ALTERATIONS ON PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY DURING SOUR GRAPE JUICE CONCENTRATE PROCESSING

ALTERAÇÕES DOS COMPOSTOS FENÓLICOS E DA ATIVIDADE ANTIOXIDANTE DURANTE O PROCESSO DE CONCENTRAÇÃO DE SUMO DE UVAS NÃO AMADURECIDAS

Ali Guler1*, Ozlem Tokusoglu2, Nevzat Artik3

1 Viticulture Research Institute, Atatürk Strt, Horozköy Ave., 45125 Yunusemre/Manisa, Turkey.
2 Celal Bayar University, Engineering Faculty, Department of Food Eng., 45140, Manisa, Turkey.
3 Ankara University, Engineering Faculty, Department of Food Eng., Ankara, Turkey.

* Corresponding author: Tel: +902362111071, e-mail: aligguler@gmail.com

SUMMARY

The alterations of phenolic compounds and antioxidant capacity of sour grape juice were investigated during the concentration process stages. Phenolics and antioxidant properties of the samples changed more at the vacuum evaporation stage than that of the other stages. After evaporation, the antioxidant capacity of the samples decreased approximately between 14.2 and 17.0 % for DPPH and ABTS methods, respectively. Besides, phenolic contents also decreased approximately as 14.8%. HPLC data on phenolics of sour grape juice during concentrate processing gave 12 polyphenols, including gallic acid, (+)-catechin, (-)-epigallocatechin, vanillic acid, (-)-epigallocatechin gallate, (-)-epicatechin, caftaric acid, caffeic acid and p-coumaric acid, which were determined as 1.05-1.83 mg/100 g, 5.40-7.83 mg/100 g, 6.35-9.21 mg/100 g, 3.01-5.18 mg/100 g, 0-1.95 mg/100 g, 2.33-3.54 mg/100 g, 12.40-37.60 mg/100 g, 1.44-2.26 mg/100 g, and 0.27-0.44 mg/100 g, respectively. Ferulic, sinapic acids and quercetin were only detected at concentrate step of the sour grape juice processing and were found as 0.18 mg/100 g, 0.28 mg/100 g and 0.76 mg/100 g, respectively.

RESUMO

As alterações dos compostos fenólicos e da capacidade antioxidante do sumo de uvas não amadurecidas foram investigadas durante as etapas do processo de concentração. Os compostos fenólicos e as propriedades antioxidantes das amostras sofreram maior alteração na fase de evaporação sob vácuo do que nas restantes fases. Após a evaporação, a capacidade antioxidante das amostras diminuiu aproximadamente entre 14.2 e 17.0% nas determinações efetuadas pelos métodos DPPH e ABTS, respectivamente. Por outro lado, os teores de compostos fenólicos também diminuíram aproximadamente 14.8%. Os resultados obtidos por HPLC revelaram a presença de 12 polifenóis, incluindo ácido gálico, (+)-catequina, (-)-epigallocatequino, ácido vanílico, (-)-epigallocatequina gallato, (-)-epicatequina, ácido cafártico, ácido cafeico e ácido p-cumárico, com teores de 1.05-1.83 mg/100 g, 5.40-7.83 mg/100 g, 6.35-9.21 mg/100 g, 3.01-5.18 mg/100 g, 0-1.95 mg/100 g, 2.33-3.54 mg/100 g, 12.40-37.60 mg/100 g, 1.44-2.26 mg/100 g e 0.27-0.44 mg/100 g, respectivamente. Os ácidos ferúlico e sinápico e a quercetina foram detetados somente na etapa de concentração do sumo de uvas não amadurecidas, com teores de 0.18 mg/100 g, 0.28 mg/100 g e 0.76 mg/100 g, respectivamente.

Key words: sour grape juice, concentrate, processing, phenolic compounds, antioxidant activity.
Palavras-chave: sumo de uvas não amadurecidas, concentração, processamento, compostos fenólicos, atividade antioxidante.

INTRODUCTION

Fruits play important role on the protection of human health due to their contents such as phenolics, minerals, vitamins and antioxidants. Grape (Vitis sp.) is one of the most produced fruits in the world. It is used for wine, juice, raisin, sour grape juice, vinegar and pekmez (grape concentrate, molasses). Sour grape is used for ‘verjuice’ or sour grape juice, consuming for salad dressing, processed vegetables and drinks as sherbet with sweeteners (Karapinar and Sengun, 2007; Nickfardjam, 2008). The sour grape juice has different names, such as ‘verjuice’, ‘verjus’ and ‘koruk’ juice, according to the producing country. Additionally, it has been traditionally produced for many years.

