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Evaluation of Roasting and Brewing effect on Antinutritional Diterpenes-Cafestol and Kahweol in Coffee

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Abstract

Coffee brew prepared from roasted coffee beans contains quite a lot of compounds which are known to influence consumers health. Among these, cafestol and kahweol are associated with lipid fraction of coffee and reported to be responsible for elevated serum cholesterol levels in people who drink more coffee. Aim : A study has been taken up to find out the influence of roasting and brewing methods on antinutritional diterpenes, in coffee brew. Methodology : Coffee bean samples were roasted at different temperatures and the brew prepared from these beans was analyzed for cafestol and kahweol profiles by using HPLC. Similarly coffee brew was prepared by mocha, filter, espresso, french press etc., and their diterpene profiles were analyzed by HPLC. Results: There was a substantial difference in cafestol and kahweol profiles in brews with highest content of cafestol and kahweol in Turkish-style and French press coffee. Similarly higher roasting temperatures and prolonged roasting times had significant influence on diterpenes profiles in roasted beans.

Index terms— Arabica, Brew, Cafestol, Coffee ground, Kahweol, Robusta

1 INTRODUCTION

offee is a non-alcoholic refreshing beverage that mainly keeps us awake and is reported to be good when used in moderate levels. Throughout the world it is consumed by up to 80% of the adult population. Earlier surveys reported consumers preference for espresso [1] over other types of coffee. The coffee bean mainly contains two major metabolites i.e. alkaloid caffeine and phenolic chlorogenic acids. It is a source of dietary minerals (such as magnesium) along with antioxidant polyphenols and in some countries coffee is the source of two-thirds of the population's antioxidant nutrient intake [2,3,4]. Drinking coffee has to be looked more from health point of view than as a just refreshing drink in view of researchers consensus on coffee drinking -longer term effect is that can raise LDL and total cholesterol [5]. The reason is likely to be the presence of two cholesterol-elevating diterpenes called cafestol and kahweol in coffee [6,7,8]. In view of their cafestol and kahweol are considered as anti-nutritional factors which are unique to coffee. Cafestol concentrations range between 0.15 -0.37% d.m. of beans in robusta and between 0.27% to 0.67% d.m. of beans in Arabica. Similarly kahweol levels are of 0.11-0.35% d.m. and $< 0.1\%$ d.m. in Arabia and robusta beans respectively [9]. Variation in the content of these two diterpenes in different Coffee species was documented **??**10] along with influence of geographical distribution [9]. Researchers have attempted to find out the changes in cafesol and kahweol content in coffee brew prepared by various methods **??**11, 12, 13, and 14]. In general brewing releases oil droplets (lipid fraction of beans) containing diterpenes from ground coffee beans which are either retained by paper filter paper or directly passes to the brew depending on method of brew preparation. The chemical composition of the roasted beans is very essential as it is one of the major factor related to quality of coffee, which, in turn, is affected by the chemical composition of the green beans and by post-harvesting processing conditions. The influence of such an important step in Coffee processing i.e. roasting on caffeine profiles, coffee flavor and aroma along with biogenic amines have been well documented **??**15, **??**6], But similar studies pertaining to roasting influence on diterpenes are not available.

In view of the above a holistic approach on coffee diterpenes-cafestol and kahweol presence in coffee beans is warranted mainly under the influence of various roasting methods and brewing methods.

