REVIEW ARTICLE

THE OCCURRENCE OF CAFESTOL AND KAHWEOL DITERPENES IN DIFFERENT COFFEE BREWS

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(Received: March 22, 2019; accepted: May 20, 2019)

ABSTRACT: Coffee is the second most consumed brew in the world, after water. Cafestol and kahweol are two diterpenes that have been identified, until now, exclusively in the coffee tree. They are co-extracted from the coffee beans that are roasted and ground for the beverage preparation. Their pharmacological properties are related to anticancer and anti-inflammatory activities, although they also increase the serum cholesterol. Several researches reported that coffee brew preparation methods influence directly the levels of the diterpenes. This paper describes a compilation of the main results published for different coffee brews and all variables related to their preparation. Major differences in the reported concentrations have been noted.

Index terms: Brew preparations, boiled, drip-filtered, espresso, moka, French press, Turkish/Greek, diterpene content, chromatography, hypercholesterolemia.

A OCORRÊNCIA DOS DITERPENOS CAFESTOL E CAVEOL EM DIFERENTES BEBIDAS DE CAFÉ

RESUMO: O café é uma das bebidas de maior consumo pela população adulta ao redor do mundo. Cafestol e caveol são dois diterpenos, até o presente, identificados exclusivamente nos tecidos do cafeeiro. Estão localizados nos frutos durante o seu amadurecimento, sendo posteriormente extraídos para a bebida durante a sua preparação a partir dos grãos torrados e moídos. Suas propriedades farmacológicas estão correlacionadas ao combate de alguns tipos de câncer e à inflamação, embora também contribuam para o aumento do colesterol sérico. Várias pesquisas relataram que o modo de preparo da bebida influencia diretamente o teor dessas substâncias na mesma. Este artigo descreve uma compilação dos principais resultados publicados sobre o teor desses diterpenos em bebidas de café, além das variáveis relacionadas ao seu preparo sobre o teor diterpênico. Diferenças grandes em suas concentrações relatadas têm sido notadas.

Termos para indexação: Modos de preparo, fervido, filtrado, espresso, moka, prensa francesa, turco/grego, teor diterpênico, cromatografia, hipercolesterolemia.

1 INTRODUCTION

Coffee is one of the most popular beverages in the world, being consumed by 80% of the adult population (SRIDEVI; GIRIDHAR; RAVISHANKEAR, 2011). In 2016/2017, more than 156 million bags (60 Kg) of coffee were consumed in the world, making it one of the most traded agricultural commodities (INTERNATIONAL COFFEE ORGANIZATION – ICO, 2018a). The habit of drinking coffee has its origins in the Arab culture, supported by the Qur'an`s prohibition of alcohol consumption. With the peak of the Enlightenment movement coffee consumption expanded mainly in the intellectual classes and turned into a social unifying characteristic that endures still this day (ASSOCIAÇÃO BRASILEIRA DA INDÚSTRIA DE CAFÉ - ABIC, 2018a). Moreover, this habit directly relates to regional and cultural factors that influence the preparation and the strength of the beverage, besides the choice of the type of beans used, the roasting level and consumer routine. Since its spread started in the seventeenth century, researchers have questioned the adverse effects of coffee consumption on health, thus attracting special attention from the public and the scientific community (PREEDY, 2015).

According to the International Coffee Organization (ICO), there are two coffee species with commercial importance, known popularly as Arabica (*Coffea arabica* Linnaeus) and Robusta (*C. canephora* Pierre ex A. Froehner) coffee (ICO, 2018b). They show great differences in their agronomic characteristics, chemical composition

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and organoleptic properties. In the ICO website, Brazil is currently the world's largest producer of coffee, being responsible for 33.1% of the total production. Of the total amount production in Brazil, approximately 96% was Arabica (ICO, 2018b). This clearly shows a preference for this variety, because it is considered a finer coffee with superior quality, aroma and flavor, allowing its use for gourmet coffees. In addition, the country is also the second largest consumer with 21,225 million bags of 60 kg/year, behind the United States, which consumes 25,775 million bags/year (ICO, 2018a).

The coffee beverage preparations

Coffee is being appreciated and disseminated worldwide and there are several ways to prepare the infusion. Among them, some stand out for being the most commonly used, and are presented below.

Turkish/Greek coffee

Originally from the ancient Ottoman Empire, the Turkish/Greek coffee (Figure 1) is prepared in a small coffee pot called *cezve* in Turkish, being traditionally made of copper. The beverage should be prepared by the addition of high-quality and medium roasted Arabica coffee beans, finely grounded (75-125 µm), cold water and, if desired, sugar, followed by twice boiling to form a foam. After preparation, one must wait about one minute for the decanting of the powder from the brew. It is served in special porcelain cups, named *zarf*. Once ready the beverage is not mixed, and it should be drunk down to the ground coffee level (YILMAZ; ACAR-TEK; ZÖZLÜ, 2017; ABIC, 2018b).

Boiled coffee

Derived from an adaptation of Turkish/ Greek coffee, and traditionally consumed by the Scandinavians, the so-called boiled coffee (Figure 2) was spread around the world and consists of a roasted and coarsely ground coffee boiled for up to 10 minutes. Subsequently, let it sit for 2 to 5 minutes before consumption (ABIC, 2018b).

