

# The Effects of Sugar Addition and Degree of Roast on the Bioactive Compounds and Antioxidant Activity of Turkish-Style Coffee Brews

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## ABSTRACT

**Introduction:** Coffee is one of the most widely consumed beverages by the people all around the world. This study was aimed to evaluate the effect of sugar addition, double effects of sugar addition and roasting degree on the polyphenol content, and antioxidant activity of Turkish-style coffee brews. **Materials and Methods:** The levels of three phenolic compounds (chlorogenic acid, caffeic acid, sinapic acid) and caffeine by reversed-phase high performance liquid chromatography (HPLC) method, as well as chromaticity parameters, absorbance value at 420 nm, total phenolic and flavonoid content, antioxidant activities with two methods (DPPH and FRAP methods) were determined in different degree of roast Turkish-style coffee brews with sugar and without sugar. **Results:** It was found that antioxidant activity of Turkish-style coffee brews was increased while the phenolic content was decreased due to the degree of roasting process. Also, the addition of sugar into Turkish-style coffee brews at cooking stage caused decrease both in antioxidant activity and polyphenol content. **Conclusion:** The study indicated that light roasted coffee brews showed greater polyphenol content and medium roasted coffee brews had greater antioxidant capacity compared to other samples. Consumption of coffee brews without sugar provided the higher health benefits.

**Key words:** Antioxidant, Coffee, Degree of roast, Sugar addition, Phenolic compounds.

## INTRODUCTION

Epidemiological studies have shown that a regular coffee consumption is directly related to the prevention of many chronic and degenerative diseases such as cardiovascular disorders,<sup>1</sup> neurodegenerative disorders,<sup>2,3</sup> the obesity and diabetes,<sup>4,5,6,7</sup> and even some types of cancers.<sup>8,9,10,11</sup> It has been reported that these health benefits are not based on only single compound. They are due to the combined effects of coffee bioactive components, including phenolic compounds.<sup>12,13</sup> The major class of polyphenols exist in coffee are hydroxycinnamic acids,<sup>14-20</sup> mostly in esterified form with organic acids, sugars, and lipids.<sup>19,20</sup> The major representative of hydroxycinnamic acids is also caffeic acid occurs in foods mainly as chlorogenic acid (5-caffeoylquinic acid, an ester of caffeic acid with quinic acid). In human diet, coffee

is known as a major source of chlorogenic acid.<sup>14-20</sup> Chlorogenic acid and derivatives have a number of beneficial health properties because of their antioxidant activity.<sup>21,22,23</sup> However, one of the other biologically active compounds in coffee is caffeine. Caffeine, which has a bitter taste, is white crystalline xanthine alkaloid. It has several effects on body metabolism such as stimulating the central nervous system and increasing blood pressure in the short term.<sup>24,25</sup>

In the world, the most important agricultural harvest is coffee, which is second only after petroleum in terms of global trade activity and value.<sup>26,27</sup> According to the International Coffee Organization, approximately 157 million bags of coffee are produced worldwide in 2015 and 2016

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years.<sup>28</sup> The genus *Coffea* (Rubiaceae family) is possessed of over sixty different species and three of them; *Coffea arabica* L. (arabicas), *Coffea robusta* L. (robustas), and *Coffea liberica* L. have economical value.<sup>29,30</sup> Especially *Coffea arabica* L. and *Coffea robusta* L. are produced commercially.<sup>27,31</sup> Arabica variety is thought to be of superior quality due to the milder and more flavorful taste formed during roasting.<sup>27,32</sup> The instant coffee industry use the Robusta coffee for the purpose of producing soluble coffee extracts.<sup>27,33</sup> Turkish-style coffee brew is a sort of boiled coffee obtained by using the Turkish coffee preparation technique and *Coffea Arabica* L.<sup>34</sup> The beans for *Coffea Arabica* L. are first roasted. The term of “roasted” covers light, medium, and dark degrees of roast, depending on its color. Specific changes occur in the chemical composition of roasted coffee. Depending on the degree of roast, the external color of the beans are changed (varying from light to dark brown), flavor is developed, the amount of dry matter loss is came about, some bioactive components is broken down and others are formed.<sup>35,36</sup> Medium roasted coffee is generally used for preparation of Turkish-style coffee brew because it is appealing to the taste buds of Turkish people. After roasting, beans are grounded or pounded to obtain the finest possible powder, and the powder has finer particles than used coffee in other preparation methods. The coffee bean powder obtained can be named as “Turkish coffee”. Turkish coffee is mixed with cold water and boiled in a special type of narrow topped small pot called a “cezve” with sugar or not sugar. It is served hot in a cup.<sup>34,37</sup> Turkish-style coffee brew is a very famous drink and consumed widely by enjoying with the same preparation technique in the Balkan and Middle East countries.<sup>38</sup>

In the literature, several studies were found about the antioxidant activity and/or polyphenolic content of coffees roasted at different temperatures and times.<sup>30,39,41-45</sup> Only one of them examined the polyphenols and differences in antioxidant content Turkish-style coffee brews.<sup>30</sup> Yet, there is no study, which determines the effect of addition of sugar into the coffee or Turkish-style coffee brew on the polyphenolic content and antioxidant activity at the cooking processes. Also, no literature found clarifying the double effects of addition of sugar in the Turkish-style coffee brew and different degree of roast of Turkish coffee on the polyphenolic content and antioxidant activity of coffee, either. This study will be the first of its kind for these reasons.

