https://dergipark.org.tr/tr/pub/food
OPEN ACCESS JOURNAL

ISSN 2667-5803

DergiPark AKADEMİK

FHTI Journal of Food Health and Technology Innovations
September Vol 4, No 8 (2021)

ASOS indeks

Proceeding Article

Antioxidant Activity and Volatile Aroma Compounds of Sour Cherry (*Prunus cerasus* L.,) Vinegar in the Fermentation Process

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Abstract

In the present study, sour cherry vinegar was produced from sour cherry juice produced from fresh sour cherries and sour cherry concentrate with rich bioactive component contents, strong antioxidant, antidiabetic, antiobesity, antimutagen, and anticarcinogen properties. In the vinegar production process, changes in total acidity, total soluble dry matter, antioxidant activity, and organic acid composition were examined. The present study mainly aimed to determine the effects of using fresh cherry juice or concentrated cherry juice in vinegar production. Tartaric, malic, and acetic acid organic acids were detected during the production stages of sour cherry vinegar.

In the antioxidant activity analysis by the DPPH method, it was determined that the percentile inhibition level of sour cherry vinegar was higher than those of sour cherry juice and cherry wine. No differences were observed in the component level in the analyses of fresh cherry juice and sour cherry vinegar samples produced from concentrated sour cherry. Therefore, considering that the harvest time of sour cherry is very short, it was concluded that sour cherry vinegar can be efficiently produced from concentrated sour cherry juice regardless of the harvest season.

Keywords: Cherry vinegar, antioxidant, DPPH, organic acid components, acetic acid

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INTRODUCTION

Sour cherry (*Prunus cerasus* L.) is a fruit belonging to the *Rosaceae* family. With its distinctive sour taste, dark red color, and flavor, it is usually consumed as fruit juice (Kirakosyan et al., 2009). The preventive effects of sour cherry, which is rich in bioactive components, on overall health, diabetes, obesity, cardiovascular and inflammatory diseases have been reported (Lara et al., 2016). Sour cherry is consumed as sour cherry juice, sour cherry juice concentrate, sour cherry jam, sour cherry marmalade, dried sour cherry, sour cherry wine. Also, sour cherry vinegar can be produced from sour cherry. Two methods are used in vinegar production as traditional (surface culture method) and industrial (deep culture method). There are two stages in the traditional method of vinegar production. The first stage is alcohol fermentation, in which the sugar content in fruits or vegetables is converted to ethanol by wine yeast (*Saccharomyces* sp.) (Garcia-Garcia et al., 2006). The second stage in which the alcohol in the environment is converted into acetic acid by the acetic acid bacteria is known as acetic acid fermentation. (Plessi, 2003; Ozturk et al., 2015). The positive effects of vinegar on health are related to the raw materials used and the fermentation conditions (Ubeda et al. 2011). Studies have shown that vinegar has anticarcinogen, antioxidant, antibacterial effects, and some other effects that positively affect cholesterol levels and strengthen the immune system. (Budak et al., 2014).

The present study aimed to produce vinegar by surface culture method using fresh sour cherry and concentrated sour cherry juice as raw materials. The changes in the chemical, antioxidant and organic acid components that make up the functional and organoleptic properties of vinegar during the fermentation process were examined.

MATERIAL AND METHODS

2.1 Materials, chemicals, and reagents

The chemicals and reagents used were obtained from Sigma-Aldrich. Rehydrated wine yeast (*Saccharomyces cerevisiae* strain, ConFermUni V yeast) was obtained from Eaton's

Begerow® Product Line Co. (Nettersheim, Germany). Cherry fruits were produced from local markets in Isparta province Eğirdir district while sour cherry concentrate was provided from Asya Meyve Suyu ve Gıda San. A. Ş.

2.2. Production of cherry juice from cherry fruit

Firstly, the stems were separated from the sour cherry fruits. The fruits were then washed and the seeds were removed. After the necessary conditions were met, the sour cherries were crushed to extract their juice and sour cherry juice (FCJ) was obtained by filtering through a multi-layered filter cloth.

