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Bioactive compounds, antioxidant activity, physical and sensory characteristics of Mırra coffee

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Abstract

Mırra is a coffee beverage widely consumed in Syria and Turkey, and often produced from roasted and ground coffee beans. Recently, it is prepared from classical instant coffee. In this study, some physicochemical, bioactive and sensory properties of Mırra samples were determined. The average viscosity, °Brix, HMF, total phenolic content, DPPH and ABTS values were 1.36 cP, 3.70, 71.60 mg/L, 3431.55 mg GA eq./L, 6.24 mmol Trol. eq./mL and 35.23 mmol Trol. eq./mL for Mırra samples made by traditional process, and 4.85 cP, 16.36, 303.3 mg/L, 11276.47 mg GA eq./L, 23.89 mmol Trol. eq./mL and 89.70 mmol Trol. eq./mL for Mırra samples made with classic instant coffee, respectively. All Mırra samples also contained high levels of caffeine (1416.93 - 4347.46 mg/L). Chlorogenic acid, 4-*O*-caffeoylquinic acid and *trans*-5-*O*-caffeoylquinic acid were identified in Mırra samples. Total chlorogenic acid contents of Mırra samples were ranged from 1097.85 to 5283.21 mg/L. In all sensory parameters, Mırras with °Brix value over 5.75 had high scores. Results show that Mırra has high antioxidant activity. However, Mırra consumption may have negative health effects for risk groups due to the high caffeine content.

Keywords: antioxidant activity; caffeine; coffee; HMF; Mırra; chlorogenic acids.

Practical Application: The usage of instant coffee for preparation of Mırra leads to formation of higher HMF and caffeine which may cause some negative health effects in risk groups.

1 Introduction

Coffee is the most popular beverage worldwide (Borrelli et al., 2002). The modality of consumption and preparation of coffee beverages is strictly associated with social habits and country cultures. Therefore, there are a lot of types of coffee beverages such as espresso, cappuccino, Turkish coffee, Ireland coffee, etc. (Desem, 2000). Differences in coffee bean varieties, post-harvest processing conditions (drying, storage, grinding and roasting) and extraction procedures result in a great chemical diversity of the coffee beverages (Hečimović et al., 2011). Recent scientific studies declare considerable evidence of positive health effects of moderate coffee consumption that is 3-4 cups of coffee per day for adults. Moderate coffee consumption decreases the risk of several degenerative (Type II diabetes, Alzheimer's and Parkinson's) and liver diseases (cirrhosis and hepatocellular carcinoma) because of its strong antioxidant activity (Higdon & Frei, 2006). Phenolics, Maillard reaction products and caffeine are the main contributor to the high antioxidant activity of coffee (Capek et al., 2014). Average total phenolic contents (TPC) of caffeinated and decaffeinated espresso coffees were found as 410 and 324 mg chlorogenic acid eq./cup (30 mL), respectively (Alves et al., 2010). Chlorogenic acid (CGA) and its derivatives are the most abounded phenolic compounds in coffee that is a major source of CGA in the human diet. The total CGA content in caffeinated and decaffeinated coffee samples ranged from 5.26 mg/g to 17.1 mg/g and 2.10 mg/g to 16.1 mg/g, respectively (Fujioka & Shibamoto, 2008).

Brown pigments (Melanoidins) and 5-Hydroxymethylfurfural (HMF) develop during thermal treatments in foods and beverages via the Maillard reactions (Bradbury, 2001). Especially, brown pigments are important contributors to the antioxidant activity of coffee (Somporn et al., 2011). Fujioka & Shibamoto (2008) noted that the brown pigments (A_{420}) in caffeinated and decaffeinated coffee beverages ranged from 0.273 to 0.458 and from 0.283 to 0.482, respectively. HMF contents in the brewed coffees (30 mL) from Caffea Arabica with different roasting degrees varied from 0.84 to 2.60 mg (Alves et al., 2010).

Coffee is a major source of caffeine in the human diet. Potential health risks of coffee are often associated with its caffeine content. There is some evidence that coffee consumption increases the risk of cardiovascular disease, diuresis and anxiety due to its caffeine content (Zulak et al., 2006). However, the high caffeine content in coffee resulted in greater antioxidant activity (Vignoli et al., 2011). Fujioka & Shibamoto (2008) noted that the caffeine content in caffeinated and decaffeinated coffee beverages ranged from 10.9 to 16.5 mg/g and 0.34 to 0.47 mg/g, respectively. Crozier et al. (2012) reported that the caffeine contents of single servings (23-70 mL cup size) of twenty different commercial espresso samples ranged from 51 to 322 mg.