Besides Turkish-style coffee brews being a quite popular drink, there are also many positive effects on human health. The degree of roast or formulation change can

modify the phenolic profile of Turkish-style coffee brew, and so, physiological effects it exhibits on human may change as well. The aim of this study is to investigate the influence of degree of roast and adding sugar on phenolic and antioxidant properties of Turkish-style coffee brew (TCB).

## MATERIALS AND METHODS

### MATERIALS

#### Samples

Four different Turkish coffee samples (green (crude), light roasted, medium roasted and dark roasted coffee) produced from the same coffee beans were purchased from local coffee shop (İlyas Gönen Coffee (Izmir, Turkey)). All of the analyses were made from green and roasted Arabica coffee beans that were roasted to three roasting degrees ( $L^*$ -values 37.28, 28.50, 15.92) at 200°C using a table-top roaster and ground very finely (<300 µm) with a Turkish coffee grinder. The moisture contents of analyzed samples were 7.99 % (green roasted Turkish coffee), 4.99 % (light roasted Turkish coffee), 2.52 % (medium roasted Turkish coffee) and 2.36 % (dark roasted Turkish coffee).

#### Brewing

All of the coffee brews were prepared with a Turkish coffee machine (Arçelik, Istanbul, Turkey), because cooking are performed with a machine at equal temperature and time. According to the standardization of brewing procedure, ground coffee (5 g) and ultra-pure distilled water (65 mL) were heated in a Turkish coffee machine. The sugar (8 g) was added into some samples at cooking stage. Then they were cooled to 25°C and finally filtered through the Whatman no. 3 filter paper (Figure 1). The green, light roasted, medium roasted,

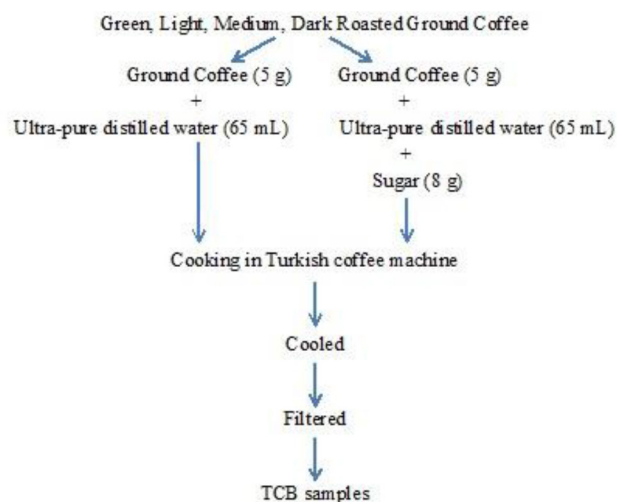


Figure 1: The brewing procedure of TCBs.

dark roasted TCBs with no sugar were named as TCB1, TCB2, TCB3, TCB4 and ones with sugar were also named as TCB5, TCB6, TCB7, TCB8, respectively.

## METHODS

### Color Measurement in TCBs

Color analysis was carried out utilizing the Hunter colorimeter (CFLX 45-2 Model Colorimeter, Hunter Lab, Reston, VA) with direct reading of the  $L^*$  (0 darkness, 100 brightness),  $a^*$  (+ redness, - greenness) and  $b^*$  (+ yellowness, - blueness) CIELAB scale parameters and calibrated using a standard black reflector plate. The  $L^*$ ,  $a^*$  and  $b^*$  values were converted to different functions of color as the total color difference ( $\Delta E^*$ ), chroma ( $C^*$ ) and hue angle ( $h^\circ$ ) values by using following the equations:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (1)$$

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2)$$

$$h^\circ = \tan^{-1} \left( \frac{b^*}{a^*} \right) \quad (3)$$

$$\Delta L^* = L_0^* - L^* \quad (4)$$

$$\Delta a^* = a_0^* - a^*$$

$$\Delta b^* = b_0^* - b^*$$

where  $L_0^*$ ,  $a_0^*$ ,  $b_0^*$  were initial values of TCB1 or TCB5;  $L^*$ ,  $a^*$ ,  $b^*$  were the values of light/medium/dark roasted coffee brews with or without sugar.

### Browning Intensity in TCBs (Abs 420 nm)

100  $\mu$ l of coffee brews were diluted up to 2 mL with ultra-pure distilled water, and then, browning intensity was determined by measuring the absorbance of samples at 420 nm in a 1.5 mL capacity cuvette (1 cm length) with a spectrophotometer (Optizen Pop, Mecasys Co., Ltd., Korea).<sup>42</sup>

### Determination of Total Phenolic Content in TCBs

The total phenolic content of coffee brews was quantified according to the method described by Singleton and Rossi (1965),<sup>46</sup> and Li *et al.* (2006)<sup>47</sup> with some modifications. 0.5 mL of the diluted coffee brew was mixed with 2.5 mL of 0.2 N Folin-Ciocalteu phenol reagent and 2 mL of 7.5% sodium carbonate. The mixture was left to stand at dark and room temperature for 30 min before measuring the absorbance of the solution at 760 nm,

using a spectrophotometer. The results were expressed as mg of gallic acid equivalents (GAE) per 100 mL for coffee brews ( $y = 0.0084x + 0.0049$ ,  $R^2 = 0.9988$ ).