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Grape includes polyphenols that have protective effect on human health (Chira et al., 2008; Xia et al., 2010; Toaldo et al., 2015). The phytochemical polyphenols have anticancer and anti-inflammatory effects in vitro (Castilla et al., 2006; Capanoglu et al., 2013). It was reported that these compounds inhibit cardiovascular diseases, some types of cancer cells, reduce plasma oxidation stress and aging effect (Meyer et al., 1997; Falchi et al., 2006; God et al., 2007; Xia et al., 2010; Tsanga et al., 2015). Many studies were performed related to the grape, wine and grape juice effects on human health although investigations on improving health of sour grape juice were fairly limited. Zibae Nezhad et al. (2012) expressed that sour grape juice had improving effect on serum levels of HDL-C but no lipid-lowering effect on triglyceride and serum levels of LDL-C. Grape polyphenols show high antioxidant properties. For this reason, many researchers tried to determine the relationship between phenolic compounds and antioxidant activity of the grape (Xia et al., 2010). It was stated that significant correlations were found among phenolic compounds and antioxidant activity of grape, grape juice, grape concentrate and wine (Castilla et al., 2006; Paixao et al., 2007; Stratil et al., 2008; Gollucke et al., 2009; Buyuktuncel et al., 2014; Lima et al., 2014; Toaldo et al., 2015).

No study related to sour grape juice processing and concentration could be found in literature although many studies were conducted on grape juice processing and concentration (Gollucke et al., 2009; Capanoglu et al., 2013; Lima et al., 2014). On the other hand, the studies that were regarding phenolic compounds and antioxidant properties of sour grape juice were fairly limited.

In the current study, the alterations of phenolic compounds and antioxidant capacity of sour grape juice were investigated during the concentration process stages. The relationship between phenolic content and antioxidant capacity was also observed at six processing stages.

MATERIAL AND METHODS

Chemicals

Folin–Ciocalteu reagent, potassium carbonate, formic acid, ethyl alcohol, potassium persulfate were purchased from Merck (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and methanol were purchased from Sigma–Aldrich (St. Louis, Missouri, USA). 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) was purchased from Amresco Inc. (Radnor, Pennsylvania, USA). Water for HPLC was purchased from Carlo Erba (Carlo Erba Reagents S.A.S., Val de Reuil, France). Potassium bitartrate (K\textsubscript{2}C\textsubscript{4}H\textsubscript{6}O\textsubscript{7}) was purchased from Bereket Chemical (Bereket Chemical, ZAG Industry Chemicals, Istanbul, Turkey).

Gallic acid, caffeic acid, p-coumaric acid, (-)-epicatechin and quercetin were purchased form Sigma–Aldrich (St. Louis, Missouri, USA). (+)-Catechin, (-)-epigallocatechin gallate, vanillic acid and caftaric acid were purchased from Fluka (St. Louis, MO, USA). (-)-Epigallocatechin and sinapic acid were purchased from Alfa Asear (Karlsruhe, Germany). Ferulic acid was purchased from Merck (Germany).

Sour grapes

Sour grape samples of Sultani seedless (\textit{Vitis vinifera} L.) grape variety used for juice production were obtained from Manisa Viticulture Research Institute vineyards. After harvest, samples were immediately transferred to the grape processing unit of the Institute.

Sour grape samples were harvested before veraison period in 2015. No diseases and insect damage were detected in the sour grape samples at harvest; the cluster and berries of sour grapes had good sanitary appearance. The soluble solid value and total acidity of sour grapes were 9.5 °Brix and 30.45 g/L (as tartaric acid equivalent), respectively. The pH value of sour grape was 2.47.