Mırra coffee is widely consumed in Syria and the southeastern Anatolian region of Turkey and spread the Europe by immigration. It is prepared with similar production steps to Turkish coffee

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from the bean of *Coffea arabica*. However, coffee bean for Mırra is more roasted, and less ground than Turkish coffee. Mırra is also prepared more concentrate than Turkish coffee and has a strong bitter taste. Recently, Mırra was started to produce by using classical instant coffee as an alternative to the traditional preparation methods. The use of classical instant coffee quickly spreads due to the ease of use and energy, time and cost saving characteristics in the last two decades. In this study, some physicochemical, bioactive and sensory characteristics of the Mırra samples prepared by traditional methods and with classical instant coffee were investigated.

2 Materials and methods

2.1 Materials

In this study, eleven different Mırra samples prepared by traditional methods (TM) and seven different Mırra samples prepared with classical instant coffee (CM) were purchased from coffee shops in Mardin and Şanlıurfa cities (southeastern Anatolia, Turkey). As control groups, Mırra samples were prepared with a traditional method in the laboratory (LM). Roasted and ground coffee for the preparation of LM sample was purchased from a local market (Artukbey Industry and Trade Co., Turkey) in Mardin city. Turkish coffee samples (TC) were also prepared in the laboratory for comparing with Mırra samples. To make Turkish coffee, roasted and ground coffee was obtained from Kuru Kahveci Mehmet Efendi Trade Co., (İstanbul, Turkey).

2.2 Preparing of Mırra in laboratory

Water (350 mL) was added on roasted and ground coffee (100 g) into a copper cup. The cup content was boiled on weak charcoal fire for 30 min and then cooled and filtered with a cheesecloth. Five gram coffee was added again on the filtrate. The content was boiled until the final volume was reached to approximately 100 mL. The content was cooled to room temperature and then filtered with a cheesecloth. Prepared Mırra beverage was kept in an amber glass container at -26 °C until analysis.

2.3 Preparing of Turkish coffee in laboratory

Turkish coffee (TC) was prepared from 10 g coffee and 120 mL water using a Turkish coffee maker (K3190 Telve, Arçelik, Turkey).

2.4 Colour measurement

Hunter color values were determined with Konica Minolta Chromameter (CR-400, Osaka, Japan). Color parameters were expressed as L (100 whiteness/0 darkness), a (+redness/−greenness), and b (+blueness/−yellowness) in Mırra samples.

2.5 °Brix

°Brix of the Mırra samples was measured by Abbe refractometer after cleared with Carrez reagents (Association of Official Analytical Chemists, 2003).

2.6 Viscosity

Viscosity was determined according to the method reported by Rizk (2016) with some modifications. Measurements were made with a Brookfield RV (Model DV-III Ultra) viscometer. The SC4-21 spindle was used in conjunction with low volume sample adapters. The viscosity values of Mırra samples were measured 20 times at 10 second intervals at 100 rpm spin rate at 20 °C. The average of the obtained data was given as viscosity value.

2.7 Brown pigment content

The brown pigment content was measured with a UV-Vis spectrophotometer (Agilent 8453, Santa Clara, CA) at 420 nm in 50 fold diluted samples (Turkmen et al., 2006).

2.8 HMF

The method developed by Alves et al. (2010) for HMF analysis was used with minor modification. Mırra samples clarified with Carrez solutions were filtered through a 0.45 µm PVDF syringe-driven filter, and injected into a HPLC system (Shimadzu, Kyoto, Japan). It consisted of LC-20 AD-VP gradient pump, SPD-M20A diode-array detector, Rheodyne 7725i valve furnished with 20 µl loop, CTO-10AS VP column oven and DGU-20A degasser. Waters Symmetry C18 (250×4 mm, 5 µm) column was utilized with mobile phases (Methanol (A) and water with % 0.5 acetic acid (B)) at a flow rate of 0.7 mL/ min. The elution profile is 0 min 5% A, 20 min 5% A, 45 min 60% A. Detection was made at 276 nm, and the column temperature was 30 °C. HMF was identified on retention time and spectral data by comparison with HMF standard. Results were expressed as mg/L.