### Determination of Total Flavonoid Content in TCBs

The total flavonoid content of coffee brews was determined using the method of Heimler *et al.* (2005).<sup>48</sup> 250  $\mu$ L of coffee brew and 1.25 mL of distilled water were mixed with 75  $\mu$ L of 5%  $\text{NaNO}_2$  solution. The mixture was stood for 6 min at dark and room temperature. Then, 150  $\mu$ L of 10%  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  was added. After it was incubated for 5 min at dark and room temperature, 0.5 ml of 0.1 M NaOH solution and 275  $\mu$ L of distilled water were added into the mixture. Thus, the volume of mixture is reached to 2.5 mL. The absorbance was measured at 510 nm using a spectrophotometer, immediately. Total flavonoid content was expressed as mg of (+)-catechin ((+)-CE) equivalents per 100 mL for coffee brews ( $y = 0.0036x - 0.0069$ ,  $R^2 = 0.9963$ ).

### Determination of Antioxidant Activity by the DPPH Radical Scavenging Method in TCBs

Detecting of the scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical in coffee brews was performed as following the methods of Chu *et al.* (2000)<sup>49</sup> and Cheung *et al.* (2003)<sup>50</sup> with some modifications. 0.5 ml of 0.1 mM DPPH radical prepared in methanol was mixed with 1 ml of coffee brews by using vortex. When the control was also prepared, coffee brew was replaced by methanol. After mixing, the absorbance (Abs) was measured immediately at 520 nm with a spectrophotometer. The scavenging activity (%) of DPPH radicals for each coffee brews was calculated using the following formula:

$$\text{The scavenging activity \%} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \quad (5)$$

### Determination of Antioxidant Activity by the FRAP Radical Scavenging Method in TCBs

The ferric reducing antioxidant power (FRAP) assay was applied to estimate the antioxidant capacity of coffee brews according to the methods of Guo *et al.* (2003)<sup>51</sup> and Xu *et al.* (2004)<sup>52</sup> with some modifications. The solution of the FRAP reagent was prepared via addition, at 1:1:10 (v/v/v), of 10 mmol/L 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) solution in 40 mmol/L HCL, 20 mmol/L  $\text{FeCl}_3$  solution and 0.3 mol/L pH 3.6 acetate buffers. FRAP solution was warmed to 37°C and then 40  $\mu$ L of the diluted coffee brew was mixed to 1.8 mL of FRAP solution and 200 of  $\mu$ L distilled water. The mixture was incubated at 37°C for 30 min. After

the absorbance was determined at 593 nm using a spectrophotometer, the calculated values were expressed as mg of reduced iron equivalents per 100 mL, based on a  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  calibration curve ( $y = 0.2448x + 0.034$ ,  $R^2 = 0.9884$ )

## The Determination of Phenolic Compounds and Caffeine in TCBs by HPLC

### HPLC Analysis

The protocol of analysis was based on the conditions used by Belguidoum *et al.* (2014).<sup>53</sup> In this context, an Agilent 1200 LC system (Agilent, Santa Clara, CA) with a diode array detector (DAD), a gradient elution pump and vacuum degasser, an auto sampler system, and a thermostated column compartment were used. ACE 5 C<sub>18</sub> 5 $\mu\text{m}$ , 100 Å, 250 x 4.6 mm column (Advanced Chromatography Technologies Ltd, UK) was utilized for separation. 1% phosphoric acid in water (solvent A) and 1% phosphoric acid in acetonitrile (solvent B) were applied as a binary gradient elution. The gradient program began at 30% B, then increased until 50% B in the course of 8 min and held for 2 min. Re-equilibration time was 10 min at 30% B. A constant flow rate of 1 mL/min was used with an injection volume of 20  $\mu\text{L}$ . The caffeine and cinnamic acid were monitored at 280 nm, while caffeic acid, ferulic acid, sinapic acid, and chlorogenic acid were monitored at 324 nm. The DAD was adjusted to collect spectral data over the range 190-400 nm. In coffee brews chlorogenic acid, caffeic acid, sinapic acid, and caffeine were identified qualitatively by comparing retention times, spectra of standards and using standard addition technique, and quantitatively by applying the external standards method ( $y = 1.7106x - 52.6952$ ,  $R^2 = 0.9982$  for chlorogenic acid,  $y = 1.8721x + 85.6517$ ,  $R^2 = 0.9964$  for caffeic acid,  $y = 2.4469x - 145.4581$ ,  $R^2 = 0.9970$  for sinapic acid and  $y = 1.4979x - 27.9205$ ,  $R^2 = 0.9999$  for caffeine).

