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Bioactive diterpenes (cafestol and kahweol) in Turkish coffees: Impact of roasting

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Abstract

While the cholesterol-raising effect of coffee has been ascribed to the presence of diterpenes, they have also been shown to present favourable health effects. Boiled-type coffees show slightly higher levels of diterpenes than those made with other brewing methods. However, there is considerable controversy regarding the effect of roasting on the contents of the diterpenes cafestol and kahweol. Therefore, the aim of the present work was to measure the contents of these diterpenes in Turkish coffees, and to determine how they are influenced by roasting. The samples used were 16 roasted and ready-ground Turkish coffees sold in supermarkets in the Turkish Republic of Northern Cyprus. The cafestol and kahweol contents of the coffee samples were analysed using liquid-liquid extraction followed by HPLC-DAD. The lipid contents of commercially roasted and ground Turkish coffee samples varied in the range of 14.32 ± 0.09 to 15.60 ± 0.09 g/100 g. The lipid contents of brewed Turkish coffee samples varied from 318 ± 2.00 to 571 ± 4.30 mg/100 mL. When compared within each commercial brand, dark roasted ground Turkish coffee samples had higher lipid contents. The average diterpene content in one cup of Turkish coffee sample was between 2.69 ± 0.28 and 13.58 ± 0.88 mg. The ranges of cafestol and kahweol contents in a cup were 1.4 ± 0.21 - 6.9 ± 0.65 mg and 1.28 ± 0.07 - 6.68 ± 0.28 mg, respectively. Within products of the same brand, the highest amount of oil was observed in dark roasted Turkish coffee beverages, and no significant differences were found in total diterpene, cafestol, and kahweol contents in coffee beverages among the different roasting levels. It is recommended that future studies perform more detailed investigations of the effect of roasting on the diterpene contents in Turkish coffees, and the impact of preparation parameters, as well as the presence of diterpene-derived compounds.

Introduction

After water, coffee is the second most consumed beverage in the world (Butt and Sultan, 2011). Coffee naturally contains a variety of compounds (Godos *et al.,* 2014). The two most important coffee species are *Coffea arabica* and *C. robusta*, and the green beans of these species contain 15 and 10% fat, respectively (Speer and Kölling-Speer, 2006). The lipid content of coffee is considered an important source of bioactive diterpene compounds, major among which are cafestol and kahweol (Moeenfard *et al.,* 2015). These compounds from the kauran family may be found in free or esterified forms that are relatively specific to coffee only (De Roos *et al.,* 1998).

Diterpenes comprise up to about 10 - 15% of the lipidic fraction of roasted coffee beans (Urgert *et al.,* 1995; Nystad *et al.,* 2010). Different preparation and brewing methods affect the concentration of diterpene compounds in the final coffee brew (Urgert *et al.,* 1995; Karabudak *et al.,* 2015; Moeenfard *et al.,* 2015). Brewing or boiling releases oil droplets that contain diterpenes from ground coffee, but they are largely removed by filtration (Abalı *et al.,* 2009; Moeenfard *et al.,* 2016). The highest levels of cafestol and kahweol have been found in unfiltered Scandinavian, Turkish, and French press (cafetiere) coffee, whereas percolated and instant coffee contain negligible amounts (Ludwig *et al.,* 2014).

Besides the preparation method, the absolute amounts of diterpenes in brewed coffee may vary according to different factors such as the coffee species, cultivar, harvest year, roasting degree, grinding (particle) size, amount of ground coffee, percolation time, water quantity and quality (pressure, temperature), coffee-to-water ratio, and extraction device (Kurzrock and Speer, 2001; Urgert

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and Katan, 1997; Moeenfard *et al.,* 2015). Boiled Turkish/Greek coffees show slightly higher levels of cafestol and kahweol than field samples. This may be due to a higher amount of finely ground Arabica coffee beans that is traditionally present in Turkish-style coffee as compared to boiled coffee and other brews (Urgert *et al.,* 1995; Boekschoten *et al.,* 2006; Sridevi *et al.,* 2011).