Sour grape concentrate production

Sour grapes were rinsed to remove the dust, soil and other impurities after harvest. Then, stalks were discarded and clusters were passed through a crusher destemmer machine (Türköz Metal Makine, Turkey). The mash was pressed in a hydraulic press (Türköz Metal Makine, Turkey) and clear ‘koruk’ juice was obtained [A]. The juice was kept at 2-4 °C cold room for 24 h for precipitation and removing rough residue [B]. Pectolytic enzyme application (Shazym Claro Pectolytic Enzyme, 10.500 PGNU/g polygalacturonase, 0.15 g/L) was performed at 50°C for 2 h [C]. Bentonite and gelatin were applied during clarification process; 10 ml/L from 10% bentonite solution and 25 ml/L from 1% gelatin solution were used at 20 °C and then ‘koruk’ juice was kept at 4 °C for 24 h [D]. At the same temperature, 5 g/L potassium bitartrate (K\textsubscript{2}C\textsubscript{4}H\textsubscript{6}O\textsubscript{7}) was added and left for 7 days for detartarization [E]. The final clarified ‘koruk’ juice was concentrated to 42-45 °Brix at 50 °C and 600 mm Hg vacuum [F]. Flow diagram of sour grape concentrate production is presented in Figure 1. The concentration processing was made in duplicate and 150 kg of sour grape was used for each replicate.

\[\text{Gallic acid + \text{caffeic acid} + \text{p-coumaric acid} + \text{(-)-epicatechin} + \text{quercetin} + \text{(+)-catechin} + \text{(-)-epigallocatechin gallate} + \text{vanillic acid} + \text{caftaric acid} + \text{ferulic acid} + \text{merck} + \text{merck france} + \text{merck gmbh} + \text{merck karlsruhe} + \text{merck turkey} + \text{merck usa} + \text{merck} + \text{merck usa}\]
It was chosen six steps for sampling during concentration process. The sampling steps for analysis were at the end of the pressing, removing rough residue, depectinization, clarification, detartarization and evaporation stages. These sampling steps were indicated with [A], [B], [C], [D], [E] and [F] letters, respectively. Six samples were taken from each concentration process steps for analysis.

**Determination of total polyphenols**

Total phenolic compounds in the samples were determined according to Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). Briefly, 100 µL of Folin-Ciocalteu solution was added to each 4 mL diluted samples and then 500 µL of 20% saturated sodium carbonate (Na₂CO₃) was added to final solution after 3 min and all was shaken. Then the samples were incubated at room temperature (24 ± 1 °C) for 30 min. At the end, 350 µL samples were transferred into a 96 well microplate and the absorbance was measured at 760 nm. 5, 10, 20, 30, 40 and 50 mg/L of standard concentrations were used for calibration curve (y = 0.0649 x + 0.0324; R² = 0.0995). Results were expressed as milligrams of gallic acid equivalents (GE) in 100 g dry matter (dry weight, DM).

**Radical scavenging activity assay**

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay was performed according to Brand-Williams et al. (1995). The principle of the method is the measurement of the reduction ability of the DPPH• radical in samples. Briefly, 3 mL of the 1 mM DPPH• solution was transferred into 10 mL tubes and 200, 400, 600, 800 and 1000 µL of diluted samples were added and bring up to 4 mL with methanol and incubated at room temperature (24 ± 1 °C) in the dark during 30 min. Methanol was used as blank. The absorbance was measured at 517 nm wavelength in a spectrophotometer (Thermo scientific, Multiskango, Finland). Percent inhibition values were calculated according to blank absorbance as described in the formula: Inhibition% = (A_DPPH−A_SAMPLE)/ A_DPPH x 100. Calculated inhibitions and the sample volumes were subjected to linear regression on the graphic, and slope of each sample and equilibrium of these slopes were obtained. EC₅₀ values were calculated based on the equation of obtained slope values (necessary volume of equate for elimination the 50% of DPPH•): EC₅₀ = [(a × sample volume) ± b]/dilution factor. A calibration curve was drawn using a standard solution of Trolox (8, 16, 32, 64 and 128 µM; y = 0.2741x + 0.4972; R² = 0.9987). Antioxidant activity was expressed as µmol Trolox in 100 g DM.