### Analytical Method Validation

The limit of detection (LOD) and limit of quantification (LOQ) were determined considering as 3:1 and 10:1 peak to noise ratio, respectively.<sup>54</sup> The LOD ranged

from 0.01 to 0.02 mg/L, when the LOQ ranged from 0.03 to 0.05 mg/L (Table 1). The recovery capacities of the method were evaluated with adding the original concentrations defined in samples of standard phenolic compound and caffeine in coffee brews with sugar or without sugar. The values of recovery were greater than 99% for all compounds, except sinapic acid (Table 1).

Additionally, five different standard concentrations were used for forming calibration curves and range of their concentrations was between 3.13 to 62.50 mg/L, approximately. Correlation coefficients in calibration curve were greater than 0.99 for all standards of phenolic compound and caffeine.

### Statistical Analysis

All experiments were carried out triplicate during this study and results were expressed as means  $\pm$  standard deviations. Statistical analyses were realized with the SPSS v.20.0 software package. One-way analysis of variance (ANOVA) was performed to determine the significant difference in all analysis between the green, light, medium, and dark roasted Turkish-style coffee brews. Duncan's multiple range tests were used to determine the significant difference of the data sets of the green and different roasting degree Turkish-style coffee brews. Pearson correlation test was utilized for assessing correlations among variables. Paired sample t-test was also carried out for evaluating the significant difference between Turkish-style coffee brews with/without sugar in all analysis. Level of significance was accepted as 95% in all statistical analysis.

## RESULTS AND DISCUSSION

### Influence of Degree of Roast and Sugar Addition on Color and Browning Intensity of TCBs.

In heat-treated foods, like coffee, one of the most visual changes is brown color development during the process.<sup>42</sup> In this study, the color of different TCBs was determined by means of the CIELab parameters ( $L^*$ ,  $a^*$ ,  $b^*$  and also  $\Delta E^*$ ,  $C^*$  and  $h^\circ$  as chromaticity parameters) and the Absorbance at 420nm (Table 2).

**Table 1: Performance characteristics of high pressure liquid chromatographic method.**

Compounds	Retention time (RT)	LOD	LOQ	Regression equation for calibration curve	R <sup>2</sup>	Recovery for TCB3	Recovery for TCB7
		[mg/L]	[mg/L]			[%]	[%]
Chlorogenic acid	2.949	0.01	0.04	$y = 1.71058036x - 52.695182$	0.9982	99.32	99.01
Caffeine	3.478	0.02	0.05	$y = 1.49787555x - 27.920499$	0.9999	101.46	100.68
Caffeic acid	3.815	0.01	0.03	$y = 1.87307244x + 85.65169$	0.9964	98.06	99.27
Sinapic acid	5.097	0.01	0.04	$y = 2.44687187x - 145.45809$	0.9970	97.10	97.39

**Table 2: Browning intensity values and chromaticity parameters of TCB samples.<sup>a</sup>**

Samples	Browning intensity (Absorbance at 420 nm)	Chromaticity parameters					
		L*	a*	b*	C*	h°	ΔE*
TCB1	0.036±0.0001g	0.96±0.03e	-0.40±0.08g	0.70±0.07e	0.81±0.09e	60.19±2.89a	-
TCB2	0.391±0.0005d	11.26±0.11a	12.17±0.16a	16.61±0.39a	20.59±0.24a	53.77±0.96b	22.75±0.25a
TCB3	0.437±0.0005b	1.93±0.10c	3.36±0.24d	2.84±0.20c	4.40±0.26c	40.25±2.18d	4.44±0.26c
TCB4	0.481±0.0012a	1.31±0.10d	1.62±0.12e	1.79±0.15d	2.42±0.17d	47.85±2.18c	2.33±0.16d
TCB5	0.043±0.0005f	1.05±0.05e	-0.48±0.06g	0.08±0.01f	0.47±0.06e	9.93±1.80e	-
TCB6	0.345±0.0004e	8.79±0.58b	11.78±0.06b	13.28±0.89b	17.75±0.68b	48.38±1.79c	19.61±0.84b
TCB7	0.414±0.0001c	1.86±0.06c	3.58±0.13c	2.74±0.10c	4.50±0.16c	37.42±0.47d	4.91±0.16c
TCB8	0.484±0.0003a	1.35±0.10d	1.33±0.18f	1.90±0.18d	2.33±0.14d	55.06±5.29b	2.59±0.15d

<sup>a</sup> Values are given as "mean ± standard deviation". Different matching letters in a column express significant differences according to Duncan test ( $p < 0.05$ ).