Recent studies have shown that consumption of Turkish coffee may increase serum cholesterol levels depending on the diterpene content (Ranheim and Halvorsen, 2005; Nystad *et al.,* 2010; Dias *et al.,* 2014; Godos *et al.,* 2014). Moreover, based on previous studies related to the roasting level of coffee beans, higher roasting temperatures and prolonged roasting can significantly affect the diterpene contents of brews (Campanha *et al.,* 2010; Rebello and van Dam, 2013). However, there is considerable controversy regarding the effect of roasting on cafestol and kahweol contents. Some researchers observed stable cafestol and kahweol contents (Urgert *et al.,* 1995; Rebello and van Dam, 2013; Toci *et al.,* 2013; Moeenfard *et al.,* 2020), whereas others reported an increase (Kitzberger *et al.,* 2013) or a decrease (Campanha *et al.,* 2010; Sridevi *et al.,* 2011) during roasting.

Therefore, the aim of the present work was to measure the contents of the diterpenes cafestol and kahweol in Turkish coffees sold at the retailer level in North Cyprus. Furthermore, the influence of roasting on the content of diterpenes in the same Turkish coffee brands at different roasting levels (intensity) was also examined. While the cholesterol-raising effect of coffee has been ascribed to the presence of diterpenes, they have also been shown to present favourable health effects such as antioxidant, hepatoprotective, anticarcinogenic, anti-inflammatory, and anti-angiogenic functions (Ludwig *et al.,* 2014; Moeenfard *et al.,* 2015).

Due to the high biological potential of coffee diterpenes, analysing the diterpene contents in a wide range of coffee samples is important. The results could be used to evaluate the daily exposure to diterpenes from coffee, and to estimate the beneficial amount for dietary and pharmacological applications (Moeenfard and Alves, 2020; Makino *et al.,* 2021). Furthermore, diterpene contents of coffee have received attention in recent years due to their relationship with cup quality (Novaes *et al.,* 2015; Barbosa *et al.,* 2019), as well as their application in coffee authentication (Moeenfard and Alves, 2020), which has been demonstrated in the characterisation of *Coffea* species (Zanin *et al.,* 2020). Thus, new supporting data related to the diterpene content, and their distribution in roasted Turkish coffee samples will help in better understanding coffee as a functional beverage.

Materials and methods

Lipid and diterpene analyses of Turkish coffees

Between January and February 2018, a total of 16 roasted and ready-ground Turkish coffees were obtained. These coffees comprised five local brands with three different roasts (light, medium, and dark), and an imported dark roast from Turkey. All of them were sold in supermarkets in the Turkish Republic of Northern Cyprus (TRNC). Three different samples were collected for each of the coffees sold, in 100 to 150-g aluminium outran or 3-layer foil quadro packs, and kept closed until further analyses.

The total lipid content of Turkish coffee powders was quantified. In addition, the total amounts of lipid and diterpene (cafestol and kahweol) were also quantified. The influence of roasting on the contents of diterpenes for the same Turkish coffee brands were determined at different roasting levels (light, medium, and dark), as well as for the general declared roasting intensity groups.

Chemicals and reagents

Cafestol and kahweol standards (98% purity) were separately purchased from ChromaDex (Irvine, CA, USA) and LTK Laboratories (MN, USA), respectively. Diethyl ether (99% purity) and HPLC-grade acetonitrile (95%) containing *n*-hexane and methyl alcohol were purchased from VWR (BDH Prolabo, Belgium). The other chemicals used were 85% purity potassium hydroxide (Merck, Germany) and sodium chloride (Panreac Quimica, Spain). HPLC analysis was done using filtered distilled water that had been vacuum purified using 0.45-µm filter membranes.

