ORIGINAL ARTICLE

A C G publications

Rec. Agric. Food. Chem. 3:1 (2023) 14-20

records of agricultural and food chemistry

Determination of Caffeine Amount of Organic and Conventional Coffees

Gülsüm Deveci[1], Elif Çelik[1], Özge Cemali[1], Teslime Özge Şahin[1], Ayşe Derya Bayazıt[1], Sena Özbay[2], Duygu Ağagündüz[1*] and Makbule Gezmen Karadağ[1]

[1] *Department of Nutrition and Dietetics, Faculty of Health Sciences, Gazi University Ankara, 06490, Türkiye*

[2] *Department of Food Technology, Kaman Vocational School, Kırşehir Ahi Evran University, Kırşehir, 40360, Türkiye*

(Received May 09, 2023; Revised June 23, 2023; Accepted June 30, 2023)

Abstract: Organic farming is a controlled and certified form of agricultural production at every stage, from production to consumption, without using chemical inputs in production. It has been suggested that there are differences in the amount of nutrients and non-nutrient compounds in foods produced by organic and conventional agriculture. In light of this information, the caffeine content of coffee, which is an important consumption product in our country and the world, has been evaluated in terms of organic and conventional coffees. For this purpose, 12 different types of organic (n=7) and conventional (n=5) coffees belonging to 5 different brands (A, B, C, D, E) offered for sale in the market were included in the sampling. The included brands were carefully selected to provide both organic and conventional options. The caffeine content of all ready-to-serve coffee samples was analyzed with high-pressure liquid chromatography (HPLC). The caffeine content of the coffees was expressed as mg/L, and the statistical analysis of the data was done with the SPSS 22.0 package program. The amount of caffeine (mg/L) in organic coffees was therefore found to be lower than that in conventional coffees when coffees are compared based on production methods (863.2181.46 mg/L, 1115.4380.88 mg/L, p<0.05, respectively). While the quantity of caffeine in organic coffee from three brands was found to be lower than that of conventional coffee (Organic and conventional, respectively; A: 694.9±31.73 mg/L and 1030.8±7.55 mg/L; D: 853.9±32.50 mg/L and 1576.4±250.94 mg /L; E: 1094.2±32.03 mg/L and 1470.7±13.45 mg/L; p<0.05), it was found that the converse was true for the other two brands when the amount of caffeine is compared according to both the production method and the brands (Organic and conventional, respectively; B: 904.9±189.54 mg/L and 831.3± 67.91 mg/L, C: 684.4±63.78 mg/L and 667.7±84.36 mg/L; p<0.05). The amount of caffeine in the coffees acquired using the conventional production method was higher than the coffee obtained using the organic production technique, and the amounts of caffeine in the coffees varied depending on the brands. This integrates the fact that the amount of caffeine in food can depend on whether or not production.

Keywords: Caffeine; organic coffee; conventional coffee; HPLC analysis.

[*] Corresponding author: e-Mail: duyguturkozu@gazi.edu.tr

The article was published by ACG Publications
https://www.acgpubs.org/journal/records-of-agricultural-and-food-chemistry January-June 2023 EISSN: 2792-0763
DOI: http://doi.org/10.25135/rfac.17.2305.2780
Available online: June 30, 2023

1. Introduction

As an alternative to conventional farming, sustainable agriculture is recognized as a promising type of production to overcome the challenges associated with conventional farming [1]. Sustainable agriculture is a form of application that meets the need for food, energy, and natural resources, while protecting soil, water, and biological diversity [2]. Organic farming is a production system that avoids, or largely excludes, the use of synthetic fertilizers, pesticides, growth regulators, and livestock feed additives in any stage, including cultivation, harvesting, classification, packing, labeling, storing, and transporting. Organic farming aims at environmental, social, and economic sustainability [3]. Long-term and excessive use of chemicals can be dangerous in some respects in terms of human and soil health, as well as environmental pollution. Therefore, farmers in developed countries are encouraged to convert their existing farms to organic agriculture [4]. Thus, the health of farmers and their families and society is eventually protected and improved. Over the last decades, organic food and farming have been growing year by year across the world. Recent data shows that 72.3 million hectares are devoted to organic farming and are processed by 2.4 million producers [5]. In Turkey, the number of organic agricultural product varieties, which was 53 in 1997, increased to 225 in 2016 [6]. Parallel to this, the data show that the demand for organic food and beverages is increasing in food markets all over the world [7,8]. It has been suggested that there are differences in the amount of nutrients and non-nutrient compounds in foods produced by organic and conventional agriculture [9]. While some scientific studies support that organic foods are healthier, more nutritious, safer, and more delicious, some studies report that organic or non-organic foods are not different from each other [10-13].