Differences in roasted degree of TCBs had significant effects ( $p < 0.05$ ) on all of chromaticity parameters. Green Turkish-style coffee brews (TCB1 and TCB5) had the lowest L\*, a\*, C\* values and the lowest b\* and h° values belonged to TCB5 sample statistically ( $p < 0.05$ ). While all chromaticity parameters of TCB2 were found to be significantly higher than other samples, h° value was the highest in TCB1 and difference was found statistically important ( $p < 0.05$ ). With increasing the roasting degree, a color was obtained darker, paler with yellow and red shades in TCBs. Similar changes in color and chromaticity parameters with roasting were also observed by Sacchetti *et al.* (2009)<sup>40</sup> and Ludwig *et al.* (2013).<sup>55</sup> The highest total color difference of TCBs was found in TCB2; the lowest amount was in TCB4 and TCB8 ( $p < 0.05$ ) in accordance with the results of Liang *et al.* (2016)<sup>56</sup> and statistically important difference was determined as well. No statistically significant difference was observed between the chromaticity parameters except the h° value as a result of sugar addition to TCBs ( $p < 0.05$ ).

The 420 nm wavelength has been used to assess the color intensity of browning reaction mixtures affecting the palatability of heat processed foods and beverage.<sup>57,58</sup> The impact of sugar addition and the degree of roast in TCBs significantly affected absorbance at 420 nm ( $p < 0.05$ ). When TCBs were evaluated in terms of roasting degree, the lowest absorbance at 420 nm was found in TCB1 and also the samples of TCB4, TCB8 exhibited the highest absorbance at 420 nm statistically ( $p < 0.05$ ). Generally, these results are in agreement with the reports of Ludwig *et al.* (2013)<sup>55</sup> and Liang *et al.* (2016).<sup>56</sup>

The most well-known Maillard reaction products are melanoids generated during the roasting process.<sup>56,59</sup> They are one of the compounds responsible for the

brown color of coffee. The sugar (retro) aldolization/dehydration and carbohydrate condensation products are also known as many other brown-colored products in roasted coffee.<sup>42,60,61</sup> These compounds are the cause of increasing in brown color as the roasting degree increases. For this reason, the absorbance value at 420 nm of TCBs increased, as the roasting degree of coffee increased. Also, it is found that addition of sugar to TCBs formed a statistically significant difference in the absorbance at 420 nm ( $p < 0.05$ ). When sugar and Turkish-style coffee brew is mixed and stirred, firstly the concentrated liquid is obtained. Then, the mixture becomes lighter with increasing temperature and ongoing stirring. The small bubbles are formed. So, the volume is increased. The same color is diffused across to a larger area. Therefore, the color got diluted and its intensity started to disappear. It is concluded that the color of TCBs with sugar is lighter than the ones without sugar. According to a study of Al-Wahab (2009),<sup>62</sup> the color difference between coffee brews with sugar and no sugar might be due to the sticky effect of sugar on the staining of coffee.

### Total Phenolic Content and Total Flavonoid Content of TCBs

The methods used for the determination of the polyphenol content are based on a redox reaction. The Folin-Ciocalteu method and the aluminum chloride colorimetric method can be considered as general methods of total polyphenol and flavonoid content determination, respectively.<sup>63,64</sup> The total polyphenol content and flavonoid content of TCBs are tabulated in Table 3. While the lowest polyphenol and flavonoid content found in the sample of TCB8 ( $209.49 \pm 3.04$  mg GAE per 100 mL TCB and  $15.50 \pm 1.11$  mg GAE per 100 mL TCB) relative to other coffee samples that had

different roasting degrees, TCB2 had statistically the highest amount of polyphenols and flavonoids content ( $337.23 \pm 3.63$  mg GAE per 100 mL TCB and  $164.67 \pm 2.50$  mg GAE per 100 mL TCB) among the roasted TCBs ( $p < 0.05$ ). According to the statistical analysis of variance, the differences found among polyphenol and flavonoid content in the samples were statistically significant ( $p < 0.05$ ). The Maillard reaction products formed during roasting process, reacted to Folin-Ciocalteu reagent and contributed to the increase in total phenolic compounds measured by Folin-Ciocalteu assay.<sup>40-67</sup> At the beginning of the thermal process, a smaller loss of polyphenols and more Maillard reaction products formation occurs for light roasted TCBs compared to green TCBs (Table 3). For this reason, total polyphenol content measured in samples exhibited a slight increase with the roasting process, and then, it was determined that the phenolic and flavonoid content with roasting process showed an inverse proportional change. The reason for the lower levels of polyphenols compared to Maillard product formation in roasted coffee can be polymerization, autoxidation or degradation of phenolic compounds during roasting.<sup>65,68</sup> In this study, the change of total phenolic and flavonoid determined in TCBs with different roasting degrees correlate with the results of Cämmerer and Kroh (2006);<sup>65</sup> Górnas *et al.* (2016);<sup>45</sup> Ludwig *et al.* (2013)<sup>55</sup> and Sacchetti *et al.* (2009).<sup>40</sup>