Turkish coffee preparation

Coffee samples were prepared in amounts of 55 ± 3.97 mL using 4.45 ± 0.51 g of coffee grinds. The coffee was brewed by boiling it twice (80 - 90°C) following the Turkish coffee preparation standards (Özgür, 2012). The pH value was fixed at 5.62 for all analyses. For each parameter, three coffee beverages were prepared, transferred to polyethylene tubes, and kept at -22°C. For the diterpene contents, all samples were subjected to extraction for four times. Their fat contents were determined as a result of double extraction.

Lipid content in Turkish coffee brews

The total lipid contents of the brewed coffees were determined in accordance with Parenti *et al.* (2014) with minor modifications. Briefly, 5 mL of brewed coffees were extracted with 5 mL of *n*-hexane for four times by stirring for 3 min over a vortex, and then centrifuged at 4,000 rpm for 15 min. The organic phase was decanted and re-centrifuged to eliminate the entire aqueous phase. The solvent was evaporated first in a water bath (at 80°C), and then in an oven at 103 - 105°C. The total lipid content was quantified by weighing the dried extract, and the result was determined in mg/mL.

Total lipid content in roasted and ground Turkish coffee powders

The coffee fat was extracted using a Soxhlet extractor according to Araújo and Sandi (2007). For this purpose, 20 g of ground coffee (particle size ≤ 500 µm) was extracted using *n*-hexane (250 mL) for 16 h. Next, the solvent was removed by a rotary evaporator (Buchi, R-210, Switzerland) at 30°C, followed by residual solvent evaporation in an oven (at 103 - 105°C). The weight of the coffee fat was calculated in g/100 g ground coffee on a dry basis.

Diterpene extraction from Turkish coffee brews

The diterpene contents in the brewed coffee were measured using a previously optimised and validated method defined by Moeenfard *et al.* (2015). Within a very short period, 2.5 mL of the brewed coffee was heated to 60°C with 2.5 mL of distilled water, and directly saponified with 3.0 g of potassium hydroxide in a water bath at 80°C for 60 min. The solution was then subjected to two consecutive extractions using diethyl ether, and cleaned using 5.0 mL of 2 M NaCl solution. The clean organic phase was dried under nitrogen stream, and stored at -22°C until HPLC-DAD analysis. The data are reported in mg/cup.

High-performance liquid chromatograph-diode array detector (HPLC-DAD) analysis

HPLC analyses were performed in a Merck Hitachi Elite LaChrom instrument (Tokyo, Japan), which was equipped with a quad pump (L-2130), an L-2200 automatic sampler, and an L-2455 UV/vis spectrophotometry diode array detector. Separation was performed by employing an end-capped Purospher STAR Lichro CART RP 18, 250 × 4-mm, 5- µm column, which was attached to a protective column (4 × 4 mm, 5 µm) of the same type. The detection wavelengths were 225 nm for cafestol, and 290 nm for kahweol.

The software EZChrom Elite v. 3.1.6 was used to acquire and analyse the data. Before the chromatographic analysis, the volume of the dried extracts of brewed coffees was adjusted to 2.5 mL with acetonitrile. They were filtered with 0.45-µm polytetrafluoroethylene (PTFE) membranes (VWR, USA), and then, 20 µL of redissolved samples were injected into the HPLC system. The mobile phase was acetonitrile/water (55/45 v/v) with an isocratic flow rate of 0.8 mL/min. The target compounds were identified by comparing spectra and retention times of the reference standard solutions. The quantitative analysis was performed using corresponding peak areas in graphs of concentrations by using external standard calibration curves.