2.3. Production of sour cherry juice from sour cherry concentrate

The sour cherry concentrate obtained from sour cherry fruits collected from the same region was provided. The sour cherry concentrate was diluted with distilled water (dH_2O) and was adjusted to 14 (%) DM. This sour cherry juice was coded as CJJ.

2.5. Preparation of the Inoculum

Inoculation procedure was prepared according to Özen et al. 2020.

2.6. Production of sour cherry vinegar

Production of sour cherry vinegar (FCV and CCV) was carried out according to the traditional vinegar production method reported by Özen et al. (2020). Vinegar production flow charts are given in Figure 1 and Figure 2. The samples were taken on days 0, 15, 30, 45, and 60

Proximate analyses

Total titratable acidity was measured according to AOAC (1992). Total soluble solids (TSS; °Brix) contents of the samples were determined using Abbe refractometer (Bellingham Stanley Limit 60/70 Refractometer, England).

2.8. Antioxidant activity analyses

The antioxidant activity values of the samples were determined spectrophotometrically using the stable radical 2,2-diphenyl-1-picrylhydrazil (DPPH). The free radical scavenging activity of a sample is given as the percentage of DPPH reduced by the determined amount of active ingredient. Accordingly, 3.9 mL of 80 µM DPPH (0.0032 g DPPH is completed with 100 mL methanol) solution to be completed to 4 ml by taking 10 µL from the samples completely dissolved (using ethanol, water, etc. suitable solvent) in a single concentration was taken and treated with 0.1 mL of sample. Only the final solution and the DPPH solution were kept in the dark for 30 minutes and the absorbance values were read against methanol at 517 nm. Total activity values are given as inhibition % (Bunea et al., 2011).

2.9. Organic acid analyses

Organic acids and their values in the samples were determined by HPLC (Shimadzu SCL-10A, Scientific Instruments, Inc., Tokyo, Japan). HPLC device consisted of a DAD detector (SPD-M20A), a system control unit (LC 20ADvp), a pump (LC 10ADvp), a gas separator (DGU 20A3), and a column furnace (CTO-10ASvp). Inertsil ODS-3V C18 (GL Sciences Inc.) column was used for organic acid determination (250x4.60 mm, 5 µm). The mobile phase used was the 5 mM H_2SO_4 solution. The pH of the mobile phase was adjusted to 3.0±0.05 using 4 N NaOH. The column furnace temperature was set at 30°C and the flow rate of the mobile phase was 1.0 mL/min. The samples were directly passed through a 0.45 µm polytetrafluoroethylene (PTFE) filter (Membrane Solutions) and 20 µL was injected into the system. Organic acid determination was carried out using a DAD detector at 210 nm wavelength. The results are given by calculating the graph equations obtained from the standards given at different concentrations. The sample mixture chromatogram of the standards used in the determination of organic acid is given in Figure 3.

2.10. Statistical analysis

Both vinegar productions were carried out in two parallels with three replicates. All analyses were repeated thrice. Analysis results are expressed as mean±standard deviation. Data were subjected to a one-way analysis of variance (ANOVA) using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). The Duncan's test was utilized to evaluate significant differences (P< 0.05) between fermentation times while the Student t-test was used to evaluate significant differences (P< 0.05) between the samples.

RESULTS AND DISCUSSIONS

3.1 Proximate composition

In the present study, sour cherry vinegar (FCV and CCV) was produced from fresh cherry juices (FCJ) and concentrated fruit juices (CCJ). During the fermentation, titration acidity and % water-soluble dry matter values were determined. These values are given in Figures 4 and 5.

While the total acidity values were statistically different from each other at the beginning of the fermentation (P< 0.05) among the samples, the values on day 30 were found to be close to each other (p>0.05). In the acid fermentation process of the second stage of fermentation, the total acidity values of both samples increased significantly ($P < 0.05$). At the end of the acetic acid fermentation, no differences were observed between the total acidity values of the vinegar. The values 4.52% in the FCV sample and 4.74% in the CCV sample met the minimum 4% acidity criterion specified in TSE. The values obtained in the present study were similar to those obtained by Budak et al (2017) and Aykin et al. (2015) in apple and pomegranate vinegar.