2 II.

3 AIM

A study has been taken up to find out the influence of roasting and brewing on cafestol and kahweol profiles in coffee beans. Various methods of coffee brew preparation that pertaining to India and to other countries viz., Espresso, drip, Mocha, Indian Filter method, Turkishstyle, French press, Drip filter method, have been selected. As per International Agency for Research on Cancer (IARC) [17] guide lines, the prescribed quantity of ground coffee was taken and accordingly the brew was prepared with known quantity of demineralised water. Later respective brews were cooled to room temperature in an ice bath and stored at 50 C until required for pH determination and analysis for cafestol and kahweol. For this experiment, the coffee ground was brewed in triplicates. The amount of ground coffee taken and the quantity of water used for obtaining known volume of brew varies with brewing method (Table **??**). In brief the methods used for brew preparation are: Espresso method uses 30 pounds of pressure for tapping 9 bars of decoction. The flow rate through the reservoir for every 10 grams of powder taken will be 60 ml per 30 sec. Crema formed for Robusta coffee was observed to be Reddish brown which is its characteristic feature,(The espresso machine Model Astoria C?, A 240 N? 40039s Mod-SAE.I2-4L, HZ 50/60. Italy N 4200). The standard volume according IARC [17] is 30 ml per cup. In percolation method Coffee grounds were made by passing the grounds through a ditting grinder-6.0, with a sieve size of 700 µm and in Mocha method Ditting grinder-2.5, with sieve size of 800 µm were used. The standard volume for mocha method according to IARC is 60 ml per cup. Similarly for filter (used commonly in India) method coffee beans were ground with Ditting grinder -1.0, with 300 µm sieve. The brew was prepared d) Extraction and HPLC analysis of cafestol and kahweol

4 III. MATERIAL AND METHODS

5 ?

Brix value was recorded.

C. arabica and C. canephora seed samples (1

6 ?

Powdered samples of 10g each was transferred into the individual thimbles and extracted with tert-butmethyl ether as a solvent for 6h by using a soxhlet. The solvent was evaporated from extract and residue was dried in oven to get constant weight. Then the residue was unsaponified after extraction [18]. HPLC analysis was performed on Shimadzu LC 20 A (Shimadzu Corp., Kyoto, Japan) equipped with CLASS-VP integrator software for data processing. HPLC Separation was performed on Nucleosil column 120-3 C18, 250/4 (Macherey -Nagel, Gmbh, Germany) with UV absorbance at 230nm for cafestol and 290nm for kahweol. The mobile phase used was Acetonitrile: water: glacial acetic acid (70/29.5/0.5 v/v) with a ~pH 3.1 and flow rate of 0.6 ml/min for 30 min [18]. The standards and their dilutions used for HPLC analysis were same as used for spectrophotometer analysis. The identification of compound was based on peak elution of compound i.e. retention time (RT) comparison and co-elution with authentic standards (Sigma-Aldrich, USA). Five replicates for each brew were analysed. by adding 30g of ground coffee powder to the 150ml boiling water and kept aside for 15min and slowly brew is collected in the vessel. To prepare brew by French press, the fine grounds were made by passing beans through a Ditting grinder-6.0, with 800 µm sieve. 150ml of water for every two table spoons of coffee powder (16g) was used in any event, coffee was measured after it was heated to the boiling. In Electric Drip Filter method, grounds were made through ditting grinder-3.5 with a sieve size of 600 µm. Amount of water and ground coffee must be measured carefully to get. The standard volume 150ml per cup [17]. The Manual filter involves pouring hot water into a filter containing the coffee ground, which then drips into the cup or carafe. Filtered water at about 95°c was used to have fast dripping to get a standard volume of 150ml per cup. For this purpose paper filter of prescribed grade was (No.4, Filter a cafe blanc, Grand Jury, France). To obtain brew by Turkish method coffee was prepared by boiling a mixture of 5g roast and ground coffee, 10 g sugar and 60ml cold water. The grounds were prepared by passing the appropriate beans through Ditting grinder-1 with a sieve size of 250 µm. The brew collected in this method was a standard volume of 30 ml per cup. The pH of respective brewed coffee sample (prepared by different methods) was measured with a pH meter (Control Dynamics, Digital pH meter, APX 17, 175). The total solids content of prepared brew samples was analysed by using refractometer. A known quantity of each brew sample in quadruplicates was used and the December IV.