**Trolox equivalent antioxidant capacity assay**

ABTS [2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] method was used as previously described by Re et al. (1999). Firstly, stable ABTS stock solution was performed by reacting 7 mM ABTS with 2.45 mM potassium persulfate (final concentration) allowing the mixture to stand in the dark for 12–16 h at room temperature before use. The ABTS•⁺ solution was diluted with methanol to the absorbance of 0.70 (± 0.02) at 734 nm and equilibrated at 30°C. 10, 20 and 30 µL of each diluted samples were pipetted and ABTS•⁺ radical solution were added to final volume of 1000 µL. Then, absorbance was measured during 6 min with one min intervals. The initial (A₀) and the end of the 6th min (A₆) absorbance were used for calculation of the absorbance inhibition percentages of each sample or standard volume. 5, 10, 15 and 20 µM standard
Trolox concentration was utilized for calibration curve ($y=3.2239x – 0.0101; \ R^2=0.9991$). Trolox equivalent antioxidant capacity (TEAC) of the samples was calculated using slope of the sample and standard. Results were expressed as µmol Trolox in 100 g DM.

**HPLC analysis for individual phenolic compounds**

Phenolic compounds were evaluated by high performance liquid chromatography (HPLC) method. ODS C18 (250 x 4.6 mm, 5µm) column for analytical separation and diode array detector (DAD) was used in the HPLC system (Agilent 1260 infinity). Detection and quantification of phenolic compounds was carried out according to Özkan and Gökturek Baydar (2006) and Caponio et al. (1999) with slight modification of mobile phase. 2.5, 5, 10, 20 and 40 mg/L standard concentrations were used for calibration curves of gallic acid, vanillic acid, caficrac acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, quercetin and (-)-epicatechin. 5, 10, 20, 40 and 60 mg/L concentrations were used for (+)-catechin, (-)-epigallocatechin and (-)-epigallocatechin gallate calibration curves. The sour grape juice samples taken from the previously defined steps were diluted with methanol and filtered through a 0.45 µm of PTFE syringe filter before the HPLC analysis. Then, the samples were injected directly into the HPLC system.

Gradient elution was used; solvents consisting of ultra-pure water: formic acid (99.8:0.2 v/v) (A) and methanol (B). Gradient elution program was as follows: the initial elution 0% B, followed 3 min by linear gradient from 0% to 5% B, 15 min linear gradient to 20% B, 2 min isocratic elution step %20 B, 10 min linear gradient elution to 25% B, 10 min elution to 30% B, 10 min elution to 40% B, 5 min elution to 50% B and 10 min linear gradient elution to 100% B. Then, 5 min 100% A elution was performed for returning to initial condition. Column temperature was set at 30°C and detection was made at 280, 320 and 360 nm. Gallic acid, (+)-catechin, (-)-epigallocatechin, vanillic acid, (-)-epigallocatechin gallate and (-)-epicatechin were detected at 280 nm wavelength. Caficrac acid, caffeic acid, p-coumaric acid, ferulic acid and sinapic acid were identified at 320 nm. Finally, quercetin was detected at 360 nm wavelength. The elution time was 65 min, the injection volume was 10 µL and the flow rate was 1mL/min. The phenolic compounds of the samples were identified by comparing their retention times and spectra with those of analytical standards. The concentration of phenolic compounds in the samples was calculated through the calibration curves and expressed as mg/100 g DM. Chromatographic analyses were performed in triplicate.

**Statistical analysis**

A two-way analysis of variance (ANOVA) was applied to the obtained results. Duncan multiple comparison test was performed to determine the differences between the average values (p<0.05 significance level was used for comparisons). Pearson correlation coefficients were calculated to prove the relationships between total phenolics and antioxidant properties.

**RESULTS AND DISCUSSION**

The results for total phenolic (TP) contents of the samples are presented in Table I, where it can be observed that the values varied from 245.62 and 288.4 mg/100 g DM at processing steps. Significant statistical differences were found between the TP values of the investigated processing steps (p<0.05). The lowest TP content was found in [F] stage (after evaporation), while the highest TP was found in [A] stage. According to Piva et al. (2008), which investigated the physical and functional alterations during grape juice cooking using three different concentrate ratios (max. 35, 60 and 70%), the TP contents in fresh and concentrated grape must samples varied from 27.1 to 1259 mg/L. Öncül and Karabıyıklı (2015) reported that TP content of ‘verjuice’ samples varied from 233.44 and 672.75 mg/L. Nikfardjam (2008) reported similar TP content ranges in ‘verjuice’ (200-1330 mg/L). Turkmen et al. (2017) revealed that TP contents of unripe grape juice extracts ranged from 436.36 and 758.52 mg/L. Hayoglu et al. (2009) reported that TP content of ‘verjuice’ samples ranged from 2374.8 to 4041.5 mg/L. TP results of the sour grape concentrate are similar to those obtained in previous studies. However, the results are lower than the results reported by Hayoglu et al. (2009). The differences could be due to the maturation of grape, applied process steps and evaporation conditions (Gollücke et al., 2009; Hayoglu et al., 2009; Sabir et al., 2010; Öncül and Karabıyıklı, 2015; Tastan and Baysal, 2015).