Coffee, one of the most consumed non-alcoholic beverages after water, contains many bioactive compounds. Coffee contains mainly caffeine but also has diterpenes, chlorogenic acid, and polyphenols [14]. Caffeine is a neuro stimulant alkaloid whose molecular formula is 1,3,7-trimethyl-1H-purine-2,6(3H,7H)-dione [15]. The amount of caffeine in coffee can vary depending on several factors, including the type of coffee bean, the roasting process, and whether the coffee is organic or non-organic [14]. Organic coffee is produced using farming practices that minimize the use of synthetic fertilizers, pesticides, and herbicides. The production of organic coffee is associated with lower environmental impact and improved food safety and quality [16]. The determination of caffeine content in coffee can be of interest to both coffee consumers and coffee producers. Many factors affect the amount of caffeine in coffee, such as the origin of coffee beans, light exposure, height above sea level, method of growing, roasting beans, fertilizer, pesticides, etc. [17-19].

This study aims to compare the caffeine amount of organic and non-organic coffees using High-Performance Liquid Chromatography (HPLC) and determine whether there is a significant difference in caffeine content between these types of coffee brews available in the Turkish market. The potential differences in caffeine content between organic and non-organic coffee have important implications for consumers who are concerned about the impact of their dietary choices on their health and the environment. So, the results of this study can help consumers make informed decisions about their coffee choices and provide valuable information for coffee producers to improve the quality of their products.

2. Materials and Methods

2.1. Sample Selection

This study included 12 coffee samples from 5 different market-available brands (A, B, C, D, E). Seven of the coffee samples were organic. The included brands were carefully selected to provide both organic and conventional options. All of the coffees under examination have the same roasting level (medium roast/medium strength) and are made entirely of Arabica coffee beans. In the selection of organic coffees included in the study, attention was paid to the presence of the "organic" logo on the packaging. “Organic” coffees with the organic logo on their packaging are brands certified by organizations authorized by the Ministry of Agriculture of the Republic of Turkey. Certification procedures are carried out in accordance with the "regulation on the principles and practice of organic agriculture" prepared by the Ministry of Agriculture of the Republic of Turkey.

2.2. Preparation of Coffee Samples

All of the study's coffee beans were ground for 60 seconds in a coffee grinder (220 V 50/60Hz 180W). The ground coffees were weighed using an analytical balance (Precisa XB 220A) and prepared using the recommended brewing methods (10 g coffee/180 mL water or 6-7 g coffee /125 mL water) described on the label information. All the coffees were brewed for 3.5 minutes using an automatic filter coffee machine (Braun Cafe House) and No. 4 filter paper. A thermometer (Selecta) was used to measure the temperature of the coffees after they had been brewed. The coffees were not stored, and they were prepared on the day of the analysis.

2.3. Preparation of Standard Solutions

Caffeine standards were prepared with bidistilled water as 1 ppm, 5 ppm, 10 ppm, 20 ppm, 50 ppm, and 100 ppm. Standard solutions were transferred into HPLC vials using a filter (PTFE Syringe Filter, 0.45 µm).

2.4. Caffeine Content Analysis

The caffeine content of all coffee samples was determined by the reverse phase HPLC method. High-performance liquid chromatography system analysis was performed using a Thermo Finnigan UV1000 detector and SpectraSYSTEM pump, C18 analytical column (Phenomenex). The method of Shrestha et al. (2016) was used in HPLC analysis [20]. The mobile phase was prepared with water:methanol ratio of 60:40. The flow rate was 1 mL/min, the column temperature was 40°C, the injection volume was 100 µL, and the wavelength was 275 nm. All analysis was carried out at room temperature. The caffeine content of coffee is expressed in mg/L. Caffeine content is calculated as cups, and 1 cup of coffee is determined as 240 mL [21].