French Press

In this preparation, the coffee is put in the French press, also known as coffee plunger (Figure 3). This method uses a particular coffee maker in which the coarsely ground powder is mixed with hot water and left for a few minutes. After that, the plunger is pushed down until it reaches the bottom of the French press (ABIC, 2018b).

Drip-filtered

The drip-filtered coffee (Figure 4), in turn, is the most popular form of coffee preparation in Brazil, being preferred in the homes of 94% of the population (GOVERNO DO BRASIL, 2018).

FIGURE 1 - Sequence of events (1-6) for Turkish coffee preparation using a coffee pot *cezve*, coffee powder and sugar.

FIGURE 2 - Sequence of steps (1-3) for boiled coffee beverage preparation.

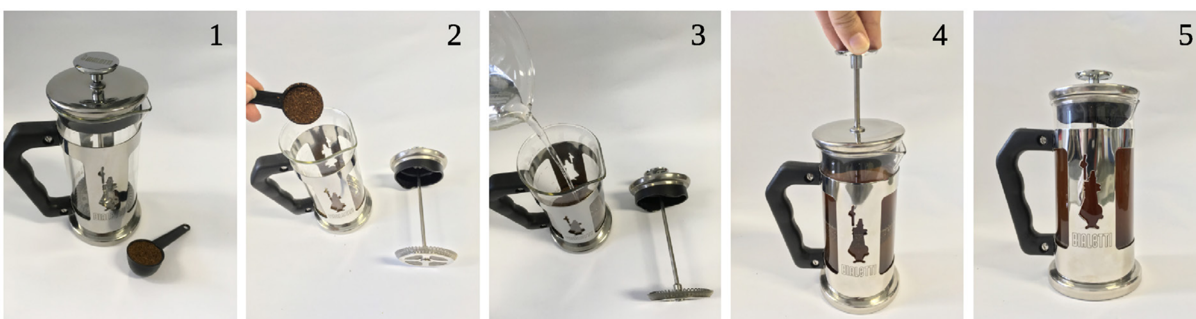

FIGURE 3 - French press coffee maker and sequence of events (1-5) for brew preparation.

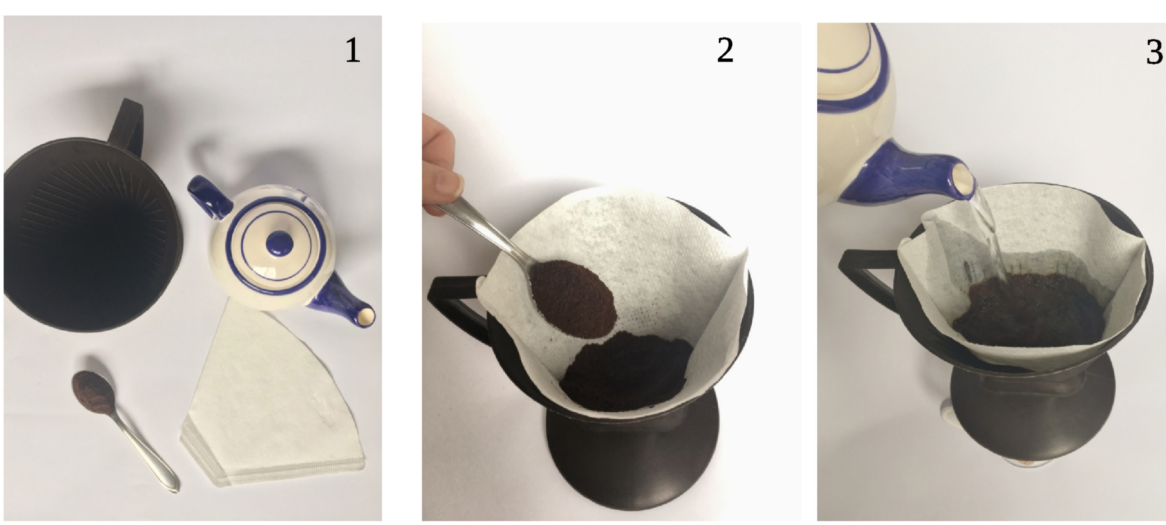

FIGURE 4 - Steps for drip-filtered coffee preparation using hot water, coffee powder, paper filter and a drip-filtered coffee maker.

Because of the great diffusion of this beverage, numerous forms of preparation are known. One of them and the most popular utilize a tapered support in which the paper filter, or cloth, is filled with the roasted and ground coffee. Hot water (93-98 °C) is poured on the powder and the filtered beverage is collected in a bottle, a tea pot or a cup (ABIC, 2018b), consumed according to taste, with or without sugar.

Espresso coffee

The espresso coffee (Figure 5) was developed in Italy in the early twentieth century and was prepared under steam pressure (ABIC, 2018b). It is a drink with intense aroma, which uses a commercial machine in which hot water (90 ± 5 °C) under high pressure (typically 9 ± 2 bar) passes through finely ground and roasted compressed coffee, in a short period of time (30 ± 5 seconds), producing a mixture (50 mL) with a strong flavor (PETRACCO, 2005). This fact, *i.e.* the pressure, explains the origin of its name, because it is squeezed, there is no relation to the speed of its preparation as people might think. The texture of this drink is more consistent compared with the others.

Espresso capsule

One protagonist that has attracted attention is the espresso capsule due to its growing consumption worldwide (WUERGES et al., 2016). The espresso coffee single-dose capsule was developed in 1970 by Nestlé® (NESPRESSO, 2017). It uses ground coffee capsules in individual portions in an automatic specific coffee maker. This system was created with the intention to produce beverages with replicate coffee quality with fast preparation.