TP content of the concentrate decreased approximately 14.8% according to the first processing stage. The decreasing was linear from [A] to [F] stages. Gollücke et al. (2009) reported that TP contents of ‘Concord’ and ‘Isabel’ grape juice concentrate decreased after heat treatment and the reduction was lower than after filtration and concentration.
TABLE I

Total phenolic content and antioxidant properties of sour grape processing samples

<table>
<thead>
<tr>
<th>Process steps</th>
<th><strong>TP</strong> (mg/100 g DM)</th>
<th><strong>DPPH</strong> (EC₅₀ (µl))</th>
<th><strong>TEAC</strong> (µmol/100 g DM)</th>
<th><strong>ABTS</strong> (µmol/100 g DM)</th>
<th><strong>TEAC</strong> ABTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressing [A]</td>
<td>288.42±6.23a</td>
<td>20.62±1.45c</td>
<td>198.00±0.82a</td>
<td>569.25±21.86c</td>
<td>20.44±0.01d</td>
</tr>
<tr>
<td>Removing Rough Residue [B]</td>
<td>274.81±2.66b</td>
<td>18.11±2.28c</td>
<td>264.59±7.44a</td>
<td>675.17±28.16b</td>
<td>24.53±0.86c</td>
</tr>
<tr>
<td>Depectinization [C]</td>
<td>277.68±3.52b</td>
<td>22.73±1.64c</td>
<td>165.91±1.97c</td>
<td>698.79±15.39b</td>
<td>26.63±0.30c</td>
</tr>
<tr>
<td>Clarification [D]</td>
<td>259.42±0.41c</td>
<td>19.35±0.71c</td>
<td>204.07±1.63c</td>
<td>728.74±22.80c</td>
<td>19.01±0.70d</td>
</tr>
<tr>
<td>Detartarization [E]</td>
<td>256.89±0.82c</td>
<td>25.64±0.34a</td>
<td>198.24±1.42b</td>
<td>652.10±43.47g</td>
<td>26.91±0.20d</td>
</tr>
<tr>
<td>Evaporation [F]</td>
<td>245.62±4.34d</td>
<td>80.78±3.70a</td>
<td>30.68±0.473</td>
<td>488.59±19.56d</td>
<td>69.38±1.30c</td>
</tr>
</tbody>
</table>

Values followed by different letters within each group and column are significantly different at p<0.05. **TP**: Total phenolic content; **DPPH**: Inhibition value for DPPH method; **EC₅₀**: Efficiency concentration for DPPH; **ABTS**: Inhibition value for ABTS method; **TEAC**: Trolox equivalent antioxidant capacity.

On the other hand, Capanoglu et al. (2013) investigated the changes of polyphenols during production of grape juice concentrate and stated that TP content of grape concentrate decreased by 84.4% dry weight basis. The ratio is considerably higher than in the current study.

Antioxidant activities of samples were detected by DPPH and ABTS assays and the obtained results were showed in Table I. Significant statistical differences were observed among processing steps for all antioxidant properties evaluated (p<0.05). DPPH and ABTS inhibitions in [A] stage (after pressing) were 20.62 and 20.44%; the inhibitions in [F] stage (after evaporation) were 80.78 and 69.68%, respectively. Turkmen et al. (2017) found that DPPH inhibitions of unripe grape juice samples varied between 64.07 and 82.64%.

TEAC_ABTS and TEAC_DPPH of the samples ranged from 1137.22 to 1672.00 µmol/100 g DM and from 488.59 to 698.79 µmol/100 g DM, respectively. Antioxidant activity of ‘verjuice’ samples were reported as 25-231 µmol TE/L for FRAP and 158-885 µmol TE/L for TEAC by Öncül and Karabıyıklı (2015). Additionally, in the same study, the values in ‘verjuice’ sauces were between 35 and 721 µmol TE/L for TEAC.