2.5. Statistical Analysis

Statistical analyzes were performed using the SPSS 22.0 package program. It was analyzed how the dependent variable, the amount of caffeine, varied depending on the independent variables, such as the cultivation method (organic vs. conventional) and the coffee brands (A, B, C, D, E). All values are reported as "Mean±SD". Statistical significance was considered when $p<0.05$. The variables were investigated by analytical methods (Kolmogorov-Simirnov/Shapiro-Wilk's test) to determine whether or not they are normally distributed. The caffeine amounts of organic and conventional coffee samples were compared using the student's *t*-test. One-way ANOVA was used to compare the caffeine amounts of organic and conventional coffees according to brands (A, B, C, D, E). The Levene test was used to assess the homogeneity of the variances. When an overall significance was observed, pairwise post hoc tests were performed using Tukey's test [22].

3. Results and Discussion

This study evaluated the caffeine contents of organic and conventional coffees belonging to five different coffee brands (A, B, C, D, E). The regression curve of the caffeine standards used (1, 5, 10, 20, 50, and 100 ppm/L) is given in Figure 1. The determination coefficient (R^2) value obtained according to the regression equation was 0.9986.

When organic and conventional coffees of various brands were examined (Figure 2), the caffeine amount of conventional coffees were found to be higher than organic coffees ($p<0.05$). While the amount of caffeine in organic coffees was 863.2±181.46 mg/L, it was determined as 1115.4±380.88 mg/L in conventional coffees.

There was a significant difference between the caffeine amount of organic ($p=0.010$) and conventional ($p<0.001$) coffees belonging to five different brands (A, B, C, D, E) (Table 1). Among organic coffees, there is a significant difference between A and E brands, and the amount of caffeine is higher in E coffees ($p=0.019$). In addition, the amount of caffeine in E coffee, which is one of the organic coffees, was higher than the C brand ($p=0.016$). The amount of caffeine belonging to the D and E brands of conventional coffees is higher than the caffeine content of the A, B, and C brands ($p<0.05$). When organic and conventional coffees of each coffee brand are evaluated within themselves, the caffeine

content of organic coffees of A, D, and E brands was significantly lower than conventional coffees (p<0.05). In contrast, the caffeine content of organic coffees of B and C brands was higher than conventional coffees (p>0.05).

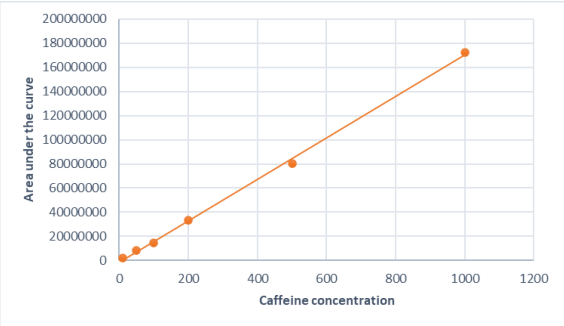

Figure 1. Caffeine regression curve, regression equation, and the determination coefficient (r^2)

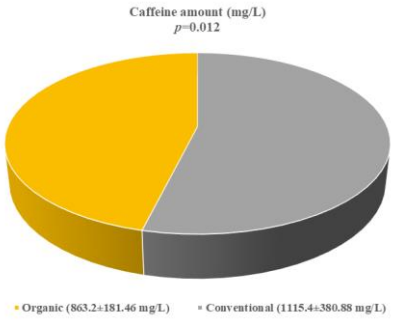

Figure 2. Caffeine amount of organic and conventional coffees (mg/L)

There is a statistically significant difference between the amount of caffeine of brands A and E and brands C and E (p<0.05). Conventional coffee: Conventional coffee; There is a statistically significant difference between the amount of caffeine of brands A and C, brands A and D, brands A and E, brands B and E brands, brands C and D, and brands C and E (p<0.05).

When examining Table 1, it can be seen that the highest caffeine level was measured in the conventional coffee of brand D, with a level of 447.7 mg/L. The lowest caffeine level was detected in the C brand coffee, also a conventional coffee. Among the organic coffees, the highest caffeine level was found in brand E, with a level of 270.1 mg/L. A significant difference was observed between conventional and traditional coffees in terms of maximum caffeine levels.