Method validation

Following injections of nine standards at 2 - 200 mL/L, calibration curves of the cafestol and kahweol were plotted. The validation parameters were determined based on standard coffee brews including the precision (in variation coefficient expressed as CV%), accuracy (expressed as recovery percentage), and detection and quantification limits. The method and instrument precisions were determined using the variation coefficient of the analyses repeated under intra- and inter-day conditions, respectively. Intra-day precision (repeatability) was evaluated by using six identical analyses carried out in the same day. In inter-day variance (reproducibility) studies, one extract of the same sample was analysed thrice over three consecutive days. The accuracy of the method was evaluated by spiking cafestol and kahweol standards (twice, 25 and 50% of the initial diterpene concentration) to the matrix (Turkish coffee brew) before the extraction process. The recovery percentage (%) was determined as the mean ratio between the realised and expected diterpene concentrations of the spiked samples. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on signal to noise ratios (S/N) of 3 and 10, respectively. The intra (intra-day) CV% and inter CV% (inter-day) were determined to be 1.56.

Statistical analysis

Differences between the different roasting types of the same brands of coffees were evaluated by one-way ANOVA at three replications with a level of significance of 95%. A *post-hoc* Tukey's test was performed to evaluate the significance of differences between roasting types. Data were expressed as mean ± standard deviation.

Kruskal-Wallis test was used to evaluate the differences among the groups of declared roasting levels. Differences were considered significant at $p \leq 0.05$. All statistical analyses were performed using IBM SPSS v. 24.0 software.

Results and discussion

Lipid contents in Turkish coffee powders

The mean lipid content of all commercially roasted and ground coffee samples varied in the range of 14.32 ± 0.09 to 15.60 ± 0.09 g/100 g (dry weight) (Table 1). The mean lipid contents of light, medium, and dark roasted coffee samples were 14.84 ± 0.34, 14.88 ± 0.38, and 15.22 ± 0.31 g/100 g, respectively (Table 2). Similar to the present study, the coffee oil content of a roasted Arabica coffee powder was reported as 15.1 ± 0.1 g/100 g following a liquid-liquid extraction procedure with HPLC-DAD analysis (Moeenfard *et al.,* 2015). Another study reported that the oil fraction of the roasted coffee samples can vary from 11 to 21 g/100 g (Toci *et al.,* 2013).

In the present work, when the coffee

Table 1. Oil contents of commercially available roasted and ground Turkish coffees.

Commercial Turkish coffee	Roasting type	Dry weight (%)*	Moisture (%)*	Oil content (g/100 g)*
Brand-1	Light	96.7 ± 0.61	2.43 ± 0.019	15.08 ± 0.09
	Medium	95.9 ± 0.78	2.39 ± 0.02	15.23 ± 0.03
	Dark	94.78 ± 0.55^{c}	2.36 ± 0.05	15.6 ± 0.09^{bc}
		p = 0.031	p = 0.103	< 0.001
Brand-2	Light	93.56 ± 0.61	2.51 ± 0.03	14.95 ± 0.07
	Medium	95.51 ± 0.67^{a}	2.48 ± 0.02	15.09 ± 0.09
	Dark	96.66 ± 0.76^{bc}	2.41 ± 0.015^{bc}	15.41 ± 0.07^{bc}
		< 0.001	p = 0.004	< 0.001
Brand-3	Light	97.41 ± 0.32	2.38 ± 0.02	15.22 ± 0.05
	Medium	97.8 ± 0.45	2.33 ± 0.025	15.28 ± 0.03
	Dark	98.3 ± 0.52	2.29 ± 0.016^{c}	15.43 ± 0.010^{bc}
		p = 0.118	p = 0.005	< 0.001
Brand-4	Light	94.38 ± 0.81	2.61 ± 0.023	14.32 ± 0.09
	Medium	95.23 ± 0.61	2.54 ± 0.04	14.39 ± 0.05
	Dark	95.91 ± 0.27	2.52 ± 0.02^{c}	14.87 ± 0.06^{bc}
		p = 0.057	p = 0.021	< 0.001
Brand-5	Light	94.73 ± 0.34	2.49 ± 0.021	14.81 ± 0.023
	Medium	95.32 ± 0.45	2.45 ± 0.019	14.79 ± 0.052
	Dark	95.39 ± 0.42	2.41 ± 0.02^{c}	14.98 ± 0.065^{bc}
		p = 0.172	p = 0.008	p = 0.006
Brand-6	Dark	97.65 ± 0.27	2.39 ± 0.014	15.24 ± 0.03