2.9 TPC

TPC was determined by the Folin-Ciocalteau method (Singleton & Rossi, 1965). Results were expressed as gallic acid equivalent (mg GA eq./L).

2.10 Simultaneously determination of CGAs and caffeine

Chromatographic analysis for the determination of chlorogenic acids and caffeine were carried out using the HPLC system (Shimadzu, Kyoto, Japan). The method reported by Colaric et al. (2005) was used with some modifications. The diluted Mırra samples were filtered through 0.2 µm PVDF syringe filter and then injected into the HPLC system. Separation of phenolic compounds was carried out using a Symmetry C18 (250 × 4.6 mm id, particle size 5 µm) column (Waters, USA) at 25°C. The method utilizes a binary mobile phase consisting of 2% acetic acid in water (A) and 0.5% acetic acid in water:acetonitrile (1:1, v/v; B). The gradient program was as follows: 0 min 90% A; 30 min 80% A; 60 min 65% A. Flow rate of mobile phase was 1 mL/min and the column temperature was 25 °C. Detection was made at 273 nm for caffeine and 320 nm for chlorogenic acids. The compounds appearing in chromatograms were identified on retention times and spectral data by comparison with standards.

2.11 DPPH and ABTS assays

DPPH assay in Mırra sample was performed using a spectrophotometric method described by Pyo et al. (2004). The ABTS assay was carried out according to the method described by Re et al. (1999). The results of both DPPH and ABTS assays were expressed as Trolox equivalent antioxidant capacity (mmol Tr. eq./mL).

2.12 Sensory evaluation

Ten Mırra samples having different Brix° values (TM-1, TM-2, TM-4, TM-6, TM-7, TM-9, TM-10, CM-1, CM-5, CM-6) were used for sensory evaluation. Ten semi-trained panelists who regularly consumed Mırra were used for the sensory evaluation of Mırra. Panelists were trained prior to evaluation to become familiar with the quality attributes of Mırra samples. Approximately 10 mL Mırra sample at 60 °C was served to panelists in a porcelain Mırra cup (~20 mL). Cups were randomly coded with 3-digit numbers. Every Mırra sample was evaluated for color, odor, consistency, aroma, flavor and overall liking using a hedonic scale (1 = extremely disliked, 2=moderately dislike, 3=Neither like nor dislike, 4=moderately liked, 5 = extremely liked) in sensory evaluation (Mendes et al., 2001).

2.13 Statistical analysis

The data obtained were analyzed using Minitab 14 program for variance analyses and descriptive statistics. Duncan multiple comparison test was applied to identify significantly different groups ($P < 0.05$). To simplify the interpretation of the relation among the twenty-one properties of ten Mırra samples used in sensory analysis, results were analysed by principal component analysis (PCA).

3 Results and discussion

3.1 °Brix, viscosity and color values of Mırra

Results of °Brix, viscosity and Hunter color (L, a and b) values of Mırra samples are given in Table 1. Average °Brix values of TM, CM, LM and TC were found to be 3.70, 16.36, 5.32 and 2.10, respectively. °Brix values of CM were higher than those of TM. °Brix value of CM has also a low variation when compared to that of TM. This may be due to higher solubility of instant classic coffee in water. Viscosity values of TM and CM samples ranged from 1.00 to 2.00 cP, and 2.50 to 7.80 cP, respectively. The high °brix and viscosity values of CM indicated that it was more concentrated than other Mırra samples. For °brix and

Table 1. °Brix, viscosity and Hunter color (L, a and b) values of Mırras.