Table 1. Caffeine amount (1 cup) of organic and conventional coffees belonging to five different brands (A, B, C, D, E) (mg/L)

Types of coffee	Brands	Mean ± standard deviation*	Minimum	Maximum	*p*
Organic (*n*=7)	A (*n*=1)	166.8±7.62	158.7	173.2	**<0.010**
	B (*n*=3)	217.2±45.49	154.3	255.2	
	C (*n*=1)	164.3±15.31	148.9	179.5	
	D (*n*=1)	204.9±7.80	195.9	209.5	
	E (*n*=1)	262.6±7.69	254.8	270.1	
Conventional (*n*=5)	A (*n*=1)	247.4±1.81	245.4	248.8	**<0.001**
	B (*n*=1)	199.5±16.30	183.6	216.2	
	C (*n*=1)	160.3±20.25	142.6	182.4	
	D (*n*=1)	378.4±60.23	339.6	447.7	
	E (*n*=1)	353.0±3.22	349.3	355.2	

*1 cup of coffee is determined as 240 mL.

In addition, the study found significant differences in caffeine levels among coffee brands in general. Moreover, caffeine levels in conventionally produced coffee were higher than those in organic coffee. Similarly, in a different study, caffeine levels were found to be 48.10 ± 3.95 mg/100 mL in organic coffee and 57.95 ± 5.39 mg/100 mL in conventionally produced coffee [23]. It is known that traditional coffee contains significantly higher levels of caffeine than organic coffee. However, organic coffee contains almost all bioactive compounds at higher levels than traditional coffee [23]. In another study examining coffee grounds, it was reported that organic coffee grounds samples contained significantly higher concentrations of bioactive and antioxidant compounds compared to conventional coffee grounds samples [24]. However, in a different study, researchers found that organic coffee

contained higher levels of chlorogenic acid and caffeine compared to conventional coffee, although this difference was not statistically significant [25].

It is observed both in the literature and in the conducted study that traditional coffee contains a higher level of caffeine than organic coffee. This is mainly due to the widespread use of nitrogen fertilizers in conventional farming systems, which can affect the formation of caffeine. Caffeine is a purine alkaloid, and nitrogen fertilization can increase the level of this alkaloid in coffee beans [26]. In samples spiked with pesticides, there is more caffeine present. This shows that caffeine and pesticides compete for sorbent adsorption [19].

However, these differences between organic and traditional coffee can arise from many reasons. This situation is due to the interactive relationship between the soil in coffee production and the coffee bean. The effects of organic or traditional coffee production on the physical, chemical, biological, and microbial diversity of the soil are known [27]. While the coffee bean affects the structure of the soil, it has been shown that different farming practices also affect the caffeine levels of coffee [28]. However, it is known that the caffeine content in coffee beans is influenced not only by the geographical location and physicochemical properties of the soil but also by the variety of the coffee bean, environmental factors [29], and the geography where the coffee plants are grown [30]. The duration of coffee storage and roasting level during production also affect the caffeine level in coffee beans, as well as the coffee type [31]. In a study, the caffeine levels of organic coffee (4.61±1.69 mg/g) were lower than conventional coffee (5.26±1.97 mg/g). However, in the storage process, the opposite was observed. The caffeine content in organic stored coffee (8.55±3.45 mg/g) was found to be higher than traditional coffee beans (5.41±2.32 mg/g) [28]. It is also known that the way in which coffee is brewed and prepared by the end consumer affects the caffeine level [23].

4. Conclusions

Coffee is an important beverage with many traditional varieties that are consumed widely throughout the world. The presence and level of caffeine, one of the most important components of coffee, is influenced by many factors. The coffee bean, production location, climate, post-harvest storage, roasting processes, and brewing methods during consumption all affect the caffeine level of coffee. In our study, we examined the effect of an important factor among these, production methods. The results of our study are consistent with the literature. Caffeine levels in traditional coffees were significantly different and higher than those in organic coffees. There are several limitations in our study. The number of samples included in the study may be a feature of the study that needs improvement. Because new studies to be conducted with a larger number of samples will provide the statistical power of the findings and the generalization of the study result. With the inclusion of organic types of existing coffee brands in the markets as well as the traditional types, it will be possible to make an evaluation in a larger sample, at least for Turkey. The coffee samples from different countries/regions suggest the possibility of different cultivation methods, and these situations will change the caffeine content. However, there is not enough information about them on the label. Among the other reasons for the difference in the amount of caffeine between traditional and organic coffees are the differences in the synthetic chemicals used during production, antioxidants, pesticides, and fertilizers. A more detailed examination of all these in other studies will enable the study to be addressed in all its aspects. This situation will be a comprehensive data source in relation to health. In addition, the absence of bioactive components in our study can be stated as a limitation. Bioactive components can also be looked at in future studies.