Moka Coffee

The term Moka, depending on the spelling of the word, designates different coffee drinks. Mocha coffee is originally a noble variety of the species *C. arabica*, coming from the port city of Mocha, located in Yemen, but mocha today is also known as a coffee drink with chocolate. With the letter k, it is a coffee prepared in a commercial Italian coffee machine, also known as moka pot coffee. This pot has three compartments (Figure 7), the filter cup is filled with roasted coffee powder – generally a medium roasting degree with total weight loss of 16%, and the bottom compartment is filled with water (NAVARINI et al., 2009). When the bottom compartment is heated, the water boils and increases the inside pressure, thus allowing the hot water to infuse through the coffee powder, and the extract is collected in the upper compartment (ABIC, 2018b; GROSS; JACCAUD; HUGGETT, 1997; NAVARINI et al., 2009).

Beverage components and increased serum cholesterol levels

With over 2,000 compounds estimated to be present in roasted coffee beans, while another 900 releasing and contributing to the aroma, coffee is a chemically complex matrix (ALVES; CASAL; OLIVEIRA, 2009; TOLEDO, PEZZA, PEZZA; TOCI, 2016). The major classes of compounds are medium and long chain polysaccharides, melanoidins, aromatic volatile compounds, caffeine, chlorogenic acids and lipids, all with a number of benefits or harms for health (FARAH, 2012).

As for lipids, several researchers agree that these are key ingredients of the aroma of coffee, besides conferring desirable characteristics to the drink like creaminess, mouthfeel, wettability, texture and aiding in the formation and maintenance of foam (CHENG et al., 2016; FOLSTAR, 1985; LITMAN; NUMRYCH, 1978). Present in significant quantities in green coffee beans, 7 to 17% (*w/w*), most lipids in coffee are contained within cells and also cover the grain as a thin external layer of wax of up to 1-2%. Lipid fraction is mainly composed of triglycerides (75-85%) followed by diterpene esters (≤ 20%) and sterols (3.2%), both of which are also present in free form as alcohols (0.4%), plus tocopherols (0.05%), phosphatides (0.3%) and tryptamine derivatives (≤ 1%) (SPEER; KÖLLING-SPEER, 2006).

During the 80's and 90's, a correlation was found between the consumption of boiled coffee and increased serum cholesterol (ARO et al., 1987; ZOCK et al., 1990). The pioneers of this study, Aro et al. (1987), showed that volunteers who consumed a high amount of boiled coffee every day during four weeks revealed an increase in serum cholesterol of 8%, but when the coffee was substituted with filtered coffee or tea, the values declined to the baseline. Several other studies have helped to clarify this relation, noting, for example, that despite the large portion of triacylglycerols in coffee, the amount ingested when drinking coffee represents only a very small portion of the total amount consumed daily, thus being irrelevant to increase cholesterol levels.

Subsequently, a study of Urgert et al. (1997)

FIGURE 5 - Event sequence (1-4) for espresso coffee preparation.

FIGURE 6 - Espresso capsule coffee maker, contains a compartment for water and another for the capsule. The machine is turned on and the espresso is immediately obtained.

found that cholesterol raising depends mainly of cafestol content, where 10 mg of pure cafestol was supplemented directly to volunteers with increases serum cholesterol levels by 8-10%, reached 17% to 63 mg daily administration. On the other hand, the consumption of 111 mg of cafestol and kahweol (C&K) mixture (55:45) per day only increased cholesterol by 2% (RICKETTS, 2007; URGERT et al., 1997).

Attempting to understand the relationship between the availability of diterpenes in roasted coffee beans and their presence in the drink, studies such as those of Masten and Tice (1999) showed that the diterpenes are relatively stable at roasting temperatures (185 to 250 °C) and during the boiling process of the beverage preparation (2 to 10 min), where they are present in oil droplets emulsified in the brew.

The coffee diterpenes – cafestol and kahweol

Known since the 1930s, the two main diterpenes of coffee, C&K (Figure 8), are pentacyclic diterpene alcohols of *ent*-kaurane skeleton biogenetically modified to produce a furan ring on carbon 3 (BENGIS; ANDERSON, 1932; ZHU; LUO; HONG, 2014). They are present in the most important coffee species, *C. arabica* and *C. robusta*, being more abundant in Arabic beans (FARAH, 2012).

C&K differ by an unsaturation between

FIGURE 7 - Steps (1-6) for preparation of moka coffee beverage.