*mean (x) ± standard deviation (SD) of triplicate (n = 3). p = One-way ANOVA with *post-hoc* Tukey's test. ^{a}significant difference ($p < 0.05$) between light and medium roasted Turkish coffees of the same brand. ^{b}significant difference ($p < 0.05$) between medium and dark roasted Turkish coffees of the same brand. ^{c}significant difference ($p < 0.05$) between light and dark roasted Turkish coffees of the same brand.

Table 2. Lipid and diterpene contents of Turkish coffees subject to the declared degree of roasting.

Degree of roasting (*n* = 16)	Light (*n* = 5)	Medium (*n* = 5)	Dark (*n* = 6)	*p*-value
Dry weight (%)	95.32 ± 1.66	95.94 ± 1.07	96.40 ± 1.37	0.42
Moisture (%)	2.48 ± 0.09	2.44 ± 0.08	2.40 ± 0.08	0.31
Lipid content (g/100 g)	14.84 ± 0.34	14.88 ± 0.38	15.22 ± 0.31	0.19
Lipid (mg/mL)	3.67 ± 0.52	3.69 ± 0.50	4.28 ± 0.93	0.33
Cafestol (mg/55 mL)	2.74 ± 2.21	2.82 ± 2.18	3.63 ± 2.55	0.31
Kahweol (mg/55 mL)	2.39 ± 1.96	2.51 ± 1.92	3.31 ± 2.43	0.28

p-values are based on Kruskal-Wallis test.

grounds were grouped based on the declared roasting levels, no significant difference was observed in the lipid contents (Table 2). On the other hand, when the coffee powders were compared based on the roasting type of each commercial brand, the lipid content (g/100 g) of dark roasted coffees in every brand was found to be significantly higher (Table 1). Dias *et al.* (2014) mentioned that an increase in the roasting intensity causes a relative increase in lipid concentration in both species of coffee. Oosterveld *et al.* (2003) also explained this behaviour as resulting from higher amounts of heat-resistant lipids present in coffee as compared to the amounts of carbohydrates and proteins.

Moreover, it has also been stated that there is an increase in lipid concentrations as a result of pyrolysis and Maillard reactions that increase the solubility of polysaccharides, and decrease sugar and proteins simultaneously. This is the result of cell wall rupture following exposure to high temperature (Redgwell *et al.,* 2002). The increase in fat content starts after four minutes of roasting, and such an increase is especially valid for Arabica varieties/species, which has been shown to be sensitive to heat.

The reason for the different fat content increase between the varieties/species was ascribed to the fact that different coffee varieties contain different quantities of carbohydrates, proteins, fats, and moisture (Dias *et al.,* 2014). Additionally, it was stated that the moisture content of coffee may vary between 5.8 and 2.3 g/100 g for Arabica varieties/species, and the moisture content decreases with the roasting process. Both in this study and other studies (Perrone *et al.,* 2012; Ranić *et al.,* 2015), the moisture content of Turkish coffees decreased with roasting, and was found to be in a similar range (Table 1).

Lipid contents in Turkish coffee brews

Based on earlier reports, the lipid content of coffee brews may vary depending on the brewing and preparation methods (Urgert *et al.,* 1995; Karabudak et al., 2015; Moeenfard *et al.,* 2016). Coffee brews that were filtered through filter paper contained less than 7 mg of lipids, whereas coffees that were brewed without filtering but through boiling or espresso processes may contain 60 - 160 mg/150 mL (0.4 - 1.07 mg/mL) of oil per cup (Speer and Kölling-Speer, 2006). In the case of filtering through filter paper, the oil primarily remains on the spent coffee grounds, and only 0.4% passes through the coffee brew of the total lipids recovered (Farah, 2012). Therefore, it was emphasised that in boiled coffee brews, the lipid concentrations are considerably higher (by 40 - 125 times) as compared to paper-filtered coffee (Ratnayake *et al.,* 1993).