ORCID
Gülsüm Deveci: 0000-0002-8047-2926
Elif Çelik: 0000-0002-8007-8515
Özge Cemali: 0000-0002-9971-8498
Teslime Özge Şahin: 0000-0002-7289-5187
Ayşe Derya Bayazıt: 0000-0003-1361-9569
Sena Özbay: 0000-0001-6024-0805

Duygu Ağagündüz: 0000-0003-0010-0012
Makbule Gezmen Karadağ: 0000-0003-3202-3250

References

[1] C. Puech, J. Baudry, A. Joannon, S. Poggi and S. Aviron (2014). Organic vs. conventional farming dichotomy: Does it make sense for natural enemies? *Agric. Ecosyst. Env*. **194**, 48-57.

[2] F. Menalled, T. Bass, D. Buschena., D. Cash, M. Malone, BK. Maxwell, P. McVay, R. Miller, R. Soto and D. Weaver (2008). An introduction to the principles and practices of sustainable farming. *Montana State Univ*. 1-4.

[3] E.A. Stockdale, N.H. Lampkin, M. Hovi, R. Keatinge, E.K.M. Lennartsson, D.W. Macdonald, S. Padel, F.H. Tattersall, M.S. Wolfe and C.A. Watson (2001). Agronomic and environmental implications of organic farming systems, *Adv. Agron.* **70**, 261–327.

[4] S.K. Yadav, S. Babu, S.K. Yadav, K. Singh, G.S. Yadav and S. Pal (2013). A review of organic farming for sustainable agriculture in northern India, *Int. J. Agron.* 1- 8. doi: 10.1155/2013/718145.

[5] H. Willer, T. Jan, M. Claudia and S. Bernhard (Eds.) (2021). The World of Organic Agriculture. Statistics and Emerging Trends 2021. *Res. Inst. Organic Agric. FiBL, Frick, and IFOAM – Organics International, Bonn.*

[6] G.A. Eryılmaz, K. Demiryürek and M. Emir (2015). Avrupa Birliği ve Türkiye'de organik tarım ve gıda ürünlerine karşı tüketici davranışları, *Anadolu Tarım Bilim. Derg.* **30**, 199-206.

[7] J.P. Reganold and J.M. Wachter (2016). Organic agriculture in the twenty-first century, *Nat. Plants* **2**, 15-221.

[8] IFOAM (2021). Annual Report 2021. Consolidated Annual Report of IFOAM Organics International & Its Action Group.

[9] M. Barański, D. Srednicka-Tober, N. Volakakis, C. Seal, R. Sanderson and G.B. Stewart (2014). Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: A systematic literature review and metaanalyses, *Br. Nutr.* **112**, 794-811.

[10] M.E. Popa, A.C. Mitelut, E.E. Popa, A. Stan and V.I. Popa (2019). Organic foods contribution to nutritional quality and value, *Trends Food Sci. Technol.* **84**, 15-18.

[11] B.A. Stracke, C.E. Rufer, A. Bub, K. Briviba, S. Seifert, C. Kunz and B. Watzl (2009). Bioavailability and nutritional effects of carotenoids from organically and conventionally produced carrots in healthy men, *Br. Nutr.* **101**, 1664–1672.

[12] T. Gomiero (2018). Food quality assessment in organic vs. conventional agricultural produce: Findings and issues, *Appl. Soil Ecol*. **123**, 714-728.

[13] M. Chowdhury, P. Castka, D. Prajogo, X. Zhao and L.C. Wood (2021). Is organic food becoming less safe? A longitudinal analysis of conventional and organic product recalls, *Sustainability* **13**, 135-140.