carbons 1 and 2 present in kahweol and absent in cafestol (Figure 8). But Robusta coffee contains a third and fourth specific diterpenes - 16-*O*-methylcafestol and 16-*O*-methylkahweol - and in this way these coffee species can be distinguished by their diterpene composition (URGERT et al., 1995; KÖLLING-SPEER; STROHSCHNEIDER; SPEER, 1999; PACETTI et al., 2012; PACETTI; LUCCI; FREGA, 2015). During the roasting of the coffee beans, degradation products of these diterpenes are produced and found in this new matrix, such as those from dehydration (several position isomers of dehydrocafestol and dehydrokahweol; new unsaturation on carbons 11,12, 13,16 and 15,16) and oxidation reactions (cafestal and kahweal) (GUERRERO; SUÁREZ; MORENO, 2005; SPEER; KÖLLING-SPEER, 2006; PACETTI; LUCCI; FREGA, 2015). The roast may also catalyze etherification reactions of these diterpenes, with 16-*O*-methylcafestol being observed in roasted Arabica beans (GUNNING et al., 2018), as well as 16-*O*-isobutyl derivatives for C&K (GUERRERO; SUÁREZ; MORENO, 2005). Isokahweol – a position isomer of kahweol with the unsaturation between carbons 5 and 6 – was also found in roasted coffee beans, such as its 15,16-dehydration and oxidation products (SPEER; KÖLLING-SPEER, 2006; PACETTI; LUCCI; FREGA, 2015). Although, these new compounds were identified in roasted coffee beans, they were never observed in the coffee brews, at least until now. The roasting conditions – temperature and time of exposure to heat – are the main factors for their production, which must to be carefully investigated for the formation kinetics for each one of these compounds.

On coffee beans, around 99.6% of these

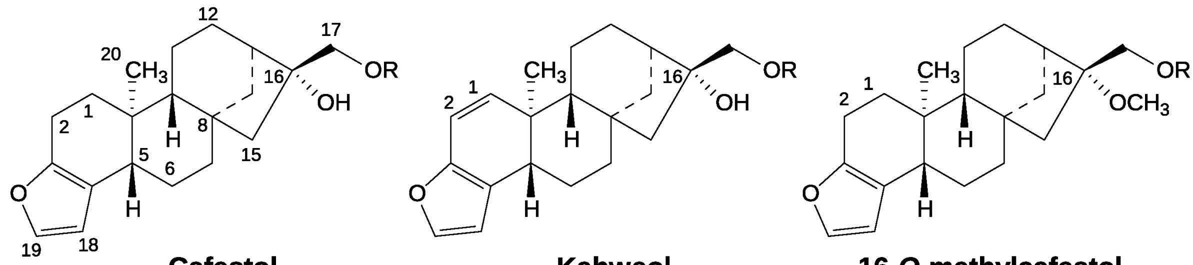

FIGURE 8 - Most common coffee *ent*-kauranes diterpenes (free form: R = H; esterified form: R = fatty acyl chain of C_{14}, C_{16}, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, C_{20}, C_{22} e C_{24}).

compounds are present in esterified form, on the hydroxyl group of carbon 17, by different fatty acids (Fig. 8) which corresponds to 20% *w/w* of the composition of coffee oil and 2.5% of the bean (KURZROCK; SPEER, 2001; SPEER; KÖLLING-SPEER, 2006; NOVAES, 2018). Due to the structural similarity of C&K esters and the absence of effective techniques to distinguish them, they are generally hydrolyzed to be concentrated on the free form, in order to simplify the C&K determination in coffee beans and brews (NOVAES, 2018).

In addition to the hypercholesterolemic activity of C&K, other effects caused by these diterpenes have been observed, like high levels of reversible transaminases, an indicator of liver damage, which can raise the level of lipids in the blood (URGERT et al., 1995); inhibition of the development of oral carcinomas, colon adenocarcinoma and breast tumors (BUTT; SULTAN, 2011; CAVIN et al., 1998; LAM; SPARNINS; WATTENBERG, 1982; MILLER et al., 1988), and further effects, such as antioxidant activity (LEE; JEONG, 2007; WOUW et al., 1994), anti-inflammatory (BERTHOLET, 1988; CARDENAS; QUESADA; MEDINA, 2011 and 2015), anti-angiogenic (CARDENAS; QUESADA; MEDINA, 2011; WANG et al., 2012), antinociceptive (GUZZO et al., 2015), and antiosteoclastogenesis (FUMMIOTO et al., 2012; SAKAI; TSUKUBA, 2015).

The diterpene content of different coffee beverages

Due to the diversity in the different coffee preparations described, the amount of C&K ingested and the frequency of consumption may allow predictions to be made by researchers and consumers. In this sense, Ratnayake et al. (1993) started this discussion when they decided to determine the lipid content and its composition in several coffee brews (Turkish, boiled, filtered and espresso) prepared from Arabica and Robusta coffee roasted beans, as well as instant coffees of different brands. Their work was based on a thin layer chromatography (TLC) analysis, which confirmed the hypothesis that the lipid content varies depending on the beverage preparation method. The espresso coffee showed the highest lipid levels and the lowest were found in the drip-filtered coffee. The researchers analyzed lipid retention in the drip-filtered beverage (0.4%), but also in the filter paper and spent coffee grounds which retained 9.4% and 90.2%, respectively. Both had the same lipid profile, showing that there is no preferential retention of these components. Diterpene esters and triglycerides were the major lipid classes in those beverages ranging from 86.6% to 92.9% and from 6.5% to 12.5% of total lipids, respectively (Table 1).

Similarly, Urgert et al. (1995) examined the level of total diterpenes in coffee beverages derived from more than 50 coffee shops and bars in Europe (Table 1). The authors were the only ones to find 16-*O*-methylcafestol in the analyzed beverages, at levels between 0.0-9.3 mg L^{-1}. Furthermore, the authors estimated the potential impact of diterpene intake on serum cholesterol levels. Variables about the beverages were studied, such as the preparation time, amount of coffee powder and the influence of the roasting process. While the time of preparation proved to have little effect, the increase in the amount of coffee powder in the beverage raised the levels of the diterpenes in boiled coffee, French press and espresso, but not in Turkish/Greek coffee, the latter being limited by the size of the coffee pot.