Sample	°Brix	Viscosity (cP)	Hunter Color Values		
			L	a	b
Traditional Mırra					
TM-1	3.90	1.13	17.51	0.22	1.10
TM-2	5.75	2.00	17.22	0.32	1.00
TM-3	1.10	1.05	17.06	- 0.04	0.68
TM-4	1.00	1.00	17.30	0.25	0.99
TM-5	2.85	1.13	17.24	0.21	0.30
TM-6	1.95	1.50	17.78	0.99	1.75
TM-7	2.80	1.28	17.03	0.22	1.01
TM-8	5.15	2.00	18.16	0.78	1.82
TM-9	4.15	1.18	17.27	0.26	1.11
TM-10	7.10	1.53	17.05	0.03	0.68
TM-11	4.95	1.13	17.23	0.15	0.99
Mean	3.70 ± 1.95^{a}	1.36 ± 0.35^{a}	17.35 ± 0.34^{a}	0.31 ± 0.30^{a}	1.04 ± 0.44^{a}
Mırra made with classical instant coffee					
CM-1	20.75	7.80	16.91	0.19	0.84
CM-2	21.50	4.50	16.84	0.62	1.23
CM-3	12.60	4.45	16.19	0.09	0.75
CM-4	13.75	3.40	17.11	0.24	0.93
CM-5	13.25	2.50	18.36	0.54	1.32
CM-6	15.70	4.78	17.40	0.60	1.18
CM-7	17.00	6.50	18.69	0.26	1.01
Mean	16.36 ± 3.59^{b}	4.85 ± 1.79^{b}	17.35 ± 0.88^{a}	0.36 ± 0.21^{a}	1.04 ± 0.21^{a}
Traditional Mırra made in the laboratory					
LM	5.32 ± 0.92^{a}	2.44 ± 0.10^{a}	16.76 ± 0.11^{a}	0.06 ± 0.01^{a}	0.75 ± 0.01^{a}
Turkish coffee					
TC	2.10 ± 0.54^{a}	1.08 ± 0.11^{a}	17.44 ± 0.22^{a}	0.38 ± 0.25^{a}	1.14 ± 0.13^{a}

Mean values are expressed as mean ± SD. Different superscript lowercase letters within the same column show significant differences at $p<0.05$, using Duncan's multiple range test.

viscosity values, the differences between TM and CM were found statistically significant (p<0.05).

Color is one of the most important characteristics of food and beverage because it contributes to consumer preference. The +a value (redness) uses in monitoring browning at food and beverage. Especially +a values of Mırra samples showed high variations compared to other color parameters (Table 1). It may be due to the differences in the roasting degree of coffee beans, brewing temperature and duration. For Hunter color values (L, a and b), the differences among TM, CM, LM and TC were statistically insignificant (p>0.05). Dmowski & Dąbrowska (2014) reported that the average CIE L*, a* and b* values of brewed coffee with dark roasted and ground coffee beans were found as 21.52, 1.36 and 1.95, respectively. Our results were lower than the findings of Dmowski & Dąbrowska (2014).

3.2 Brown pigment and HMF content of Mırra

Brown pigments and HMF contents of Mırra samples were given in Table 2. Brown pigment values ($A_{420\ nm}$) of TM and CM ranged from 0.03 to 0.51 and 0.38 to 1.72, respectively. The differences between CM and other samples were found statistically significant (p<0.05). Brown pigments value ($A_{420\ nm}$) in filter coffee was found to be 0.1 by Bravo et al. (2013). Except for TC, average brown pigment values of all Mırra samples were higher than the data reported by Bravo et al. (2013). Our results for TM and LM were similar with the findings of Fujioka & Shibamoto (2008) (0.273-0.458). HMF values of TM and CM ranged from 0.96 to 202.69 mg/L and 43.69 from 658.94 mg/L, respectively. There were significant differences between HMF values of CM and other coffee samples (p<0.05). Brown pigment and HMF contents of TM and CM showed high variation. This can be due to the lack of a standard application in Mırra preparation and using different raw materials. Especially CM samples had very high HMF content compared to other Mırra samples. The high HMF content of CM may be associated with the high HMF content of using classical instant coffee. Arribas-Lorenzo & Morales (2010) showed that soluble coffee has higher HMF content than commercial ground coffee samples marketed in Spain. Genotoxic, mutagenic, carcinogenic, DNA-damaging, organotoxic and enzyme inhibitory effects of HMF and its derivatives have been confirmed (Shapla et al., 2018). Therefore high HMF content of CM can lead to health problems for concumers.

3.3 Phenolic content and antioxidant activity of Mırra

The average TPCs of TM, CM, LM and TC were found as 3431.35, 11276.47, 5917.63 and 1214.56 mg GA eq./L, respectively (Table 2). All Mırra samples had high TPC. However, the highest TPC was found in CM. Bravo et al. (2013) noted that TPCs of spent coffee extracts obtained by different extraction systems ranged

Table 2. Some chemical contents and antioxidant activities of Mırras.