The changes of antioxidant activities during concentrate processing are presented in Figure 2. TEAC_DPPH and TEAC_ABTS values in the samples increased compared to [A] stage at [B], [C] and [D] processing stages. The increase varied between 14.58 and 28.12% for TEAC_DPPH and 0.72 to 18.06% for TEAC_ABTS. After [D] stage, antioxidant activity started to decrease. The lowest antioxidant activity results were found in [F] stage. After evaporation [F], the TEAC_DPPH and TEAC_ABTS values decreased 14.2 and 17.0% compared to [A] stage, respectively.

Figure 2. TEAC changing during sour grape processing.

Alteração de TEAC durante o processamento do sumo de uvas não amadurecidas.

Piva et al. (2008) reported that antioxidant activities of fresh and concentrated grape juice samples varied from 925 to 2100 µM/L. They concentrated the grape juice until 35, 60 and 70% ratios. The antioxidant activities in grape must concentrates decreased compared to initial fresh must values in dry weight bases. The reduction was 24.7 and 27.0% for 60 and 70% concentration ratios, respectively (Piva et al., 2008). It was reported that the antioxidant activity of the grape juice concentrate decreased between 83 and 92% during concentration stages compared to the
initial stage. The reduction ratios from pasteurization to concentration stages were 3.6 and 10.4% for ABTS and DPPH methods, respectively. Moreover, it was expressed that antioxidant capacity showed some variation through the concentration process of grape juice (Gollucke et al., 2009). The current findings obtained from the sour grape concentrate have slightly differences with those of previous studies. These differences could be due to the maturity of the grapes because sour grapes are unripe grapes and they have different chemical and physical characteristics comparing to mature grapes. Gollucke et al. (2009) have also expressed that antioxidant capacity showed some variation through the concentration process of grape juice. Öncül and Karabıyıklı (2015) have also reported the change of antioxidant components in grape depending on maturation. Additionally, grape varieties, heat treatment in processing and other processing conditions may affect these parameters.

Correlations between TP and antioxidant parameters in the sour grape concentrate process are shown in Table II. Significant correlations were found among TP, inhibitions of DPPH and ABTS, EC\text{DPPH} and TEAC\text{DPPH} (p<0.01). The highest positive and the most significant correlation was observed between TP and TEAC\text{ABTS} (r = 0.999, p<0.01). The negative significant correlation was found between TP and EC\text{DPPH} (r = - 0.919, p<0.01). Additionally, important correlations were obtained between DPPH and ABTS parameters. Lima et al. (2014) found that the correlations between the total phenolic contents and the DPPH and ABTS antioxidant activities in grape juice samples were 0.94 and 0.84, respectively. In other study, Burin et al. (2010) reported the positive correlations found between total phenolic content and antioxidant activity (DPPH methods) of grape juice samples. Öncül and Karabıyıklı (2015) have also showed similar correlation results. The correlations between TP and antioxidant parameters are consistent with the literature.

### Table II

Correlations of between total phenolic compounds and antioxidant properties in the sour grape concentrate

<table>
<thead>
<tr>
<th></th>
<th><strong>TP</strong></th>
<th><strong>DPPH\text{inh}</strong></th>
<th><strong>DPPH EC\text{50}</strong></th>
<th><strong>TEAC\text{DPPH}</strong></th>
<th><strong>ABTS\text{inh}</strong></th>
<th><strong>TEAC\text{ABTS}</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TP</strong></td>
<td>1</td>
<td>0.994</td>
<td>-0.919</td>
<td>0.998</td>
<td>0.991</td>
<td>0.999</td>
</tr>
<tr>
<td>DPPH\text{inh}</td>
<td>0.994</td>
<td>1</td>
<td>-0.929</td>
<td>0.992</td>
<td>0.989</td>
<td>0.992</td>
</tr>
<tr>
<td>DPPH EC\text{50}</td>
<td>-0.919</td>
<td>-0.929</td>
<td>1</td>
<td>-0.921</td>
<td>0.905</td>
<td>-0.911</td>
</tr>
<tr>
<td>TEAC\text{DPPH}</td>
<td>0.998</td>
<td>0.992</td>
<td>-0.921</td>
<td>1</td>
<td>0.986</td>
<td>0.998</td>
</tr>
<tr>
<td>ABTS\text{inh}</td>
<td>0.991</td>
<td>0.989</td>
<td>0.905</td>
<td>0.986</td>
<td>1</td>
<td>0.992</td>
</tr>
<tr>
<td>TEAC\text{ABTS}</td>
<td>0.999</td>
<td>0.992</td>
<td>-0.911</td>
<td>0.998</td>
<td>0.992</td>
<td>1</td>
</tr>
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</table>

