	6.53	65.11±1.39	19.34±0.63	0.13	0.57	0.13	0.69	84.37
C-BCJ	3.60	2.40	132.00±2.26	54.85±0.49	0.14	0.62	0.17	0.74	76.56
C-BCW	3.65	2.40	73.95±0.07	6.69±0.18	0.19	1.02	0.24	1.14	74.67
C-BCV	3.77	4.00	35.13±0.53	6.27±0.13	0.24	1.27	0.33	1.40	71.52

[a] The total titratable acidity (g/100 mL) of BCJ and BCW samples is expressed as citric acid equivalent, while that of BCV samples is expressed as acetic acid equivalent.

In the study, L*, a*, b*, C* and h° values of black carrot juices were measured (Table 1). In the CIE L* a* b* system, the L* value is defined as the degree of lightness and this value varies between 0 (black) and 100 (white). The measured L* value is quite close to zero, which indicates that the color is black. Although positive a* values indicate red color, Özkan (2009) reported that the measurement method was not suitable for determining the reflectance color values (L*, a*, b*, C*, and h°) of black carrot juice at different production stages. Consequently, he stated that it would be more precise to measure the reflectance color values after adjusting the pH of black carrot juice to between 1.5 and 2, where anthocyanins exhibit the most redness.

Change in total phenolic content and antioxidant activity during the alcoholic and acetic acic fermentation processes

The total phenolic contents of the BCJ, BCW and BCV samples were determined by Folin-Ciocalteu method and the corresponding results are given in Table 2. The total phenolic contents of BCJ (9820 mg/L) and the black carrot juice obtained from the commercial company (C-BCJ) (5625 mg/L) in this study were higher than those reported by previous studies. In other studies, the total phenolic content was 3000 mg GAE/L (Khandare et al., 2011); 1785 mg GAE/L (Ekinci et al., 2016) in different black carrot juice samples. The reported total phenolic content of BCJ samples varied amongst different studies,

probably due to the differences in total solid content. Considering that the BCJ used in this study had a higher solid content (217.57 g/L) compared to that of other studies (generally around 10-12°Brix), it was an expected result that the total phenolic concentrations would be higher. For instance, the total phenolic content of BCJ with a °Brix of 11.02 was reported to be 3037 mg GAE/L (Dereli et al., 2015). In addition, the magnitude of total pheolic content in BCJ was higher than those reported for other phenolic-rich raw materials (e.g. pomegranate juice, cherry juice, blueberry juice) that have been used for vinegar production. The total phenolic content values reported for pomegranate, cherry and blueberry juices were 1387 mg GAE/L (Ordoudi et al., 2014), 854.79 mg GAE/L (Budak, 2017) and 867 mg GAE/L (Su and Chien, 2007), respectively. It was also found that the BCW as well as BCVs (regardless of the type of inoculum) produced in this study exhibited significantly higher total phenolic content as compared to their commercial counterparts (C-BCW and C-BCV).

Table 2. Changes in total phenolic content during acetification

Samples	Total phenolic content (mg GAE/L)	Total phenolic content (mg GAE/g; on dry weight basis)	Antioxidant activity (EC_{50} DPPH; μL sample)
BCJ	9820±254.56^{a}	45.13±1.17^{g}	2.90±0.07^{e}
BCW	9460±28.28^{b}	89.06±0.27^{e}	3.14±0.08^{de}
G-BCV	7720±135.06^{c}	129.90±2.48^{a}	3.38±0.13^{d}
AP-BCV	6640±245.60^{d}	102.01±3.92^{d}	3.86±0.18^{c}
AL-BCV	7620±140.29^{c}	113.21±1.67^{b}	3.21±0.05^{de}
M-BCV	7583±109.85^{c}	116.47±1.70^{b}	3.42±0.22^{d}
C-BCJ	5625±35.36^{e}	42.61±0.27^{g}	4.37±0.14^{b}
C-BCW	5313±17.68^{e}	71.84±0.24^{f}	4.39±0.18^{b}
C-BCV	3800±28.28^{f}	108.17±0.81^{c}	6.81±0.03^{a}

Data are expressed in mean ± standard deviation.
Means with a different lower case letter in the same column are significantly different ($P < 0.05$).