The absolute amount of lipid content of a 100-mL coffee brew was observed to vary from 180 to 400 mg. Variation in the grinding, roasting, blending, and brewing methods could represent confounding variables (Farah, 2018). Ranić *et al.* (2015) found that Turkish coffee brews made from strong and weak infusion levels yielded lipid amounts of 153 and 87.8 mg/100 mL, respectively.

Generally, from one brand to another, the samples in the present work yielded lipid amounts that varied from 318 ± 2.00 to 571 ± 4.30 mg/100 mL (3.18 ± 0.02 - 5.71 ± 0.043 mg/mL) (Table 3). Moreover, when compared within each commercial brand, the dark roasted coffee brews showed higher content (Table 3). In another study, it was stated that the boiling time has a slight effect on increasing the lipid concentration of a coffee brew, and the lipid saturation reaches 1 g/L in the early stages of boiling. It was also mentioned that using high pressure (8 - 12 bar) and steam in coffee preparations produces more lipids from coffee grounds (Ratnayake *et al.,* 1993).

Table 3. Lipid and diterpene contents of commercially available roasted and ground Turkish coffees.

Commercial Turkish coffee	Roasting type	Total lipid (mg/mL)[#]	Cafestol (mg/cup*)[#]	Kahweol (mg/cup*)[#]	Total diterpene (mg/cup*)[#]
Brand-1	Light	3.25 ± 0.06	1.79 ± 0.09	1.67 ± 0.07	3.46 ± 0.07
	Medium	3.28 ± 0.04	1.82 ± 0.10	1.77 ± 0.03	3.59 ± 0.13
	Dark	3.32 ± 0.02	2.02 ± 0.10[bc]	1.87 ± 0.21	3.89 ± 0.31
		0.218	0.049	0.246	0.129
Brand-2	Light	3.67 ± 0.02	1.98 ± 0.21	1.78 ± 0.01	3.76 ± 0.22
	Medium	3.62 ± 0.04	1.94 ± 0.34	1.84 ± 0.19	3.78 ± 0.53
	Dark	3.92 ± 0.03[bc]	2.25 ± 0.31	1.93 ± 0.054	4.18 ± 0.40
		< 0.001	0.428	0.337	0.395
Brand-3	Light	4.49 ± 0.06	6.68 ± 0.29	5.87 ± 0.21	12.55 ± 0.50
	Medium	4.51 ± 0.03	6.71 ± 0.31	5.93 ± 0.09	12.64 ± 0.40
	Dark	5.13 ± 0.06[bc]	6.83 ± 0.27	6.17 ± 0.12	13.00 ± 0.39
		< 0.001	0.806	0.107	0.452
Brand-4	Light	3.78 ± 0.06	1.79 ± 0.07	1.32 ± 0.11	3.11 ± 0.18
	Medium	3.74 ± 0.02	1.98 ± 0.10	1.46 ± 0.17	3.44 ± 0.27
	Dark	3.98 ± 0.04[bc]	1.90 ± 0.18	1.59 ± 0.03	3.49 ± 0.21
		0.001	0.224	0.274	0.155
Brand-5	Light	3.18 ± 0.02	1.40 ± 0.21	1.29 ± 0.07	2.69 ± 0.28
	Medium	3.32 ± 0.07[a]	1.60 ± 0.06	1.54 ± 0.23	3.14 ± 0.29
	Dark	3.61 ± 0.05[bc]	1.70 ± 0.08	1.59 ± 0.04	3.29 ± 0.12
		< 0.001	0.864	0.081	0.053
Brand-6	Dark	5.71 ± 0.04	6.90 ± 0.65	6.68 ± 0.23	13.58 ± 0.88

[#]mean (x) ± standard deviation (SD) of triplicate ($n = 3$). *Coffee cup size: 55 mL. p = One-way ANOVA with *post-hoc* Tukey's test. [a]significant difference ($p < 0.05$) between light and medium roasted Turkish coffees of the same brand. [b]significant difference ($p < 0.05$) between medium and dark roasted Turkish coffees of the same brand. [c]significant difference ($p < 0.05$) between light and dark roasted Turkish coffees of the same brand.