Total soluble dry matter values of the samples decreased significantly in the first 15 days of fermentation ($P < 0.05$). No differences were observed between days 30, 60, and 90. The sugar content in the cherry juice decreased on day 15 due to the activity of the yeasts.

3.2. Antioxidant analysis by the stable radical 2,2-diphenyl-1-picrylhydrazil (DPPH) method

Antioxidant activity determination according to the DPPH method was determined in terms of inhibition %. In the antioxidant analysis carried out according to the days, the production from the cherry yielded the inhibition values 31.89, 35.95, 37.32, 82.36, and 80.85 %, respectively while the inhibition values in production using concentrated sour cherries were; 52.08, 46.75, 47.29, 83.83, 80.51 %, respectively. In the samples on days 45 and 60, DPPH analysis results were significantly higher than those of the other days ($p<0.05$). The DPPH results are given in Figure 6. Casedas et al. (2016) determined that the DPPH % inhibition value in cherry juice was 54. In the present study, the inhibition % value of sour cherry juice was determined in the range of 31.89-52.08.

3.3. Organic acid analyses

Tartaric acid, malic acid, and acetic acid were prominent in the samples at the production stages of sour cherry vinegar from sour cherry juice and concentrated sour cherry juice. Table 1 shows the data related to the organic acid contents in the samples determined by days.

The malic acid in sour cherry juice was determined to be 12361.04 and 13481.9 mg/L in the FCJ-0 day and CCJ-0 day samples, respectively. In the FCJ-15 day and CCJ-15 day samples, the malic acid value was found to be the highest in all fermentation stages with 15776.48 and 13710.71 mg/L, respectively. Malic acid values in the FCV-60 day and CCV-60 day samples were determined to be 2648.99 and 1089.92 mg/L, respectively. The malic acid levels on days 45 and 60 were significantly different from those of the other samples. The dominant organic acid in sour cherry juice was malic acid, while the dominant organic acid in vinegar was acetic acid. Acetic acid values in FCV-60 day and CCV-60 day vinegar samples were determined to be 2663.09 and 2978.71 mg/L, respectively. The acetic acid level in the samples on days 45 and 60 following the onset of acetic acid fermentation was found to be significantly different from those of the fruit juice and wine samples.

Çevik (2013) found malic acid in the range of 5.64-40.4 mg/L in the organic acid analysis they performed in 11 different sour cherry juices. The results obtained in the present study were determined to be 12361.04 and 13481.9 mg/L in production from sour cherry and in production from concentrated sour cherry juice, respectively.

Budak et al. (2010), in the production of grape vinegar using different production techniques, have reported the concentration of acetic acid in the range of 143.6 -82.81 g/L.

The tartaric acid value was determined in the range of 3795.37- 4951.58 mg/L in the samples taken during the fermentation stage obtained from sour cherry juice and the tartaric acid value was determined in the range of 2257.58- 4378.46 mg/L in the samples taken during the fermentation stages obtained from the concentrated cherry juice.

Lower levels of lactic acid were detected than the other organic acids determined in the samples. In the cherry juice samples, the lowest lactic acid value was 12.29 mg/L in the FCJ-0 day sample, while the highest was 29.69 mg/L in the FCW-30 day sample.

Examining the organic acid contents of vinegar produced from apple and wine collected from the markets of different countries, wine vinegar was found to be rich in terms of tartaric acid with 1.53 g/L, and apple cider vinegar was determined to be rich in terms of malic (0.94 g/L) and lactic acid (0.72 g/L). was determined (Gerbi et al., 1998). In the present study, the tartaric acid level was determined in the range of 3795.37- 4951.58 mg/L in the samples taken during the fermentation stage obtained from sour cherry juice, while the tartaric acid level was determined in the range of 2257.58- 4378.46 mg/L in the samples taken during the fermentation stages obtained from concentrated sour cherry juice.