	$A_{420\ nm}$	HMF	TPC	DPPH	ABTS
Traditional Mırra					
TM-1	0.11	1.07	2696.84	6.40	12.44
TM-2	0.37	202.69	6906.61	13.86	46.96
TM-3	0.09	114.55	1626.44	2.52	9.42
TM-4	0.03	82.80	1245.69	2.03	86.50
TM-5	0.08	43.31	455.46	0.51	2.74
TM-6	0.23	42.21	5527.30	10.62	22.88
TM-7	0.22	1.32	3594.83	5.08	16.89
TM-8	0.33	186.02	5994.25	11.07	44.47
TM-9	0.14	0.96	2625.00	4.02	81.59
TM-10	0.51	48.58	4262.93	7.73	33.58
TM-11	0.17	64.20	2811.78	4.84	30.07
Mean	0.21 ± 0.14^{a}	71.60 ± 0.70^{a}	3431.55 ± 427.76^{b}	6.24 ± 0.88^{a}	35.23 ± 5.87^{a}
Mırra made with classical instant coffee					
CM-1	1.37	268.11	15836.21	32.26	142.16
CM-2	1.72	406.95	18889.37	48.64	171.45
CM-3	0.38	256.10	4449.71	8.35	27.70
CM-4	1.11	266.24	12244.25	25.93	96.99
CM-5	0.79	222.86	8659.48	18.56	60.15
CM-6	0.73	43.69	10836.21	21.33	57.08
CM-7	1.67	658.94	8020.11	12.19	72.28
Mean	1.11 ± 0.50^{b}	303.3 ± 189.7^{b}	11276.47 ± 1258.26^{d}	23.89 ± 3.48^{c}	89.70 ± 13.47^{b}
Traditional Mırra made in the laboratory					
LM	0.31 ± 0.06^{a}	3.05 ± 0.23^{a}	7378.35 ± 158.20^{c}	16.22 ± 0.23^{b}	75.90 ± 8.13^{b}
Turkish coffee					
TC	0.06 ± 0.01^{a}	15.49 ± 0.34^{a}	1214.56 ± 35.12^{a}	1.68 ± 0.12^{a}	4.45 ± 1.45^{a}

Mean values are expressed as mean ± SD. Different superscript lowercase letters within the same column show significant differences at p<0.05, using Duncan's multiple range test.

from 10.20 to 17.44 mg GA eq./g. The TPC of Turkish coffee was found as 2389 mg catechin eq./L by Karakaya et al. (2001). The antioxidant activities of coffee samples were determined by DPPH and ABTS assays. High antioxidant activities were observed in all Mırra samples. Especially CM samples showed the highest antioxidant activity values. Antioxidant activities of coffee samples decreased in the following order CM>LM>TM>TC. The TPCs of samples followed the similar order. The higher antioxidant activities of CM samples may be due to their higher Maillard reaction products ($A_{420\ nm}$) and TPCs. For TPCs and antioxidant activities, the differences among Mırra samples were found statistically significant ($p<0.05$). The antioxidant activity of commercial soluble coffees noted by Marcucci et al. (2017) varied between 0.4 and 37.0 g Trol. eq./100 g.

CGA and its derivatives are the main components of phenolic fractions of coffee (Clifford, 1999). In this study, CGA, 4-CGA and 5-CGA were identified and quantified in Mırra samples by HPLC (Table 3). While CGA was the major compound, the amounts of 4-CGA and 5-CGA were generally close to each other. CGA, 4-CGA and 5-CGA contents of TM and CM were 712.69-1130.52, 221.61-663.14, 163.56-673.04 mg/L, and 946.34-2113.63, 446.46-1536.82, 401.81-1632.77 mg/L, respectively. The highest CGA contents were identified in CM samples. However, several TM samples had high CGA contents as well as CA samples. This can be related to the CGA content of ground and instant coffee used in Mırra preparation. 4-CGA, 5-CGA and total CGA contents showed significant differences between TM and other samples ($p<0.05$). TPC, CGAs contents and antioxidant activity values of both TM and CM showed high variation. It should be due to the lack of a standard application in Mırra production and differences in raw material. Ludwig et al. (2012) found that the CGA contents of the espressos prepared from Guatemala and Vietnam coffees ranged from 10.6 to 31.0 mg/L. Our results were significantly higher than the findings of Ludwig et al. (2012). It should be noted that Mırra is a more concentrated beverage than espresso. Fujioka & Shibamoto (2008) reported that the total CGA contents of filter coffee samples prepared from regular and decaffeinated coffee ranged from 5.26 to 17.1, and 2.10 to 16.1 mg/g ground coffee, respectively.