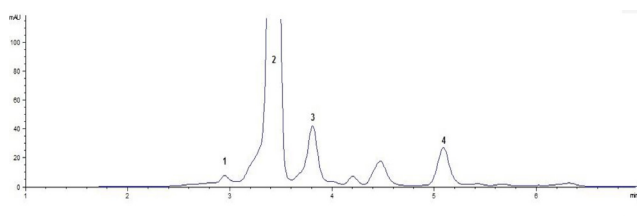
In comparison to TCBs prepared only with water and the addition of sugar, sugar significantly decreased total phenolic and flavonoid content of TCBs ( $p < 0.05$ ). This result is similar with the results obtained by Sharma *et al.* (2008)<sup>69</sup> whom reported that the total polyphenol content and antioxidant activity of tea samples are found to be higher in black tea sample compared to tea with milk, tea with sugar, and tea with sugar and milk samples. Similarly, Lončarić *et al.* (2016)<sup>70</sup> reported that a freeze-dried sour cherry puree with addition of sucrose had lower content of total phenolic, flavonoid and anthocyanin compared to control and other samples with addition of maltose and trehalose. Actually, results could be explicated by the importance of water content, its interactions between compounds in food matrix, and behavior of disaccharides in water. Water can be treated as the reactant rather than as the solvent at lower water activities (0.05-0.43), and this situation results in the increase of degradation rate of the polyphenols. Higher water activities are inducing conditions for diffusion-limited reactions. Therefore, combined effect of enzymatic activity, oxidation, and molecular mobility, which are the results of more available water are resulting degradation of sensitive compounds.<sup>70,71</sup> Finally, poly-

phenol degradation occurred due to the water content of TCBs.

### Antioxidant Activity by the DPPH and FRAP Radical Scavenging Method of TCBs.

The reliable, quick and simple methods are needed for properly estimating antioxidant capacity of TCB. Therefore, two most commonly used methods were used to investigate antioxidant activity of samples. Free radical scavenging potential of TCBs with different roasting degrees were evaluated using the methods of DPPH and FRAP are presented in Table 3. Findings pointed out that there is significant difference in the antioxidant activity at different roasting degrees ( $p < 0.05$ ). In the DPPH assay, TCB3 sample exhibited the highest antioxidant activity ( $65.59 \pm 0.41$  %), while the lowest antioxidant activity was determined in TCB8 ( $37.41 \pm 0.52$  %). In the FRAP assay, the lowest value ( $247.47 \pm 0.93$  mg FeSO<sub>4</sub> per 100 mL TCB) was noted for TCB5 and the highest value ( $451.78 \pm 0.31$  mg FeSO<sub>4</sub> per 100 mL TCB) was also observed for TCB7. In this study, it was determined that the radical scavenging activity of all TCBs, except dark roasted coffee brews, increased with the roasting process. Because the compounds occur the result of Maillard reaction exhibited antioxidant properties. These diminished the loss of the natural antioxidants and increasing the whole antioxidant properties of coffee brews<sup>72</sup>. Also, the isomerization of chlorogenic acid occurred and was partially converted to lactones.<sup>45,73,74</sup> Chlorogenic acid lactones are compounds that had antioxidant activity. Dark roasted coffee brews (TCB4 and TCB8 samples) did not exhibit the highest antioxidant activities compared to other samples due to the fact that the highest concentration of the Maillard products had in medium roasted samples.<sup>75</sup> Results obtained in this study agreed with those previously reported by several researchers.<sup>40,41,44,45,72,75</sup>

When the effect of adding sugar into TCBs on antioxidant activity determining with DPPH method was investigated, a significant difference between TCBs with sugar and with no sugar was obtained ( $p < 0.05$ ). The radical scavenging activities of TCBs with sugar were found to be lesser compared to TCBs without sugar. Due to the water content of TCBs, polyphenols of coffee were degraded.<sup>70,71</sup> Because of this reason, the measured antioxidant activity was also decreased. In contrast to the DPPH method, there was not a significant effect of adding sugar into the TCBs on antioxidant activity determining with the FRAP method ( $p > 0.05$ ). Although the mechanism that relates to antioxidant activity is an electron transfer in both methods (DPPH and FRAP methods), FRAP cannot detect compounds



**Figure 2: HPLC chromatogram of TCB2 sample. Peaks: 1, chlorogenic acid; 2, caffeine; 3, caffeic acid; 4, sinapic acid.**

that act by radical quenching (H transfer), particularly thiols and proteins.<sup>76,77</sup> These situations caused difference between results obtained by DPPH and FRAP methods. It is known that FRAP values had a poor relationship to other antioxidant activity methods.<sup>76</sup>

### The Phenolic Compounds and Caffeine Determining by HPLC in TCBs

According to the results of HPLC analysis, three phenolic compounds and caffeine was quantified (Figure 2). Sinapic acid displayed the highest amount in overall coffee brews, followed by caffeic acid and chlorogenic acid, generally. All of the phenolic compounds and caffeine presented different amounts in TCBs with different roasting degrees, depending on the initial concentration of them in green coffee and roasting process conditions. They were degraded partly or completely during the roasting process.