CONCLUSIONS

Sour cherry is one of the important fruits that have beneficial health effects due to its organic acid components and antioxidant properties. In the present study, it has been predicted that, due to its different taste and aroma, sour cherry vinegar will appeal to consumers who are searching for alternatives.

Cherry vinegar gains attention due to its tartaric, malic, and acetic acid content in the production stages. The dominant organic acid in sour cherry juice was malic acid whereas the dominant organic acid in vinegar was determined to be acetic acid. In the antioxidant activity analysis by the DPPH method, it was determined that the inhibition level of sour cherry vinegar in % was higher than sour cherry juice and cherry wine. No differences were observed in the analyses of the sour cherry vinegar samples made from two different raw materials. Therefore, considering that the harvest time of sour cherry is very short, it was determined that sour cherry vinegar can be produced from concentrated sour cherry juice regardless of the harvest season. The results of this research will provide valuable information for producers who want to produce sour cherry vinegar on an industrial scale throughout the year and contribute to the economy.

Acknowledgement

I would like to thank Tuğba Kök Taş and Bilge Ertekin Filiz for their support of this research.

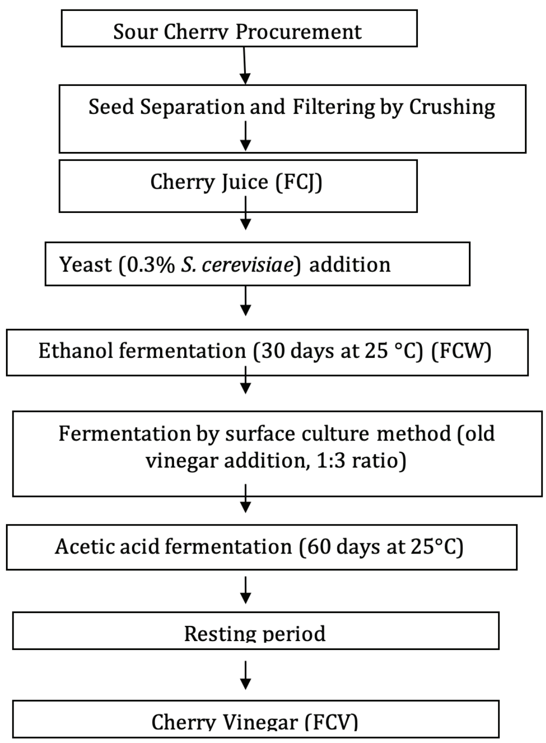

Figure 1. Vinegar production flow chart from cherry fruit

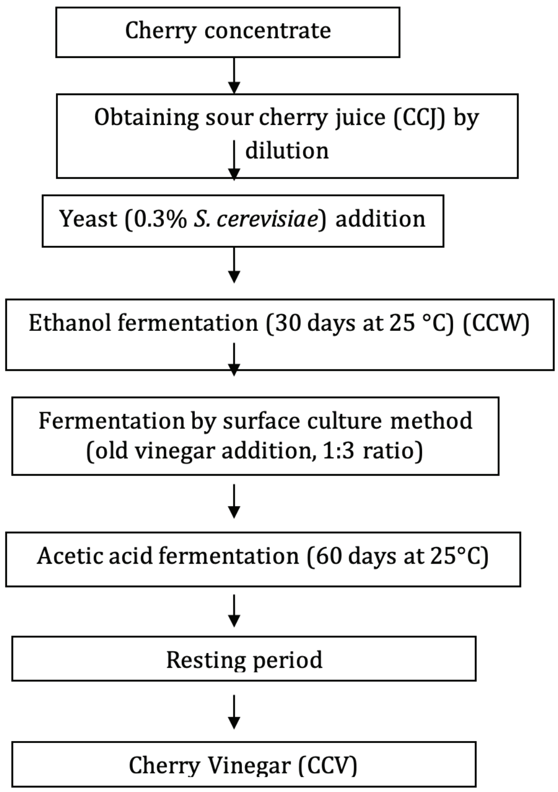

Figure 2. Flow chart of vinegar production from sour cherry concentrate

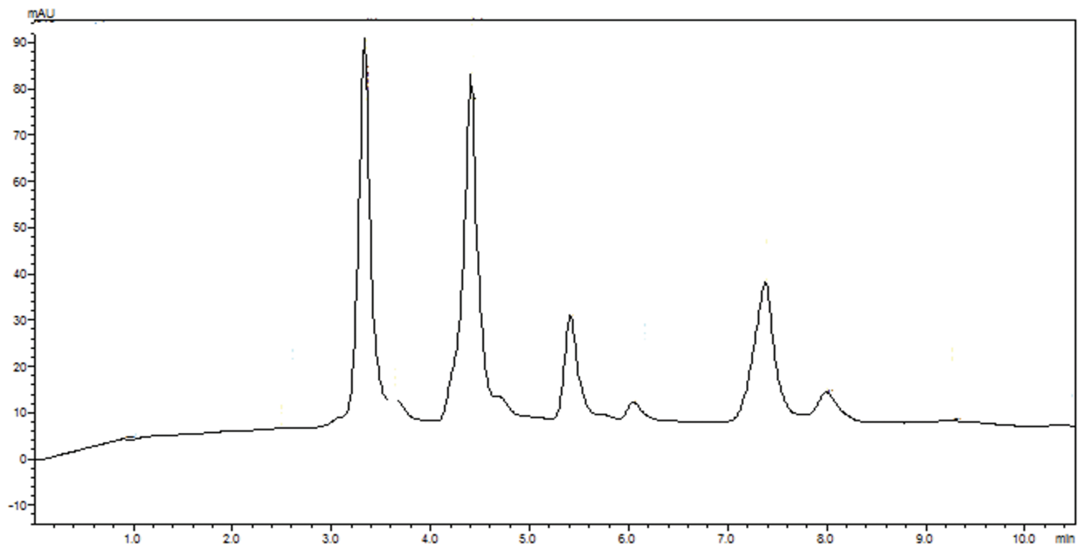

Figure 3. Sample mix chromatogram of organic acid standards

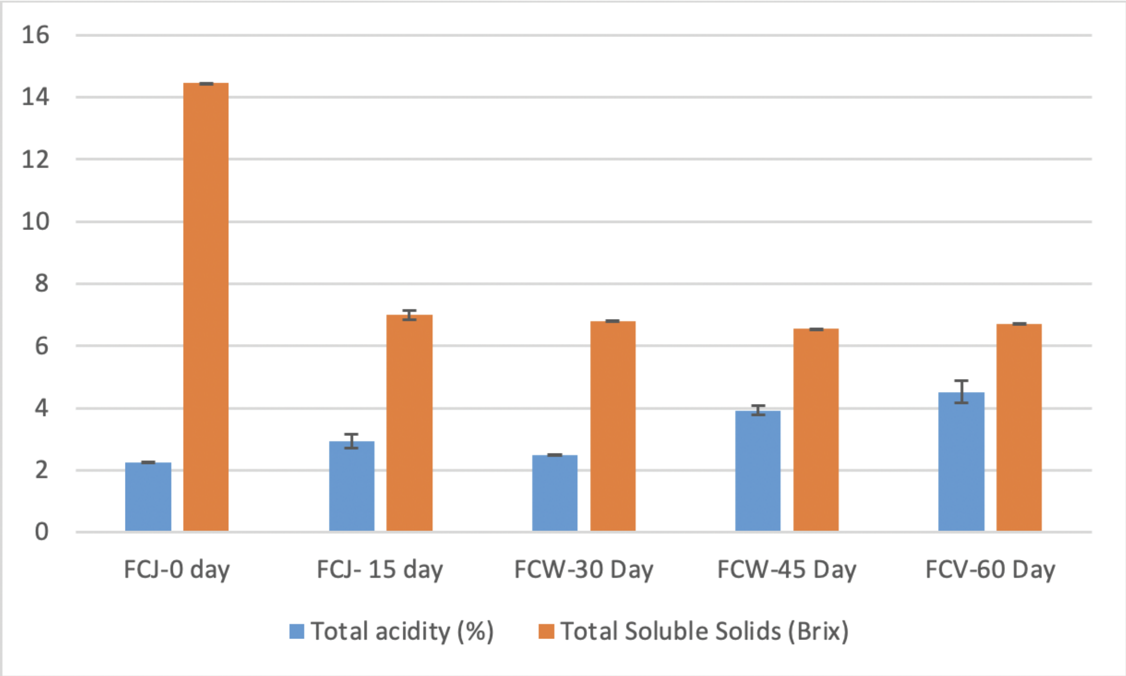

Figure 4. Juice (FCJ), wine (FCW) and vinegar (FCV) produced using fresh sour cherry juice

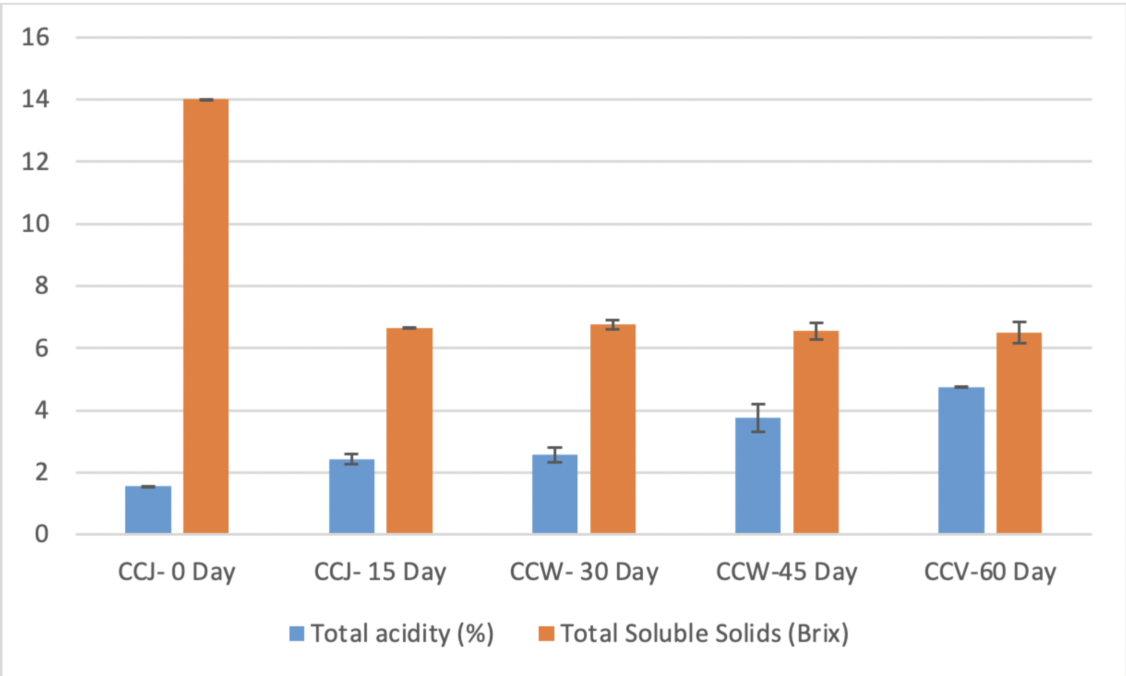

Figure 5. Juice (CCJ), wine (CCW) and vinegar (CCV) produced using concentrated sour sherry