TABLE 1 - Diterpene content in different types of coffee beverages according to published data.

References	Brewing method	Cafestol ($mg\ L^{-1}$)	Kahweol ($mg\ L^{-1}$)	Total diterpenes ($mg\ L^{-1}$)
Ratnayake et al. (1993)*	Boiled	-	-	68.3
	Espresso	-	-	161.0
	Drip-filtered	-	-	0.7
	Instant coffee	-	-	0.2 – 1.3
	Turkish/Greek	-	-	55.0
Urgert et al. (1995)*	Boiled (n = 14)	5.3 - 80.7	7.3 - 97.4	12.6 - 178.8
	Espresso (n = 10)	1.3 - 19.3	1.3 - 26.0	2.6 - 47.3
	French press (n = 5)	15.3 - 36.7	17.3 - 53.3	32.6 - 91.4
	Turkish/Greek (n = 11)	3.3 - 66.7	0.7 – 71.4	4.0 - 147.4
Gross, Jaccaud and Huggett (1997)	Boiled	48.3 ± 3.8	48.0 ± 2.5	96.3 ± 3.8
	Drip-filtered	0.12 ± 0.02	0.14 ± 0.03	0.26 ± 0.03
	Espresso	17.3 ± 1.5	17.1 ± 1.4	34.4 ± 1.5
	Espresso capsules	1.2 – 3.4	1.7 – 3.5	2.9 – 6.9
	Instant coffee	1.9 ± 0.05	1.9 ± 0.01	3.8 ± 0.05
	Moka	37.5 ± 1.3	38.5 ± 0.9	76.0 ±1.3
	Turkish/Greek	88.7 ± 4.0	89.9 ± 4.1	178.6 ± 4.1
Del Castillo, Herraiz and Blach (1999)	Boiled	615 ± 5	666 ± 7	1,281 ± 7
	Drip-filtered	11 ± 6	25 ± 9	36 ± 9
	French press	279 ± 3	266 ± 11	545 ± 11
Sridevi, Giridhar and Ravishankar (2011)	Drip-filtered	8.9 ± 6.3	2.5 ± 2.0	11.4 ± 6.3
	Espresso	100.0 ± 13.3	85.0 ± 7.5	185.0 ± 13.3
	French press	131.3 ± 10.7	114.7 ± 2.7	246.0 ± 10.7
	Moka	114.3 ± 7.5	76.7 ± 14.5	191.0 ± 14.5
	Turkish/Greek	121.7 ± 12.0	138.3 ± 5.0	260.0 ± 12.0
Silva et al. (2012)	Drip-filtered	0.3	0.0	0.3
	Espresso	4.0 - 16.0	1.2 – 8,0	5.2 – 24,0
	French press	4.0	0.1	4.1
	Instant coffee	0,3 – 1.3	0.1 – 0.4	0.4 – 1.7
	Vending machine	8.0	2.0	10.0
Zhang, Linforth and Fisk (2012)	Boiled (light roast)[a]	48.2 ± 3.5	-	-
	Boiled (dark roast)[b]	25.9 ± 3.5	-	-
	French press (light roast)[a]	53.3 ± 0.7	-	-
	French press (dark roast)[b]	29.0 ± 0.5	-	-
	Moka (light roast)[a]	32.7 ± 0.7	-	-
	Moka (dark roast)[b]	19.2 ± 0.4	-	-
	Turkish/Greek (light roast)[a]	41.3 ± 0.1	-	-
	Turkish/Greek (dark roast)[b]	22.8 ± 0.1	-	-
Erny, Moeenfard and Alves (2015)	Boiled	222.4	727.7	950.1

Moeenfard et al. (2015a)	Espresso (6.5 – 9.5 g)	12.7 – 17.3	19.2 – 25.2	31.9 – 42.5
	Espresso (30 – 60 mL)	11.9 – 21.7	17.4 – 34.1	29.3 – 55.8
	Espresso (100 – 500 µm)	15.5 – 24.0	22.5 – 34.9	38.0 – 58.9
	Espresso (10 – 30 s)	15.7 – 20.1	23.5 – 30.7	39.2 – 50.7
	Espresso (70 – 90 °C)	12.3 – 16.1	18.5 – 24.3	30.8 – 40.4
	Espresso (7 – 14 bar)	13.9 – 18.5	20.2 – 25.8	34.1 – 44.3
Moeenfard et al. (2015b)	Drip-filtered	0.5 ± 0.1	0.2 ± 0.1	0.7 ± 0.1
	Espresso	26.5 ± 0.2	32.8 ± 0.3	59.3 ± 0.3
	Espresso capsule	37.2 ± 1.9	55.1 ± 1.9	92.3 ± 1.9
	Instant coffee	2.0 ± 0.1	2.5 ± 0.3	4.5 ± 0.3
	Vending machine	14.5 ± 0.1	15.7 ± 0.1	30.2 ± 0.1
Moeenfard, Erny and Alves (2016)**	Boiled	128.8	563.6	692.1
	Drip-filtered	2.5	1.0	3.5
	Espresso	29.9	70.6	100.5
	Espresso capsules	5.8 – 22.9	26.4 – 223.6	32.2 – 246.5
	French press	43.6	171.1	214.7
	Instant coffee	0.9	1.2	2.1
	Instant decaffeinated	1.0	1.1	2.1
	Instant espresso	0.7	1.1	1.8
	Moka	19.9	68.3	88.2
	Pod espresso	7.3	22.3	29.6
	Vending machine	24.3	64.7	89.0
Wuerges et al. (2016)	Espresso capsules	3.2 – 26.2	4.0 – 29.6	7.2 – 55.8
Rendón, Scholz and Bragagnolo (2017)	Drip-filtered	2.3 ± 0.3	1.0 ± 0.2	3.3 ± 0.3
	French press	30 – 62	49 – 53	79 – 115
Rendón, Scholz and Bragagnolo (2018)	Drip-filtered	1.6 – 2.5	0.7 – 1.4	2.3 – 3.9
	French press	43.9	33.7	77.8