n=6 (p<0.01). **TP:** Total phenolic content; DPPH\text{inh}: Inhibition value for DPPH method; DPPH EC\text{50}: Efficiency concentration for DPPH; ABTS\text{inh}: Inhibition value for ABTS method; TEAC: Trolox equivalent antioxidant capacity.

The values of the 12 phenolic compounds studied during sour grape processing stages are presented in Table III. The HPLC chromatogram obtained for the sour grape juice concentrate of the 12 investigated phenolic compounds is shown in Figure 3. Regarding individual phenolic compounds, caftaric acid was the major compound, with values varying from 12.40 to 37.60 mg/100 g in sour grape juice samples during the concentrate processing. In a study that have investigated phenolic compounds in ‘verjuice’ samples from different countries it was also reported that caftaric acid was the major phenolic compound in the grape juice and wine (Toaldo et al., 2015; Yamamoto et al., 2015, Padilha et al., 2017; Alexiandre-Tudo et al., 2018). The caftaric acid values in these studies varied from 73.4 to 365.5 mg/L in grape juices and 6.6 to 167.4 mg/L in wines. The findings related to caftaric acid in the current study are accordance with these literature’s results.

The contents of gallic, vanillic, caffeic and p-coumaric acids during the sour grape processing stages ranged from 1.05 to 1.83 mg/100 g, 3.01 to 5.18 mg/100 g, 1.44 to 2.26 mg/100 g and 0.27 to 0.44 mg/100 g, respectively. Ferulic acid, sinapic acid and quercetin were detected only at [F] step of sour grape juice processing and their contents were 0.18 mg/100 g, 0.28 mg/100 g and 0.76 mg/100 g, respectively. (-)-Epigallocatechin gallate results in the samples varied from and 0.76 to 1.95 mg/100 g.
### TABLE III
Phenolic compounds contents in sour grape processing stages

<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>1.31±0.06a</td>
<td>1.83±0.02a</td>
<td>1.05±0.06a</td>
<td>1.73±0.01b</td>
<td>1.56±0.01c</td>
<td>1.76±0.01e</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>3.05±0.01a</td>
<td>3.01±0.39a</td>
<td>3.16±0.18b</td>
<td>4.88±0.07a</td>
<td>5.00±0.05a</td>
<td>5.18±0.03a</td>
</tr>
<tr>
<td>Caftaric acid</td>
<td>30.55±0.26b</td>
<td>37.60±0.28a</td>
<td>14.15±0.10b</td>
<td>12.40±0.10f</td>
<td>16.97±0.16d</td>
<td>14.98±0.22d</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>2.26±0.03a</td>
<td>2.11±0.19a</td>
<td>1.60±0.05b</td>
<td>1.58±0.14a</td>
<td>1.70±0.02b</td>
<td>1.44±0.03b</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>0.32±0.01ab</td>
<td>0.33±0.02b</td>
<td>0.32±0.01bc</td>
<td>0.40±0.02a</td>
<td>0.44±0.05a</td>
<td>0.27±0.02a</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.28±0.02</td>
</tr>
<tr>
<td>(+)-Catechin</td>
<td>5.96±1.50</td>
<td>6.94±0.94</td>
<td>6.53±0.10</td>
<td>7.82±0.16</td>
<td>7.83±0.13</td>
<td>5.40±0.04</td>
</tr>
<tr>
<td>(-)-Epigallocatechin</td>
<td>6.35±0.66</td>
<td>8.03±0.49</td>
<td>7.08±1.73</td>
<td>6.81±0.09</td>
<td>7.22±0.07</td>
<td>9.21±0.20</td>
</tr>
<tr>
<td>(-)-Epigallocatechin gallate</td>
<td>1.95±0.01</td>
<td>1.52±0.02</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.76±0.09</td>
</tr>
<tr>
<td>(-)-Epicatechin</td>
<td>2.79±0.33a</td>
<td>3.06±0.03ab</td>
<td>3.54±0.22a</td>
<td>3.24±0.24bc</td>
<td>3.43±0.08ab</td>
<td>2.33±0.42d</td>
</tr>
<tr>
<td>Quercetin</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.76±0.06</td>
</tr>
</tbody>
</table>

Values followed by different letters within each group and row are significantly different at p<0.05; n.d.: Not detected.