3.4 Caffeine content of Mırra

Coffee is the major source of caffeine which is the most widely consumed psychoactive drug in the world. Amounts of caffeine in Mırra samples are given in Table 3. The average caffeine contents of TM, CM, LM and TC were found as 1416.93, 4347.46, 4027.89 and 596.54 mg/L, respectively. All Mırra samples, especially CM, had considerably high amount of caffeine. CM contained approximately 8 and 3 times more caffeine than TC and TM, respectively. The differences among caffeine contents of Mırra samples were found statistically significant ($p<0.05$). Jeon et al. (2017) reported that the caffeine contents of homemade

Table 3. The amount of caffeine and CGAs in Mırras (mg/L).

Sample	Caffeine	CGA	4-CGA	5-CGA	Total CGA
Traditional Mırra					
TM-1	1488.24	834.70	376.66	332.92	1544.30
TM-2	2911.27	1130.52	663.14	673.04	2466.70
TM-3	888.13	798.82	304.11	245.49	1348.42
TM-4	579.19	745.10	250.71	189.24	1185.05
TM-5	486.10	712.69	221.61	163.56	1097.85
TM-6	2592.34	1020.02	426.96	463.37	1910.35
TM-7	1507.24	833.18	370.58	332.20	1535.97
TM-8	2742.52	1044.14	547.91	510.51	2102.56
TM-9	1359.52	806.49	340.32	306.73	1453.53
TM-10	1092.44	768.77	274.62	209.03	1252.42
TM-11	1111.89	1096.96	554.97	536.34	2185.41
Mean	1416.93 ± 181.98^{a}	890.12 ± 151.67^{a}	393.78 ± 141.22^{ab}	360.22 ± 164.44^{ab}	1643.86 ± 453.72^{a}
Mırra made with classical instant coffee					
CM-1	6199.63	2113.63	1536.82	1632.77	5283.21
CM-2	6504.59	1986.80	1200.89	1128.40	4316.09
CM-3	2346.36	946.34	446.46	401.81	1794.61
CM-4	5279.26	1464.38	982.67	1011.38	3458.43
CM-5	3654.59	1165.42	658.59	660.27	2484.28
CM-6	4855.16	1539.54	1006.85	1098.48	3644.87
CM-7	2312.41	1179.57	632.61	573.51	2385.69
Mean	4347.46 ± 513.09^{c}	1482.28 ± 134.03^{a}	923.56 ± 96.64^{b}	929.52 ± 107.49^{b}	3338.17 ± 1174.06^{b}
Traditional Mırra made in the laboratory					
LM	4027.89 ± 46.80^{bc}	882.31 ± 8.29^{a}	397.98 ± 9.78^{ab}	347.27 ± 6.76^{ab}	1627.55 ± 35.10^{a}
Turkish coffee					
TC	596.54 ± 46.82^{a}	477.55 ± 13.70^{ab}	596.50 ± 22.17^{ab}	397.19 ± 10.63^{ab}	2073.33 ± 63.84^{a}

Mean values are expressed as mean ± SD. Different superscript lowercase letters within the same column show significant differences at $p<0.05$, using Duncan's multiple range test.

Table 4. Sensory evaluation of Mırras.

Sample	°Brix	Color	Consistency	Odor	Aroma	Flavor	Overall Liking
Traditional Mırra							
TM-4	1.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.10 ± 0.32	1.10 ± 0.32	1.00 ± 0.00
TM-6	1.95	2.30 ± 1.34	1.60 ± 0.97	2.30 ± 1.16	1.80 ± 1.03	1.40 ± 0.97	1.80 ± 0.92
TM-7	2.80	2.50 ± 1.08	2.20 ± 0.92	2.60 ± 1.26	2.40 ± 1.17	2.50 ± 1.18	2.50 ± 1.18
TM-1	3.90	1.60 ± 0.52	1.30 ± 0.48	1.60 ± 0.52	1.40 ± 0.70	1.40 ± 0.51	1.40 ± 0.52
TM-9	4.15	1.70 ± 0.48	1.50 ± 0.53	1.80 ± 0.63	1.50 ± 0.71	1.40 ± 0.52	1.40 ± 0.52
TM-2	5.75	2.80 ± 0.63	2.80 ± 0.42	3.10 ± 0.88	2.90 ± 0.57	2.80 ± 0.63	2.90 ± 0.57
TM-10	7.10	2.70 ± 0.68	2.40 ± 0.52	2.40 ± 0.52	2.40 ± 0.70	2.00 ± 0.67	2.50 ± 0.53
Mean		2.09 ± 0.67^{a}	1.83 ± 0.65^{a}	2.11 ± 0.69^{a}	1.93 ± 0.65^{a}	1.80 ± 0.64^{a}	1.93 ± 0.71^{a}
Mırra made with classical instant coffee							
CM-5	13.25	3.70 ± 1.16	3.70 ± 1.16	3.70 ± 1.16	3.70 ± 1.06	3.30 ± 1.16	3.60 ± 0.84
CM-6	15.70	3.80 ± 0.92	3.70 ± 0.95	3.90 ± 0.74	4.10 ± 0.57	4.30 ± 0.68	4.00 ± 0.67
CM-1	20.75	4.30 ± 0.68	4.10 ± 0.99	4.00 ± 1.25	3.70 ± 1.25	4.10 ± 0.99	4.00 ± 0.94
Mean		3.93 ± 0.94^{b}	3.83 ± 1.02^{b}	3.87 ± 1.04^{b}	3.83 ± 0.99^{b}	3.90 ± 1.03^{b}	3.87 ± 0.82^{b}