a – light roast (190 °C for 10 min); b – dark roast (190 °C for 50 min); * 16-*O*-methylcafestol content added on Total diterpenes column (0 – 0.67 mg L-1 in boiled, 0 – 9.3 mg L-1 in Turkish/Greek, 0 – 1.3 mg L-1 in French press, and 0 – 2.0 mg L-1 in espresso); ** Original results expressed.

The roasting degree showed no influence in contrast to previous works (NACKUNSTZ; MAIER, 1987). The analyses were performed using a gas chromatograph (GC) with splitless injection after saponification and silylation of the diterpenes. The highest values were found for boiled and Turkish/Greek coffee, similar to those found by Ratnayake et al. (1993). Diterpenes content in filtered, instant and percolated coffees were insignificant.

Gross, Jaccaud and Huggett (1997) disagreed with the method of analysis of total diterpenes used by Urgert et al. (1995), suggesting that it was not sufficiently sensitive and specific because of the heat-sensitive working conditions of GC and also due to the lack of accuracy in the obtained contents. Subsequently, a method was developed based on reversed-phase high-performance liquid chromatography (HPLC), which resulted in similar diterpene contents than those found by Urgert et al. (1995), which confirms the use of both chromatography techniques. They studied turkish/greek, boiled, moka, espresso, espresso capsules, drip-filtered and instant coffees. Once again, it was confirmed that the preparation method of the beverage is a determinant of diterpene levels (Table 1).

Thereafter, Del Castillo, Herraiz and Blach (1999) tried to improve coffee diterpenes quantification, since it involves laborious sample preparation steps such as saponification, fractionation, extraction of lipids and the formation of silyl derivatives for GC analysis. The authors performed a pre-separation of the sample by HPLC to collect a coffee diterpene fraction, and a subsequent analysis by GC without derivatization, which led to diterpene decomposition in the inlet system (PTV in the split mode), even under optimized temperature conditions. The data are summarized in Table 1, with diterpene quantification in three different coffee beverages, showing a higher diterpene content in boiled coffee, followed by French press and drip-filtered brew (1,281 mg L^{-1}, 545 mg L^{-1} and 36 mg L^{-1}, respectively).

Sridevi, Giridhar and Ravishankar (2011) studied the importance of the coffee roasting conditions on the C&K profiles, before the beverage preparation methods. Thus, coffee beans were roasted at different temperatures and C&K analyzed by HPLC-UV. Higher roasting temperatures had a negative influence on the diterpenes bean profile, reducing the level up to 70% when compared with the light roast level. As expected, there were substantial differences in the profile of the diterpenes among the beverages analyzed with minimum and maximum in unfiltered and paper-filtered methods (Table 1).

Silva et al. (2012) found out there was a lack of a complete validation of the few published analytical methods for the quantification of C&K in coffee drinks. The authors determined the linearity, precision, recovery and limits of detection and quantification as performance parameters in HPLC. Thus, coffee samples were analyzed and the results of C&K levels were obtained and compared to the values reported for the same coffee types. As analytical techniques the authors also used HPLC, but with diode array detection (DAD), justifying their choice by the known thermolability of these diterpenes. The beverage that presented the highest levels of C&K was the espresso coffee, while the lowest values were found in filtered and instant coffee, as seen in Table 1.

Zhang, Linforth and Fisk (2012), in turn, noted that the methods previously described in the literature reported different values for cafestol concentration according to the preparation method. This is due to the extraction parameters, irregular grinding, coffee and water ratio, temperature and preparation technology of the beverages (such as coffee pads). Therefore, the authors fixed these variables to study the yield of cafestol extraction, expressed as its percentage (%) in *C. arabica* roasted and ground beans, under different roasting intensities, and in four types of coffee beverages: boiled, Turkish/Greek, French press and moka. The French press with lightly roasted beans had the highest amount of cafestol, while dark roasted moka showed the lowest value (Table 1).

Recently, Portuguese researchers from University of Porto (UP) approached the subject in four studies after noticing some gaps in the literature. The first one, by Moeenfard et al. (2015a), studied the impact of espresso preparation parameters on diterpene profiles, using HPLC-DAD. The parameters evaluated were: water and coffee powder amount, particle size, percolation time, water temperature and pressure. Although, the authors did not discuss the synergism among the variables to reduce the diterpene content, it appeared to be possible to reduce their content in up to 50%, as presented in Table 1.