7 RESULTS AND DISCUSSION

a) Influence of roasting on cafestol and kahweol A perusal of table 1 indicates the significant influence of roasting methods on diterpene profiles in coffee beans. In Arabica coffee the highest concentrations free form of cafestol and kahweol were observed in light roast followed by medium and high roasts (Table 1). In light roast 622±5.29mg

of cafestol and 453±8.62mg of kahweol per 100gms were found. As the roasting temperature increases there was a significant fall in both cafestol and kahweol profiles. Accordingly in full city roast there was ~56% reduction in cafestol and 61% reduction in kahweol compared to normal roasted beans. A similar trend was noticed in robusta beans though the diterpene profiles were less compared to Arabica. The highest percentage of cafestol (363.3±8.0mg per 100g) and kahweol (313±4.93mg per 100mg) respectively were found in light roast. But the difference in cafestol concentrations among medium, high and normal roasting was insignificant. In robusta full city roast the cafestol and kahweol profiles showed ~44% and 10% increase respectively compared to full city roast of Arabica beans (Table 1).

8 b) Influence of brewing method on free form of cafestol and kahweol

The method of coffee brewing had significant influence on total solid content in coffee brew of both Arabica and Robusta coffee (Table **??**). The total solid content given as ?brix in Table **??** for different volumes of brews in various methods of brew preparation. Highest solid content was evident in espresso for both Arabica (5.4±0.4) and Robusta (4.5±0.26), followed by brew prepared by Turkish-s style, mocha as per weight to brew obtained. In all other methods viz., filter paper, drip method the solid content was less. Analysis of cafestol and kahweol profiles in brewed samples (Table **??**) indicates significant variation in their content, with highest levels of cafestol (19.7±1.6mg) in French press coffee followed by Turkish style (7.3±0.72mg) per cup. The kahweol levels are more or less same in both French press and Turkish style coffee which were maximum compared to brew prepared by other methods. In filter paper, Indian filter and drip method based brews, the cafestol and kahweol levels were found to be very less.

The absorption maximum for cafestol and kahweol were 220 and 280 nm respectively in spectrophotometric method and the sensitivity of detection of respective standard samples was good. In the present study we have used this only for screening the samples for the presence of cafestol and kahweol. The levels of free diterpenes cafestol and kahweol were analysed and quantified by HPLC in both Arabica and Robusta coffee beans. The elution of kahweol was good at 290 nm with a retention time of 7.5 min and for cafestol at 230nm, RT was 11.97 min. Diterpene extracts from beans and brew when subjected to HPLC analysis, in all the samples cafestol and kahweol (free forms) were detected at respective retention times in accordance with reference standards. Initially in order to standardize the cafestol and kahweol identification, the resolution of both these compounds in all extracts was evaluated as well as their simultaneous determination. The maximum resolution was evident at 230 nm for cafestol and 290 nm for kahweol, as both these compounds are having maximum structural similarity except the presence of one double bond in the kaurene ring of kahweol.