Data are expressed as mean ± SD. Different superscript lowercase letters within the same column show significant differences at p<0.05, using Duncan's multiple range test.

brewed coffees with the medium-roast Costa Rican coffee and Brazilian coffee were 576.96 and 460.74 mg/L, respectively. While the caffeine content of TC was in good agreement with values reported by Jeon et al. (2017), those of Mırra samples were higher than findings of Jeon et al. (2017).

Although the high caffeine content supports the greater antioxidant activity of coffee, it may cause some negative health effects. Passmore et al. (1987) noted that both systolic and diastolic pressures significantly increased after 360 mg caffeine dose. In another study, it was concluded that 125 mg of caffeine increased anxiety (Green & Suls, 1996). A standard cup of coffee is often assumed to provide approximately 100 mg of caffeine. The amount of caffeine in 240 mL of 14 different brewed coffee samples purchased at coffee shops in the US ranged from 72 to 130 mg. Caffeine in espresso coffees ranged from 58 to 76 mg in a single shot (McCusker et al., 2003). People consume a low amount of the Mırra in a small container (~25 mL). However, dietary exposure to caffeine from CM consumption can be higher than 100 mg/a cup. Therefore, risk groups (older people, pregnant women, patients with chronic heart failure, etc.) should be careful when consuming Mırra. The use of decaffeinated coffee in Mırra preparation will reduce the possible negative health risk of caffeine.

3.5 Sensory evaluation of Mırra samples

The results of sensory evaluation of Mırra samples having different °Brix values were given in Table 4. Results showed that all sensory scores increased by increasing the °Brix value. TM-4 had the lowest °Brix value and scores in all sensory parameters, CM-1 and CM-6 have the highest °Brix values and sensory scores. Mırra samples with soluble solid content higher than 5 °Brix generally had high scores. CM showed higher scores than TM. CM-1 sample (20.75 °Brix) for color, consistency and odor, and CM-6 sample (15.70 °Brix) for aroma and flavor had the highest scores. For all sensory parameters, the differences between TM and CM were significant (p<0.05). TM has been replaced with CM during the last two decades. This is generally thought to be related to the easy preparation process of the CM. However, this study showed that more likes of CM can be the main factor in this replacement.

3.6 Principle component analysis

Principle component analysis (PCA) is one of the most frequently used data decompositions techniques. The reduction of the number of variables and detection structure in the relationship between variables are the main applications of PCA (Kirazcı & Javidipour, 2008). PCA was used in the classification of some properties of Mırra and selected Mırra samples. Using PCA based on the correlation matrix, eigenvalues, percentages of variation, and load coefficients of the first three principal components were calculated for all studied properties. PCA results are presented in terms of loading and score plots (Figure 1). It was found that the first three principal components accounted for 58.27, 19.06 and 9.88% of the variations, respectively. The cumulative proportion of the variation approached 87.21% of the total variance. The traits contributing to high variation in the first PCA component (PC 1) were viscosity, °Brix, A_{420}, HMF, caffeine, TPC, DPPH and sensory evaluation parameters. The effects of all these traits on variation were similar. Traits contributing to the second PCA component (PC 2) were a and b from hunter color values, CGA and CGA derivatives. The third PCA component (PCA 3) was related to the L from hunter color values and ABTS. According to these results, a and b from Hunter color values were positively correlated with only CGA and their derivatives, and negatively correlated with only ABTS. There was also a negative correlation between b and $A_{420,}$ L and ABTS. All other parameters except hunter color values were positively correlated to each other at high level. However, only ABTS was not correlated with CGA. The results suggest that DPPH may be more useful for assaying the antioxidant activity in Mırra than ABTS, because DPPH shows a good correlation with all antioxidant compounds.