Another study from the UP by Erny, Moeenfard and Alves (2015) showed that most studies on the subject were performed after saponification of kahweol and cafestol esters. But to better understand the composition of the diterpene esters in boiled Arabica coffee drinks, the authors performed the quantification by HPLC-DAD combined with spectral deconvolution, since the kahweol and cafestol esters eluted together. It has been found, following the optimization process, that the kahweol esters can be detected selectively adjusting the wavelength of the detector to 290 nm, allowing their quantification to be made separately from the cafestol esters with maximums at 225 nm (values summarized in Table 1).

On the third study from the UP, Moeenfard et al. (2015b) extended the universe of the previous studies, but in contrast to the preceding work they returned to the saponification process and optimized it. In this manner a simpler approach was used, also based on HPLC-DAD to quantify diterpenes and their palmitate ester contents in some coffee drinks: espresso, espresso capsule, filtered, instant and vending machine coffee. The best conditions to maximize the yield of total extract were achieved by direct saponification of 2.5 mL of coffee beverage, together with 3 g of KOH (21.4 mol L^{-1}) in a water bath at 80 °C. The highest content of C&K and their palmitate esters were found in the espresso capsule, which was the only coffee beverage analyzed made with 100% Arabica coffee beans, which has higher diterpene content than Robusta beans, present in the other samples.

In 2016, Moenfaard, Erny and Alves (2016) expanded the previous work with the analysis of the major C&K esters (linoleate, oleate, palmitate and stearate) in twelve classical and commercial coffee brews. Thus, they studied the influence of the brewing procedure over the content of diterpene esters, using the same sample preparation and analytical conditions described by Erny, Moeenfard and Alves (2015). Total free C&K content were stoichiometrically converted from their respective esters. Once again, boiled coffee had the highest diterpene content, while filtered and instant brews showed the lowest concentrations. Same profile distribution among diterpene esters was found in all coffee beverages analyzed, similar conclusion found by Ratnayake et al. (1993) for total lipid profile among the brews.

In 2016, Wuerges et al. (2016) analyzed the C&K content in commercial espresso capsule brews. Four types of commercial capsules were evaluated, differing by amount and coffee species composition (100% Arabica or blend of Arabica and Robusta), roast and ground levels. The chromatographic conditions were adapted for ultra-performance liquid chromatography (UPLC) and the compounds were identified by comparing retention times associated to UV spectra. Kahweol and cafestol contents are summarized in Table 1.

The last studies were published in 2017 and 2018 by Rendón, Scholz and Bragagnolo. In the first, the authors replied the question about the relative amount of C&K retained on the paper filter (11.1-13.6%) and on the drip-filtered coffee brew (0.09-0.15%). The higher diterpene content found is on spent coffee (86.3-88.8%), solid particles of the ground and roasted coffee beans kept on the paper filter (RENDÓN; SCHOLZ; BRAGAGNOLO, 2017), and not on the paper filter as stated in some works (ARO et al., 1987; YILMAZ; ACAR-TEK; S€OZLÜ, 2017). This result is in agreement with Ratnayake et al. (1993) who found similar distribution profile of diterpenes on filter, brew and spent coffee grounds. The focus of the second paper was on the physical characteristics of the paper filters, where the high porosity was a determinant factor to increase the diterpene passage into the brew. Particle size of the roasted and grounded coffee bean was also evaluated and directly correlated to diterpene and total solid permeation through the filters (RENDÓN; SCHOLZ; BRAGAGNOLO, 2018). To support their results, the authors also quantified C&K in French press coffee brew, which has similar mechanism for diterpene extraction without influence of paper filter.

DISCUSSION

Table 1 summarizes all scientific results present in the literature about C&K content in coffee beverages. The peculiar extraction mechanisms of different coffee makers can extract diterpenes over a broad range of concentrations (0.26 - 1281 mg L^{-1}). From the same beans, filtered (3.5 mg L^{-1}) and boiled coffee (692.1 mg L^{-1}) have a ratio between the brews of almost 1 to 200 (MOEENFARD; ERNY; ALVES, 2016). Although all the authors agreed that boiled coffee drinks have the highest concentrations of diterpenes, while filtered ones have the lowest, there is an intersection in these values when their ranges are compared (filtered coffee, 0.26 - 36 mg L-1), where the lower values from the boiled brew (12.5 mg L-1) seem to cross the higher values from the filtered coffee brew (Table 1) (DEL CASTILLO; HERRAIZ; BLACH, 1999; URGERT et al., 1995). This variation can result from the lack of uniformity among the sample preparations and origins, as well as on variations on the analyses employed by different authors, besides aspects of the infusion, preparation time and pressure, the latter in the case of espresso. In addition, the chemical compounds in coffee vary according to geographic region and edaphoclimatic conditions (GARRETT et al., 2013; MASTEN; TICE, 1999; TISCORNIA et al., 1979). This was confirmed for C&K content and other bioactive compounds, determined in several cultivars of Coffea at different latitudes, altitudes and average annual temperatures, and also observed for genetic variations of these cultivars (KITZBERGER et al., 2013; FINOTELLO et al., 2017). Urgert et al. (1995) verified these variations, since their samples were commercially purchased in different coffee shops in Egypt, Finland, Greece, Italy, Netherlands, Norway and Spain. Their results achieved a disparity in kahweol content in the same Turkish/Greek beverages by up to 100 times (0.7 – 71.3 mg L^{-1}) (Table 1).