[14] K. Kroll, M. Gantner, A. Tatarak and E. Hallmann (2020). The content of polyphenols in coffee beans as roasting, origin and storage effect, *Eur. Food Res. Technol.* **246**, 33–39.

[15] N. Amaresh, A.R. Mullaicharam and M.A. El-Khider (2011). Chemistry and pharmacology of caffeine in different types of tea leaves, *Int. J. Nutr. Pharmacol. Neurol. Dis.* **1**, 110-115.

[16] J.S. Santos, M.L.P. Santos and M.M. Conti (2010). Comparative study of metal contents in Brazilian coffees cultivated by conventional and organic agriculture applying principal component analysis, *J. Braz. Chem. Soc.* **21,** 1468–1476.

[17] E. Olechno, A.Puścion-Jakubik, M.E .Zujko and K. Socha (2021). Influence of various factors on caffeine content in coffee brews, *Foods* **10**, 1208.

[18] D.J. Gonthier, J.D. Witter, A.L. Spongberg and S.M. Philpott (2011). Effect of nitrogen fertilization on caffeine production in coffee (Coffea arabica), *Chemoecology* **21**, 123-130.

[19] M.T.S. Trevisan, R.W. Owen, P. Calatayud-Vernich, A. Breuer and Y. Picó, (2017). Pesticide analysis in coffee leaves using a quick, easy, cheap, effective, rugged and safe approach and liquid chromatography tandem mass spectrometry: Optimization of the clean-up step, *J. Chromatogr. A* **1512**, 98-106.

[20] S. Shrestha, S.K. Rijal, P. Pokhrel and K.P. Rai (2016). A simple HPLC method for the determination of Caffeine content in tea and coffee, *J. Food Sci. Technol. Nepal* **9**, 74-78.

[21] "TÜRKİYE BESLENME REHBERİ (TÜBER) 2022" Sağlık Bakanlığı, Halk Sağlığı Genel Müdürlüğü, Sağlık Bakanlığı Yayın No:1031, Ankara 2022

[22] M. Hayran and M. Hayran (2011). Sağlık araştırmaları için temel istatistik, *Ankara, Turkey: Omega Arastirma* 242-253.

[23] M. Górecki and E. Hallmann (2020). The antioxidant content of coffee and its in vitro activity as an effect of its production method and roasting and brewing time, *Antioxidants* **9,** 308.

[24] C. Ozuna, S. Mulík, B. Valdez-Rodríguez, M.D.R. Abraham-Juarez and C.L. Fernández-López (2020). The effect of organic farming on total phenols, total flavonoids, brown compounds and antioxidant activity of spent coffee grounds from Mexico, *Biol. Agric. Hortic.* **36**, 107-118.

[25] D. do Carmo Carvalho, M.R.P.L. Brigagão, M.H. dos Santos, F.B.A. de Paula, A. Giusti-Paiva, and L. Azevedo (2011). Organic and conventional Coffea arabica L.: A comparative study of the chemical composition and physiological, biochemical and toxicological effects in Wistar rats, *Plant Foods Hum. Nutr.* **66**, 114-121.

[26] D.J. Gonthier, J.D. Witter, A.L. Spongberg and S.M. Philpott (2011). Effect of nitrogen fertilization on caffeine production in coffee (Coffea arabica) *Chemoecology* **21,** 123-130.

[27] K. Velmourougane, (2016). Impact of organic and conventional systems of coffee farming on soil properties and culturable microbial diversity, *Scientifica* 1-9. doi:10.1155/2016/3604026.

[28] K. Król, M. Gantner, A. Tatarak and E. Hallmann (2020). The content of polyphenols in coffee beans as roasting, origin and storage effect, *Eur. Food Res. Technol.* **246,** 33-39.

[29] K.A. Anderson and B.W. Smith (2002). Chemical profiling to differentiate geographic growing origins of coffee, *J. Agric. Food Chem.* **50**, 2068-2075.

[30] B.T. Gebeyehu and S.L. Bikila, (2015). Determination of caffeine content and antioxidant activity of coffee, *Am. J. Appl. Chem*. **3**, 69-76.

[31] M. Poroch-Seriţan, C.B. Michitiuc and M. Jarcău (2018). Studies and research on caffeine content of various products, *Broad Res. Artif. Intell. Neurosci*. **9**, 29-35.