Diterpene contents in Turkish coffee brews

The preparation method is defined as one of the most important factors in determining the diterpene content of a coffee brew (Sridevi *et al.,* 2011; Farah, 2012). Boiled coffee had the highest diterpene ester content (4.8 - 18 mg/150 mL), while filtered and instant brews (0.1 - 0.4 mg/150 mL) had the lowest (Ratnayake *et al.,* 1993). Higher concentrations of cafestol and kahweol in coffee brews prepared by the Turkish method are supported by many studies(Urgert *et al.,* 1995; Gross *et al.,* 1997; Sridevi *et al.,* 2011). The higher content is due to the fine solid particles present in Turkish-style coffee as compared to boiled coffee and other brews (Urgert *et al.,*1995; Gross *et al.,* 1997; Sridevi *et al.,* 2011).

Moreover, in the case of filtered coffee beverages, the biggest portion (87.45%) of coffee kahweol remains in the coffee grind residue since very little fat is extracted by hot water. Furthermore, 12.41% of the remainder is retained by the paper

filter, and 0.15% passes to the coffee beverage. Similarly, the greater part of the coffee cafestol is retained by the spent coffee due to the low extraction of the lipid fraction by the hot water (Rendón *et al.,* 2017).

Urgert *et al.* (1995) highlighted that Turkish/Greek coffee may contain 1 to 10 mg of cafestol per cup, which can correspond to 2 - 20 mg/cup of the total amount of diterpenes (cafestol and kahweol). In addition, Gross *et al.* (1997) found that the average total diterpene amount in a cup of Turkish coffee was 10.7 mg. In the present work, we determined that the average diterpene content in a cup of Turkish coffee to range between 2.69 ± 0.28 and 13.58 ± 0.88 mg (Table 3). Also, the ranges of cafestol and kahweol contents of the coffees in one cup were 1.4 ± 0.21 - 6.9 ± 0.65 and 1.28 ± 0.07 - 6.68 ± 0.28 mg, respectively (Table 3).

The concentrations of total diterpenes in the coffee brews assessed in the present work agree with many other studies (Urgert *et al.,* 1995; Gross *et al.,* 1997; Ranheim and Halvorsen, 2005; Boekschoten *et al.,* 2006; Sridevi *et al.,* 2011; Moeenfard *et al.,* 2015). However, it is not entirely correct to fully compare the diterpene contents obtained in the present work with those in the literature since many studies used different amounts of coffee powder or water to prepare coffee, ground coffee particle size, colour of the roasting, volume of the brewed coffee, or preparation time. This means that the amount of diterpene in each coffee brew can be different.

The amounts of diterpenes obtained from the coffees analysed in the present work varied among brands. Although the main reason for the wide data range is not fully explained, it is believed that the main cause might be such factors as Turkish coffees having different degrees of grinding and different blends of coffee bean varieties, thereby influencing the results. Robusta coffee beans have lower quantities of cafestol, and no kahweol in comparison with Arabica, and coffees with coarser particle size do not help in the extraction of diterpenes, which support this hypothesis. Steam pressure, the duration of contact with the ground coffee, and the efficiency of the coffee machines also influence the amounts of diterpene quantities (Ratnayake *et al.,* 1993; Sridevi *et al.,* 2011). For future studies, it is recommended that the amounts of lipids and diterpenes be analysed in Turkish coffees prepared with coffee machines. It should be taken into consideration that in the present work, the pH levels of the coffee brews prepared from roasted coffee beans were in the range of 5.5 - 5.65, which were consistent with other studies (Gross *et al.,* 1997; Moeenfard *et al.,* 2015).