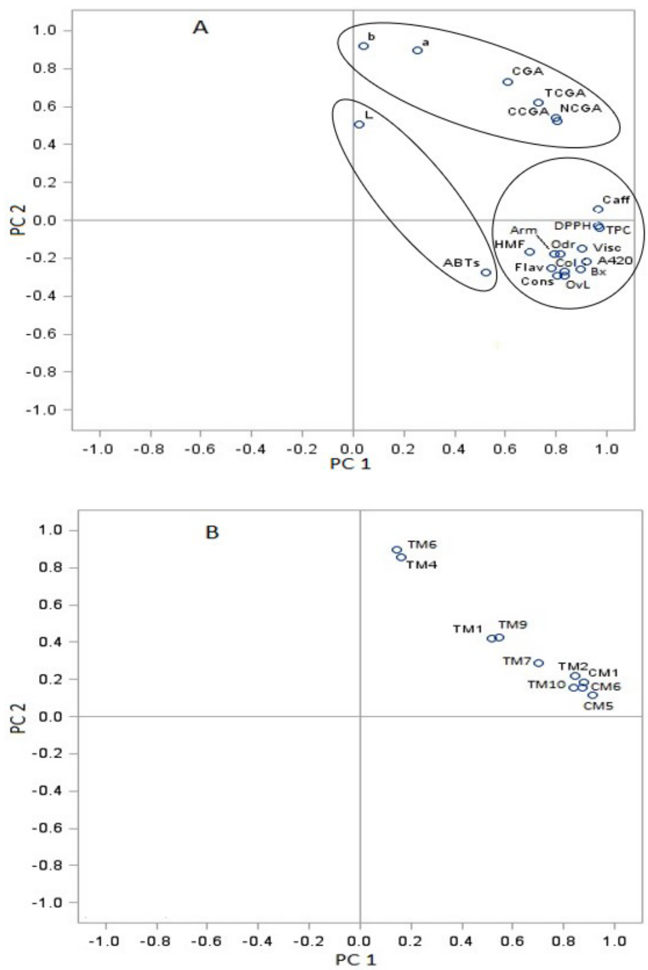

Figure 1. Loadings and scores plots of principal components analysis. (A) Loadings plot for results of properties; (B) Scores plot for the ten Mırra samples. (PC 1: first principle component; PC 2: second principal component; L: lightness from Hunter color values; a: (+) redness / (-) greenness from Hunter color values; b: (+) yellowness / (-) blueness from Hunter color values; TPC: total phenolic contents; DPPH: DPPH-scavenging activity; ABTs: ABTS-scavenging activity; CGA: chlorogenic acid; CCGA: 4-*O*-caffeoylquinic acid; NCGA: *trans*-5-*O*-caffeoylquinic acid; TCGA: total chlorogenic acid; Caff: caffeine; HMF: 5-hydoxymethylfurfural; A420: brown pigments; Bx: °Brix; Visc: viscosity; Col: color; Odr: odor; Cons: consistency; Arm: aroma; Flav: flavor; OvL: overall liking).

4 Conclusion

In this study, some physicochemical, bioactive and sensory properties of Mırra coffee were investigated. High phenolic and antioxidant activity in terms of health is a desirable feature in foods and beverages. All Mırra samples had high TPC, CGAs and antioxidant activity. Especially CM had higher TPC, CGAs and antioxidant activity than TM. The phenolic content and the antioxidant activities of the samples showed similar trends. Especially, for the measurement of antioxidant capacity in Mırra, DPPH may be a more useful method than ABTS. Mırra contains very high level of caffeine which may cause some negative health effects in risk groups. Therefore, Mırra consumers should consider the high caffeine content of Mırra. Mırra production from decaffeinated coffee can also be a good alternative for the risk groups. The high HMF content of Mırra sample, especially CM, should also be considered in terms of health. The variation in the studied properties are high. The high variability among Mırra samples may be due to the usage of different raw materials and lack of the standard applications in Mırra preparation. Therefore, further studies are needed for process standardization, geographical indications and determination of its possible effects on health.

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