The total phenolic content of BCJ (9820 mg/L) was found to be higher than that of BCW (9460 mg/L) and BCV (ranging from 6640 to 7720 mg/L) samples in this study ($P < 0.05$), indicating an overall reduction by only 4% and 21-32% due to alcoholic and acetic acid fermentation processes, respectively. A similar decreasing tendency was also reported for red and white cherry juices during vinegar production (Budak, 2017). In case of commercial black carrot products used in this study, there was a non-significant, but slight difference between C-BCJ and C-BCW samples, whereas the total phenolic content of C-BCV was significantly lower than those of C-BCJ and C-BCW. The results of this study were in agreement with Kharchoufi et al. (2018) who showed slight decrease (only around 10%) in total polyphenol content in pomegranate juice after both alcoholic and acetic acid fermentation. Similarly, Ordoudi et al. (2014) reported that the initial total phenolic content of pomegranate juice was completely retained after alcoholic fermentation and was only reduced by 10% as a result of acetification process. Hornedo-Ortega et al. (2017) showed a decrease in total phenolics due to acetification process (91%) to a larger extent than alcoholic fermentation (19%) during strawberry vinegar production. In case of lactic acid fermentation, Ekinci et al. (2016) reported a significant reduction by 71% in total phenolic content of fresh black carrot juice (1785 μg GAE/mL) after processing into fermented black carrot juice, shalgam (517 μg GAE/mL).

Significant changes in total solid content occur in the vinegar production process due to the consumption of sugars by fermentative yeast and acetic acid bacteria. Therefore, in order to make comparisons more reliable, the total phenolic content results were also given on a dry weight

basis. When total phenolic content results are expressed on a dry weight basis, it is clear that an increase in total phenolic content was observed during the transition from BCJ to BCW as well as from BCW to BCV (Table 2).

The changes in antioxidant activity as measured by the DPPH method during BCV manufacturing are shown in Table 2. In accordance with total phenolic content data (on weight-to-volume basis (mg/L)), vinegar processing resulted in decreases in total antioxidant capacity, determined using DPPH. Also, a strong correlation was found between total phenolic content (mg GAE/L) and antioxidant activity (EC_{50}) (r= -0.89). In the case of pomegranate vinegar production, Kharchoufi et al. (2018) also reported that the antioxidant activity as measured by the DPPH assay decreased by 17.6% after alcoholic fermentation and by a further 9.1% after acetic acid fermentation. It was observed that the EC50 value, which shows antioxidant activity, increased by alcoholic fermentation of BCJ into BCW and acetic acid fermentation of BCW to BCV. There is an inverse relationship between EC50 value and antioxidant activity. A decrease occurred in the antioxidant activity of vinegars, as high EC50 value indicates low antioxidant activity. In consistent with the total phenolic results, it was also found that the BCW as well as BCVs (regardless of the type of inoculum) produced in this study showed significantly higher antioxidant capacity as compared to their commercial counterparts (C-BCW and C-BCV).

Monitoring of Acetic Acid Fermentation

The interim product with 3.72 g/100 mL (v/v) alcohol (BCW) that was obtained by the alcoholic fermentation of BCJ was divided into four batches (data not shown). Then, each batch was inoculated with four different vinegar starters at a 25% (v/v) inoculum size to conduct acetic acid fermentation by surface culture acetification. In order to monitor the progress of acetic acid fermentation, pH and titratable acidity were measured at 5-day intervals. Figure 1 represents the pH values and total titratable acidity (as g/100 mL acetic acid) during the course of acetic acid fermentation of BCW. In the present study, surface film formation (mother of vinegar) was observed after 5 days in samples incubated at 30 °C, and acetic acid fermentation was terminated when the alcohol concentration dropped below 0.5% (v/v) after 35 days. In his study on the production of vinegar from Dimrit grapes, Ünal (2007) reported that on the eighth day of acetic acid fermentation, a film formed on the surface of all samples and that the fermentation lasted 37 days in two of the trials and 47 days in one.

As seen in Figure 1A, during the course of acetification, there was not a considerable increase or decrease in the pH values of the vinegar groups produced by using four different inoculums. No significant difference was found between the pH values of day 0 and day 35.