Effect of roasting on diterpene contents of Turkish coffees

It has been stated that the diterpene extraction yield of roasted and ground coffee is dependent not only on the coffee brewing method, but also on the roasting duration. Furthermore, light roasted Turkish coffee brews yielded higher cafestol, while dark roasted brews had lower extraction yields (Zhang *et al.,* 2012). Sridevi *et al.* (2011) emphasised that as the roasting temperature increases, there are significant reductions in both cafestol and kahweol concentrations. Furthermore, it was observed that higher roasting temperatures and longer roasting times had a more significant influence on diterpene profiles in Arabica coffee beans.

Rendón *et al.* (2017) reported that the greatest cafestol content was found in light roasted coffee. Furthermore, with particle size of the coffee powder below 500 μm (more solid content), the finely ground coffee particles increased the contact surface with hot water, thus leading to an increase in diterpene amounts. Moeenfard *et al.* (2015) determined that the diterpene content of espresso coffees is influenced by various brewing parameters such as water quantity, amount of ground coffee, grinding size, percolation time, water temperature, and pressure. Furthermore, the diterpene contents significantly increased by changing the particle size and the water quantity. In addition, it was found that in comparison with all parameters, coffees that are heated up to 70°C have lower diterpene content (1.2 mg/40 mL), due to lower diterpene extraction efficiency (1.4%)

Some researchers have expressed that the amounts of kahweol and cafestol are retained during roasting (Urgert *et al.,* 1995; Campanha *et al.,* 2010). On the other hand, others have stated that these diterpenes dehydrate during roasting, thus producing dehydrocafestol and dehydrokahweol (derivatives) or isokahweol and dehydroisokahweol (functional isomers) (Dias *et al.,* 2014; Moeenfard and Alves, 2020). Dias *et al.* (2014) reported that the level of diterpenes in both species of coffee is influenced by the intensity of roasting. The degradation of diterpenes (general losses from 60 to 75% on a lipid basis) and the development of dehydrokahweol and dehydrocafestol occurred simultaneously after the eighth minute of commercial roasting at 230°C. However, it was also emphasised that the amounts of cafestol and kahweol (mg/100 g of coffee) remained during roasting, and were related to the increase in lipid concentration (Dias *et al.,* 2014; Moeenfard *et al.,* 2020).

A similar trend was also noticed in the

present work, where within the products of the same brand, the highest amount of oil was observed in dark roasted coffees. Furthermore, no significant difference ($p \geq 0.05$) was found in total diterpene, cafestol, and kahweol amounts in coffee beverages among the roasting levels (Table 3). Future studies should perform more detailed investigation of the effect of roasting on the diterpene amounts of Turkish coffees, as well as the presence of diterpene-derived compounds.

Conclusion

Since the coffee species can change the lipid and diterpene content of coffee brews, it is suggested that future studies analyse the presence of 16-*O*-methylcafestol (16-OMC) to determine whether or not robusta coffee beans are used, where in particular, Turkish coffee should be prepared from Arabica beans only. In order to accurately interpret the effect of roasting on the amounts of lipid and diterpenes of coffee, it is suggested that future studies fix the grinding size and the type of Turkish coffee beans used. The particle size of the ground coffee has an influence on the quantity of the biologically active compounds present in the final brews. Thus, to determine the quality of the ground coffee, it might be important to add the ground particle size to the Turkish Food Codex (TGK) standards. It is noteworthy that there should also be investigations of other newly reported diterpenes such as arabiol, caffeadiol, dehydrocafestol, and dehydrokahweol, which are assumed to be present in great concentrations in coffees. Consequently, it should be taken into account that the diterpene content can change by varying the preparation parameters such as the use of sugar, fresh coffee, or different devices. Therefore, it is recommended that the effect of these parameters be explored in future studies.

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