The lowest diterpene concentrations were detected in filtered and instant brews (Table 1). During the preparation of any hot coffee beverage, oil droplets of free floating lipids are extracted from the powder by the hot water (91-96 °C), forming a second lipid layer soluble in the medium (MESTDAGH; GLABASNIA; GIULIANO, 2017; RATNAYAKE et al., 1993). However, diterpenes are very little soluble in the hot water and its highest content are confined in the interstices of spent coffee beans suspended in the beverage, which are further retained on the paper filter (RENDÓN; SCHOLZ; BRAGAGNOLO, 2017 and 2018). In instant coffees, a loss also occurs during industrial aqueous extraction followed by filtration (GROSS; JACCAUD; HUGGETT, 1997; RATNAYAKE et al., 1993). Moreover, the vast majority of soluble coffees are produced from Robusta beans, which have a lower level of diterpene content than Arabica beans (FARAH, 2012; SPEER; KÖLLING-SPEER, 2006; WUERGES et al., 2016).

While in unfiltered brews of boiled and Turkish/Greek coffee the highest diterpene levels were detected (Table 1). Both uses boiling water (100 °C at standard pressure), where the solubility of diterpenes is higher than the lower temperatures applied to other types of coffee beverages (91-96 °C). In additional, at boiling temperature the water molecules have more kinetic energy and lower viscosity, which means the boiled water can penetrate easier into coffee particles, including intercellular spaces, to solubilize compounds otherwise inaccessible(MESTDAGH; GLABASNIA; GIULIANO, 2017). In Turkish coffee higher amounts of fine particles are present in the brew, resulting in a larger amount of solid floating material, which provides for the main diterpene content (RATNAYAKE et al., 1993; YILMAZ; ACAR-TEK; S€OZLÜ, 2017). On the other hand, the boiled coffee undergoes elevated physical and thermal stress imposed on the bean due to the 10 min of boiling for the coffee brew preparation, delivering more oil and its diterpenes into the brew (ZHANG; LINFORTH; FISK, 2012). In general, longer preparation times allow continued solute extraction until their depletion.

For espresso, moka and French press coffee, the soluble lipids and solid material easily pass through the metal filter such as the diterpenes retained (RATNAYAKE et al., 1993). For espresso coffee, the diterpene levels are dependent on steam pressure (8 – 12 bar) and coffee-to-water ratio (GROSS; JACCAUD; HUGGETT, 1997; MOEENFARD et al., 2015a; RATNAYAKE et al., 1993; SILVA et al., 2012). The pressure forces the oil inside the cells to migrate to the surface of coffee particles, where they are carried out by the hot water (MESTDAGH; GLABASNIA; GIULIANO, 2017). The last one shows a great influence on the diterpene content, however it is made according to the taste of the consumer. Thus, the results should be also expressed by variation of the amount of coffee powder in mg of diterpene recovered per g of beans and not only in mg L^{-1} of beverage.

Espresso capsule, vending and pod espresso are examples of homemade and commercial espresso single-dose coffee machines, which became a growing trend in the world coffee market (WUERGES et al., 2016). These new machines also extract coffee diterpenes in discrepant values, ranging from 2.9 to 246.5 mg L^{-1}. This reinforces once again that further studies are still necessary for a better understanding of the subject, since there are several variables between the capsules, like weight, roast and ground levels and coffee type.

The roasting process tends to decrease C&K contents in coffee beans (NACKUNSTZ; MAIER, 1987). While Urgert et al. (1995) found no influence in the diterpenes profile, Sridevi, Giridhar and Ravishankar (2011), and Zhang, Linforth and Fisk (2012) showed an inverse response of diterpene concentrations to increased roasting levels, with losses of up to 70% from light to dark roast (Table 1), which has been confirmed by several other authors that worked with diterpene content in green and roasted coffee beans (DIAS et al., 2014; KÖLLING-SPEER et al., 1997; KÖLLING-SPEER, STROHSCHNEIDER, SPEER, 1999; SPEER; KÖLLING-SPEER, 2006; SPEER; KURZROCK; HRUSCHKA, 1999; PACETTI et al., 2012).

The grinding is also another important factor in diterpene content, since thin powders allow the release of internal material from the suspended particles into the beverage (MOEENFARD et al., 2015a; RENDÓN; SCHOLZ; BRAGAGNOLO, 2018). On the other hand, thin powders may fill the pores of the filter or coffee maker and thus offer greater permeation resistance.

CONCLUSIONS

This compilation presents the results from the literature about C&K content in different coffee beverages. As shown, the brewing preparation mode is one of the variables, being the last one before the coffee consumption and, perhaps the most important, that may change the diterpene concentration in coffee brews (Turkish/Greek, boiled, French press, drip-filtered, espresso, espresso capsule, and moka). It is important to consider that these types of beverages are strongly present in their home countries. Differences in roasting level, type of coffee machine and amount of each ingredient may cause variations in the final beverage and influence the diterpene content. Future research should be developed considering the thermodynamic properties of the solutes and the mass transport restrictions from the solid phase to the liquid phase.

ACKNOWLEDGEMENT

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES), Conselho Nacional Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Rio de Janeiro (FAPERJ), Empresa Brasileira de Pesquisa Agropecuária em Café (Embrapa Café) and Programa de Pós-Graduação em Ciências de Alimentos (PPGCAL/UFRJ). F.J.M. Novaes thanks CAPES for providing post-doctoral fellowship PNPD/CAPES 2019 at Chemistry Institute of Federal University of Rio de Janeiro (Process 88887.320176/2019-00).

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