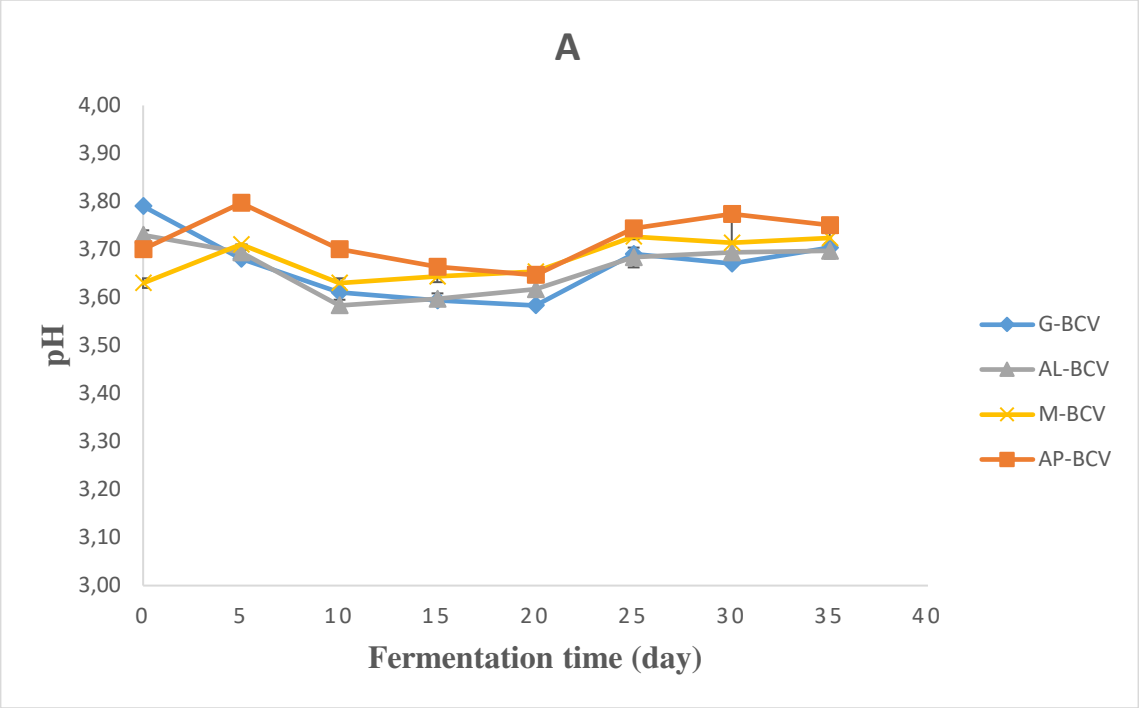
As shown in Figure 1B, an increase was observed in the total acidity values of G-BCV, AP-BCV, AL-BCV and M-BCV samples until the 35th day when fermentation is terminated. Since the acidity of black carrot juice was high, it could be said that the high initial acidity values were due to the raw material. During the course of acetic acid fermentation, the highest increase in acidity was observed between the 5th and 10th days of fermentation.

Properties of Marketable BCVs

The pH, total dry matter, reducing sugar content and total phenolic contents of marketable BCVs which were reconstituted to 4 g/100 mL acidity, were varied between 3.70 and 3.75, 35.11 and 41.46 g/L, 9.37 and 13.86 g/L and 4232 and 4655 mg GAE/L, respectively (Table 3). According to the Turkish Standards Institution TS 1880 EN 13188 vinegar standard, the total acid content (as free acetic acid in water) of the vinegar produced in our country should not be less than 40 g per liter. Therefore, in the final product, water was added to adjust the acid concentration to 4%.

The pH values of four different groups of BCV obtained by applying the slow method vary between 3.70 and 3.75. The pH of C-BCV obtained from the factory was found to be 3.77, which would be close to these values (Table 3 and 1). Ünal (2007) reported that the pH of wine vinegars produced from grapes varied between 2.68 and 2.85. Akbaş (2008) stated in his study

that the pH of grape vinegars varied between 2.63 and 3.27. Gerbi et al. (1998) reported that the average pH value in apple cider vinegar was 3.00. The reason why the pH values of BCVs were significantly higher than the pH values of other vinegars could be explained by the high dry matter ratios of black carrot vinegars and, thus, the presence of components with more buffering properties in this high dry matter composition. The dry matter contents of the vinegars produced in this study were found to be higher than those found in other studies by Ünal (2007) and Akbaş (2008) (10.85-12.60 g/L and 8.75-17.5 g/L, respectively).