The data obtained from HPLC analysis were presented in Table 3. Significant differences were detected between individual polyphenol and caffeine concentrations of TCBs had different degree of roast ( $p < 0.05$ ). The amount of whole phenolic compounds and caffeine determined in Turkish-style coffee brews, except chlorogenic acid, decreased with roasting process and increased with roasting degree, even caffeic acid degraded completely in dark roasted coffee. Only, the amount of chlorogenic acid and caffeine increased slightly at the beginning of the roasting process and then it decreased with the increasing roasting degree. During intense roasting, phenolic acids content increased as a result of the thermal instability of them and finally degraded. For example, chlorogenic acid partly converts to their lactones with dehydration of chlorogenic acid and the formation of intramolecular bonds.<sup>45,73,74</sup> Similar observations to this study in related with phenolic compounds change with roasting degree of coffee were reported by others as well.<sup>41,45,63,78,79</sup> Also, the roasting process reduced caffeine content of coffee.<sup>41,79</sup> This observation about caffeine change in TCBs are in agreement with previous findings, which

confirmed the roasting results with the change of caffeine amount by several reports.<sup>41,63,79</sup>

According to the results of Table 3, the sugar addition during the cooking process of the Turkish-style coffee brews was caused the decrease of the main phenolic compounds. A statistically significant decrease was found in the phenolic compounds, except sinapic acid, detected in the TCBs with sugar addition ( $p < 0.05$ ). The amount of sinapic acid did not exhibit statistically significant change between TCBs with sugar or without sugar ( $p < 0.05$ ). The reasons of these differences seem to be embedded in the degradation of chlorogenic acid, caffeic acid and caffeine depending on water content of TCBs on the sugar addition, whereas sinapic acid is stable against this type of degradation.

### Correlations Related with Results Obtained from Analyses

According to Pearson correlation test, statistically significant correlations were found between  $b^*$ ,  $\Delta E^*$  values of TCBs and  $h^{\circ}$  value of TCBs ( $p < 0.05$ ). While statistically significant correlations were observed between caffeine content of TCBs and total flavonoid content of TCBs, statistically significant negative correlations were obtained between browning intensity of TCBs and total flavonoid content of TCBs ( $p < 0.05$ ). Also, statistically significant correlations were determined between  $h^{\circ}$  value, caffeine content of TCBs with antiradical activity by using DPPH method of TCBs ( $p < 0.05$ ). Statistically significant negative correlations were obtained between  $h^{\circ}$  value,  $\Delta E^*$  value, and caffeine content of TCBs with antiradical activity by using FRAP method of TCBs ( $p < 0.05$ ). Additionally, statistically significant correlations were found between antiradical activity by using DPPH method of TCBs and chlorogenic acid content of TCBs ( $p < 0.05$ ). Statistically significant correlations were determined between  $h^{\circ}$  value and caffeine content of TCBs with caffeic acid content of TCBs ( $p < 0.05$ ). The negative correlations of between browning intensity of TCBs and sinapic acid content of TCBs were obtained as statistically significant ( $p < 0.05$ ). While all of the obtained statistically significant correlations has been between 0.30 and 0.61, statistically significant negative correlations has been between -0.54 and -0.72 ( $p < 0.05$ ). Whole correlations seemed as poor ( $p < 0.05$ ). No statistically significant correlations were obtained among other analyses results of all coffee samples ( $p < 0.05$ ). These correlations which confirm the conclusion obtained from the experimental data that the change of polyphenols, antioxidant activity, and color characteristics, are related with changing

Table 3: Bioactive compound contents and antioxidant activities of TCB samples<sup>a</sup>

Samples	Total phenolic content [mg GAE/100 mL]	Total flavonoid content [mg (+)-CE/100 mL]	DPPH scavenging capacity [%]	FRAP scavenging capacity [mg FeSO <sub>4</sub> /100 mL]	Phenolic compound determined by HPLC			
					Chlorogenic acid [mg/100 mL]	Caffeine [mg/100 mL]	Caffeic acid [mg/100 mL]	Sinapic acid [mg/100 mL]
TCB1	291,10±2.86c	154,94±1.11b	59,47±0.21c	334,72±1.24f	4,84±0.05c	117,33±0.14e	14,77±0.05a	26,21±0.05a
TCB2	337,23±3.63a	164,67±2.50a	64,13±0.21b	344,65±1.24e	8,26±0.02a	142,81±0.09a	13,73±0.02b	14,60±0.02c
TCB3	317,88±1.19b	123,28±2.22d	65,59±0.41a	413,89±1.55c	4,65±0.03d	133,95±0.05b	5,48±0.01e	9,13±0.00e
TCB4	220,74±3.93f	36,33±3.61e	49,93±0.21g	337,51±1.55f	3,76±0.01g	107,16±0.02f	ND g	6,44±0.02g
TCB5	259,43±3.57e	135,36±2.36c	51,42±0.10f	247,47±0.93g	4,01±0.00f	97,34±0.16h	8,90±0.06c	15,22±0.05b
TCB6	310,74±3.45b	141,89±1.39c	56,15±0.31e	403,34±0.93d	6,90±0.05b	122,96±0.19c	8,01±0.00d	13,19±0.00d
TCB7	276,45±3.10d	116,06±4.44d	57,69±0.41d	451,78±0.31a	4,38±0.00e	122,05±0.22d	3,59±0.02f	8,95±0.00f
TCB8	209,49±3.04g	15,50±1.11f	37,41±0.52h	439,04±0.62b	3,39±0.00h	98,52±0.10g	ND g	6,18±0.01h