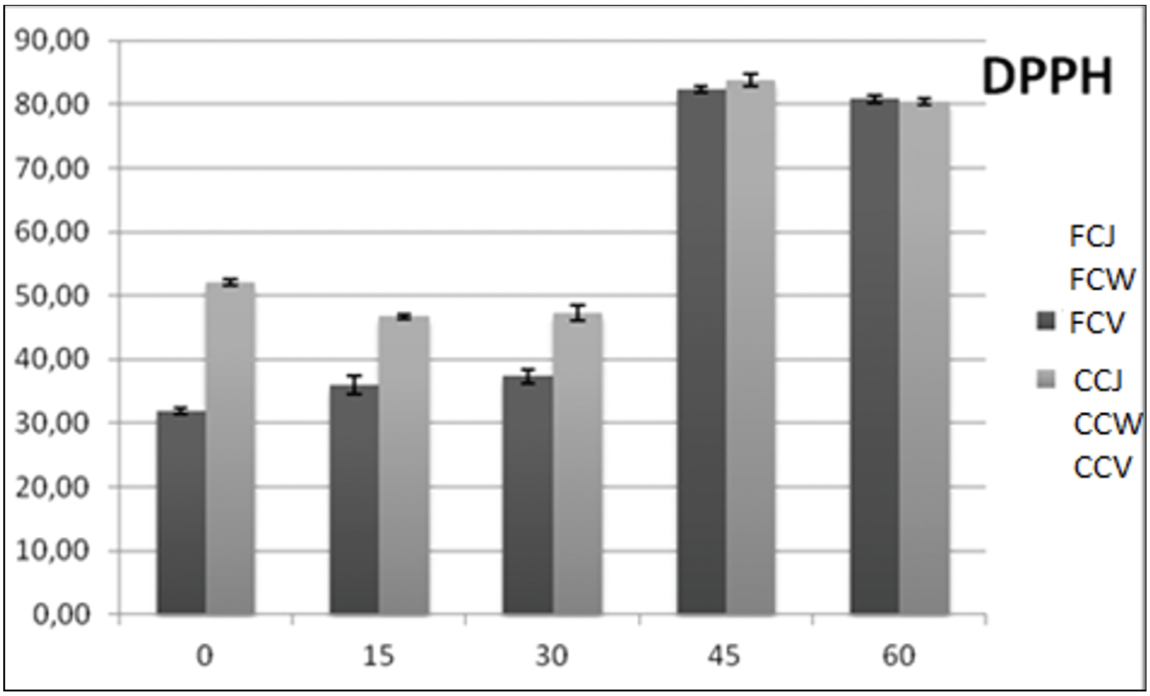

Figure 6. DPPH results of vinegar samples produced from our cherry and concentrated sour cherry

Table1. Organic acid results in vinegar samples

Samples	Fermentation days	Tartaric acid (mg/L)	Malic acit	Lactic acid (mg/L)	Acetic acid (mg/L)
FCJ	0	4951.58±122.47[a]	12361.04±311.27[a]	12.29±0.99 [a]	339.01±11.16[a]
	15	4741.20±109.69 [a]	15776.48±571.20[a]	27.07±0.50[c]	335.63±10.16[a]
FCW	30	3795.37±117.59 [a]	13655.7±535.37 [a]	29.69±0.90[c]	262.23±58.08[a]
	45.	4457.17±177.15 [a]	8114.55±530.50[c]	20.99±1.52[b]	3233.80±107.04[b]
FCV	60	4585.77±199.92 [a]	2648.99±554.51[b]	24.64±2.87[b]	2663.09±128.29[b]
CCJ	0	4378.46±104.05 [b]	13481.9±1450.31[c]	30.74±0.93[a]	389.12±51.81[a]
	15	3413.21±158.45 [ab]	13710.71±476.81[c]	66.31±1.96[b]	175.46±21.43[a]
CCW	30	2257.58±177.31 [a]	9766.21±309.03[b]	41.34±0.21[ab]	124.64±30.54[a]
	45	3972.58±103.89 [ab]	2063.87±192.74[a]	43.60±0.56[ab]	2694.09±114.03[b]
CCV	60	3690.49±124.52 [ab]	1089.92±90.74[a]	23.66±1.46[a]	2978.71±110.44[b]

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