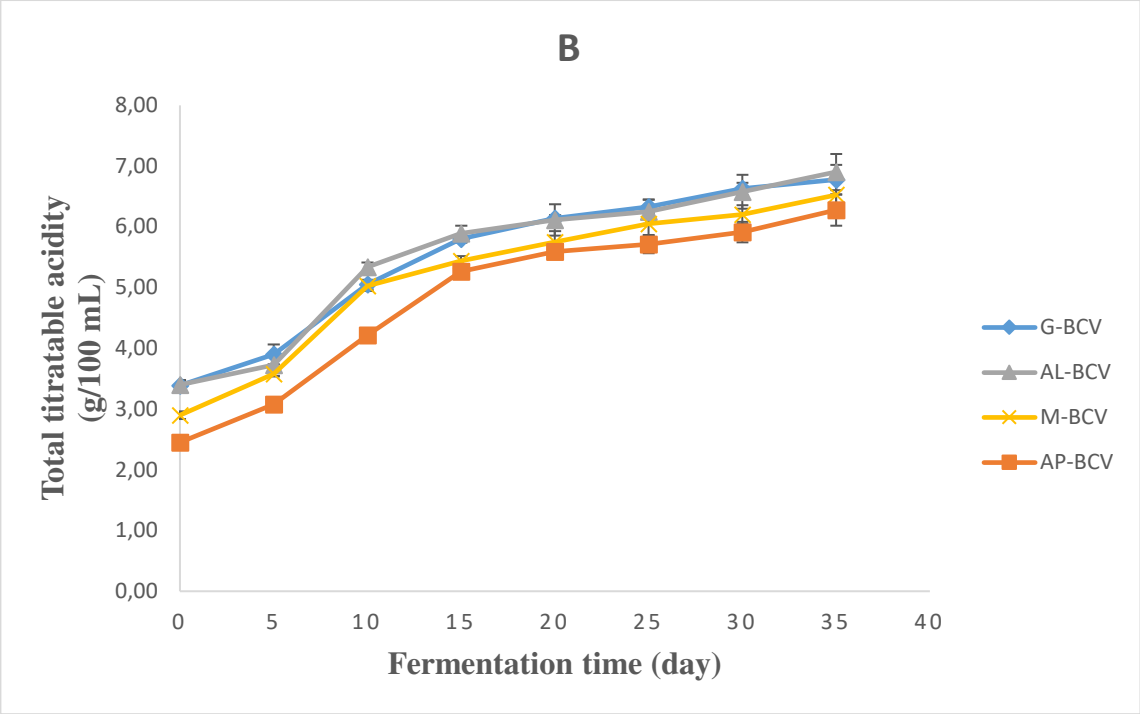


Figure 1. A) pH values and B) total titratable acidity (as g/100 mL acetic acid) during the course of acetic acid fermentation of BCW

Table 3. Values for black carrot vinegars available for sale

Samples	pH	Total solid content (g/L)	Reducing sugar (g/L)	Total phenolic content (mg GAE/L)
G-BCV	3.70±0.02^{b}	35.11±0.89^{b}	10.11±0.55^{bc}	4563±251.73
AP-BCV	3.75±0.01^{a}	41.46±1.05^{a}	13.86±0.70^{a}	4232±28.49
AL-BCV	3.70±0.02^{b}	39.08±3.14^{a}	9.37±2.17^{c}	4421±257.03
M-BCV	3.72±0.01^{b}	39.93±0.84^{a}	11.87±0.83^{ab}	4655±270.84

The pH, total dry matter, reducing sugar content, and total phenolic contents of BCVs were determined after adjusting the acid concentration to 4 g/100 mL, in accordance with the Turkish Standards Institution TS 1880 EN 13188 vinegar standard.
Data are expressed in mean ± standard deviation.
Means with a different lower case letter in the same column are significantly different (P <0.05).

Total phenolic content was not affected by inoculation with any of the seed vinegars since the difference between the total phenolic contents with respect to the four different vinegar starters (grape, apple, alcohol vinegar, and the mixture of grape and apple vinegar) was statistically insignificant ($P > 0.05$). Although not statistically significant, in comparison with other final vinegars, AP-BCV had lower phenolic content, which might be related to the differences in their pH and total solid content.

CONCLUSION

The primary aim of this study was to determine the effects of fermentation processes involved in vinegar production on the content of total phenolics and antioxidant activity of BCJ. In addition, the effect of different vinegar starters on

the quality of vinegars was also evaluated. BCVs were successfully produced from industrially processed BCJCs in order to take advantage of their nutraceutical components. The results obtained indicated that processing the BCJ into black carrot wine (BCW) led to an overall reduction of only 4% in total phenolic contents, and a further decrease of 21-32% in total phenolic content was observed in black carrot vinegars (BCVs) due to the acetification process, on a weight-to-volume basis (mg/L). Moreover, when the total phenolic calculations were made on a dry-weight basis to compensate for changes in dry matter content due to fermentation processes, an increase in total phenolic content was observed throughout the vinegar processing. With regard to the effect of different seed vinegars, the difference in total phenolic contents was statistically insignificant. In conclusion, the results of this study pointed out the potential of BCV to obtain an added-value product that will increase the variety of functional products on the market.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

AUTHORS' CONTRIBUTIONS

This study was a part of master thesis of Süeda Öztürk, approved in Ankara University, Graduate School of Natural and Applied Sciences, Department of Food Engineering, Ankara, Turkey. Süeda Öztürk performed the experiments and interpreted the results. The manuscript was written by Simel Bağder Elmacı. Simel Bağder Elmacı also participated in laboratory analyses. Filiz Özçelik made the experimental design and contributed to the writing and revision of this manuscript.

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