(+)-Catechin and (-)-epigallocatechin values were found between 5.40 and 7.83 mg/100 g and 6.35 and 9.21 mg/100 g in the samples, respectively. No statistical differences were observed for catechin and (-)-epigallocatechin values among the processing stages. Statistical differences were observed for all the evaluated phenolic compounds, except ferulic acid, sinapic acid and quercetin among the processing steps (p<0.05). Since (-)-epigallocatechin gallate, ferulic acid, sinapic acid and quercetin were not detected in the samples collected at some processing stages, the corresponding statistical analysis was not performed.

The average contents of the (+)-catechin, (-)-epicatechin, quercetin, gallic, caffeic and p-coumaric acids were 2.1 - 21.3 mg/L, 0.4 - 4.0 mg/L, 0.1 mg/L, 36.6 - 70.6 mg/L, 6.6 - 19.8 mg/L and 1.8 - 2.1 mg/L.

![Figure 3. HPLC-DAD chromatogram of the phenolic compounds of the sour grape concentrate.](image-url)


*Cromatograma HPLC-DAD dos compostos fenólicos no concentrado de sumo de uvas não amadurecidas.*

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in the ‘verjuice’ samples and from 2.20 to 500.52 mg/L, 0.6 to 10.84 mg/L, 0.04 to 4.27 mg/L, 1.8 to 16.96 mg/L, 1.43 to 41.2 mg/L and 0.07 to 11.23 mg/L in the grape juice samples according to literature (Nikdardjam, 2008; Natividade et al., 2013; Lima et al., 2014; Toaldo et al., 2015; Yamamoto et al., 2015; Margraf et al., 2016; Padilha et al., 2017; Cosme et al., 2018). Moreover, it is reported that (-)-epigallocatechin gallate, (-)-epigallocatechin, ferulic acid, sinapic acid and vanillic acid are from 0.60 to 6.20 mg/L, 0.9 to 368.2 mg/L, 0.15 to 5.20 mg/L, 0.4 to 2.8 mg/L and 20.00 to 444.92 mg/L in the grape juices which produced in Brazil (Natividade et al., 2013; Lima et al., 2014; Toaldo et al., 2015).

The contents detected for gallic, caffeic and p-coumaric acids in the current work are lower than those found by Nikdardjam (2008), but (+)-catechin and (-)-epicatechin values are in accordance with the previous findings. The results for (+)-catechin, (-)-epicatechin, gallic, caffeic and p-coumaric acids are in accordance with the results of previous studies (Natividade et al., 2013; Lima et al., 2014; Toaldo et al., 2015; Yamamoto et al., 2015; Margraf et al., 2016; Padilha et al., 2017; Cosme et al., 2018).

(-)-Epigallocatechin gallate is an important substance for human health. In a study, Legeay et al. (2015) investigated the beneficial properties of (-)-epigallocatechin gallate to prevent metabolic syndrome. Additionally, it has reported that (-)-epigallocatechin gallate has higher antioxidant capacity than other catechins (Rice-Evans, 1999; Legeay et al., 2015). Natividade et al. (2013) stated that (-)-epigallocatechin gallate average content in Vitis Labrusca (‘Isabel Precoce’), hybrid (BRS Cora and BRS Violeta) and Vitis vinifera (‘Tempranillo’, ‘Syrah’, ‘Alicante Bouschet’ and ‘Moscato Cannelli’) grape juices are 0.63-6.20 mg/L, 1.77 mg/L and 0.60-2.39 mg/L, respectively. In the current study, changes found for (-)-epigallocatechin gallate during processing stages were from 0.76 to 1.95 mg/100 g and the contents are in accordance with the mentioned literature (Natividade et al., 2013). On the other hand, this compound was not detected at [C], [D] and [E] processing stages.

**REFERENCES**


Hayoğlu I., Kola O., Kaya C., Özer S., Türkoğlu H., 2009. Chemical and sensory properties of verjuice, a traditional Turkish beverage.