<sup>a</sup> ND = not detected. Values are given as "mean ± standard deviation". Different matching letters in a column express significant differences according to Duncan test ( $p < 0.05$ ).

roasting degree of coffee and they are similar with some results found in the literature.<sup>45</sup>

According to correlation matrix based on the relations among the analyses results of TCBs with sugar and with no sugar; high correlations (0.90–0.99) observed between the samples with sugar and without sugar, from the data obtained via the analysis of total polyphenol content, total flavonoid content, antiradical activity (by using DPPH method), the phenolic compounds and caffeine (determined by HPLC), b\* and C\* values, and browning intensity ( $p < 0.05$ ). While the lowest correlation is observed between the antiradical activities by using FRAP method of TCBs with sugar and with no sugar, correlations of L\* values, a\* values and  $\Delta E^*$  values are 0.77, 0.78, 0.89, respectively ( $p < 0.05$ ). Negative and poor correlation (-0.50) is observed when h\* value of TCB with sugar compared to h\* value of one with no sugar ( $p < 0.05$ ).

All of the observation indicates that the change in phenolic content, antioxidant activity and color properties of TCBs is more dependent on the roasting process than the addition of sugar.

This paper is about the determination of sugar addition effect on the polyphenol content and antioxidant activity of TCBs. Also, it meant to display double effects of roasting degree and sugar addition. There is also no study about the effect of sugar addition on coffee brew's phenolic compounds and antioxidant activities. Only, two studies have found on sugar addition. One of them is about sugar adding at roasting process<sup>42</sup> and the other is about sugar addition in tea brew.<sup>69</sup>

## CONCLUSION

Drinking coffee is an important indoor/outdoor social activity for many people. Turkish-style coffee brews which is traditionally prepared from *Coffea arabica* L. beans, also quite famous and consumed widely in the Balkan and Middle East countries. In this study, effects of sugar addition on the polyphenol content and antioxidant activity of TCBs were investigated in different degrees of roast TCBs for the first time. In the literature several studies have found related with the determination of polyphenol and antioxidant content of Turkish-style coffee brew. Both the phenolic content of TCBs and the effects of roasting degree and sugar addition on the phenolic compounds and antioxidant activity changes were determined in this study. Also, chromaticity parameters and browning intensities of TCBs were evaluated with changing roasting degree and sugar addition. Actually, TCBs are rich polyphenolic extracts which their antioxidant and phenolic compounds were



extracted with hot water. Because they are made by boiling in water and their coffee grounds (telve) are not consumed. For this reason, they had been analyzed without applying to an extra extraction method. Generally, TCBs from different roasting degrees exhibited notable phenolic content and antioxidant potential, which were influenced by the roasting degree and the addition of sugar into it. The phenolic compounds, which present in Turkish-style coffee brew, are partly destroyed by the roasting process and other antioxidant compounds, like melanoidins, can be formed. So, antioxidant activity of TCBs can increase and phenolic content of TCBs can decrease due to the degree of roasting process. If the degree of roast increases continuously, destruction of the phenolic compounds cannot be possible to compensate the formation of other compounds after a certain stage. According to result of analysis, light roasted TCBs show greater polyphenol content while medium roasted TCBs show greater antioxidant capacity compared to other samples. Phenolic compounds are essential dietary components for human health, and the obtained data in this study show that TCBs have phenolic compounds with potential antioxidant activity. In addition, TCBs should be consumed without sugar to benefit from their health-promoting activities in maximum rate.

## CONFLCIT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

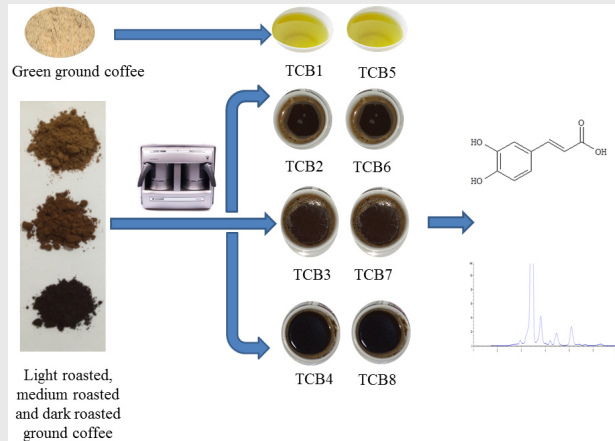
LC: liquid chromatography.

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## PICTORIAL ABSTRACT



## SUMMARY

- Coffee is one of the most widely consumed beverages by the people all around the world and has many bioactive compounds such as polyphenols.
- This is the first study which determines the effect of addition of sugar into the coffee brew on the polyphenolic content and antioxidant activity at the cooking process. Also, no literature found clarifying the double effects of both sugar addition into the coffee brew and different roasting degree of *Coffea Arabica* L. on the polyphenolic content and antioxidant activity of coffee, either.
- Therefore, samples of coffee brews with sugar and with no sugar, which prepared by Turkish-style have investigated in terms of their polyphenol contents, antioxidant activities, browning intensities and color parameters.

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