Both cafestol and kahweol profiles varied in different coffee brews. Similarly reduction in free diterpenes profiles in both Arabica and Robusta ground coffee that prepared by roasting at various temperatures was observed in our study, and the increase in roasting temperatures might be a reason. Because changes in roasting time and temperatures seem to effect CGA and caffeine contents significantly in the final coffee products [19,20] especially at higher roasting temperatures. Such variations may explain the major differences in pH and CGA content found among the commercial coffee tested. According to earlier report [21] there was a significant reduction in CGA levels by ~60% for light, 67% for medium, 88% for dark and 96.5% for very dark roast in Arabica coffee. A similar trend was also noticed in our study wherein, both cafestol and kahweol were found to be reduced by 56% and 61% in Arabica full city roast and 44% and 10% in Robusta full city roast respectively. Though there were any substantial evidences and reports available for this reduction at higher roasting temperatures, the same reasons for a similar observations for caffeine and CGA [21] might also be a reason for cafestol and kahweol in our study. The total solids content (0 brix) showed variation in different brews. In our study, the variation in total solids content of espresso and other methods is evident and also there was a difference in total solids content between Arabica and Robusta coffee. This may be attributed to the coffee ground coarse. A similar observations in total solids content in different brews was reported while analysing caffeine content in Coffee brew [22,23]. Moreover, the extraction of coffee metabolites such as caffeine was dependent upon the time of brewing. The longer brew time implies longer contact time between the water and coffee grounds leading to more complete caffeine extraction of compounds and more solids, though the more solid matter pertains to fine ground coffee compared to coarse ground coffee [24]. The initial moisture content of the green coffee beans prior to roasting was ~12.5% and the pH of the coffee brew prepared from roasted seeds in our study was in the range of 5.5-5.65 which is in concomitant with earlier reports (23). In general the minor change in pH of the brew happens due to change in roasting time. In the present study, for preparing brews we have followed normal method of roasting (240 C, 600sec) due to this there was no significant change in pH of the brew [23]. In general several differences exist in the preparation of coffee which may influence consumption of different metabolites of coffee such as caffeine and CGA [20]. In addition the prepared coffee brew volume, ground coffee to water ratio also would influence the coffee metabolites in brew [24]. A similar effect on diterpenes profiles is the reason for ? variation of cafestol and kahweol profiles in different coffee brews in our study. Apart from this, increase in the amount of coffee ground taken for preparing brew certainly responsible for quantitative variation of cafestol and kahweol in respective brew samples in our study. The method of preparation of the brew is a critical determining factor in determining the daily intake of these diterpenes from coffee consumption [7]. Higher concentrations of cafestol

and kahweol in coffee brews prepared by French press and Turkish method in our study is further supported by a similar studies with reference to caffeine and CGA in Coffee [20,24] wherein, the levels of caffeine are more in boiled coffee than filtered coffee though again the particle size of ground coffee also maters for this. Total diterpene content of brewed coffee was reported earlier, but the methods used were of inadequate sensitivity or specificity for application to measurement of individual diterpenes in brews **??**12,24]. Urgert et al. **??**13] developed a simple and sensitive method of reverse phase HPLC method using solid-phase extraction procedures for cafestol and kahweol analysis. The higher levels of cafestol and kahweol in Turkish and French press coffee in our study was in accordance with earlier report [7] wherein, both the diterpenes per cup were at higher level in boiled Scandinavian coffee (7.2 mg each of cafestol & kahweol) and Turkish coffee (5.3 mg of cafestol & 5.4 mg of kahweol) respectively. This may be due to higher amount of fine particles (solids) present in Turkish-style coffee, compared with boiled coffee and other brews [7]. Similarly insignificant levels of cafestol and kahweol were detected in our study in filter paper method, which is in accordance with earlier observations [25]. Thus the method by which coffee brew is prepared and decanted may have a great influence on its diterpene content **??**13]. The levels of cafestol and kahweol in espresso were more in our study compared to that of gross et al [7], but in concomitant with the study of Ratnayake et al **??**12] The reasons for this not clear, but might be due to the influence of steam pressure, contact of steam with content used for making brew, the roasting temperature used for roasting etc. The preference for specific type of brew varies with individual cultural preferences. Especially the pure coffee brew is a choice of many consumers in the World, but in India majority prefer coffee blended with chicory, hence, the levels of cafestol and kahweol in filter coffee brew would be obviously less. In mocha brew which is favored for its taste, contains more levels of these two diterpenes due to the presence of crema (rich in lipid fraction). So the influence of cafestol and kahweol on consumers health depends on the type of brew and the quantity of brew consumed. coffee and its effect on liver function enzymes. Journal of lipid research 1994, 35: 721-733. ground coffee and its contact timing, efficacy of filter used. Though the values of cafestol and kahweol in our study in different brews were slightly more than that of earlier report [7] the trend was same. This variation may be attributed to coffee ground particle size, water [1]

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Figure 1: Table 1 :

.1 ACKNOWLEDGEMENT

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Values are mean± S.D. of three samples *Coffee cup sizes : 150 ml for filtered, electrical dip, 60 ml for Turkish, Espresso, Mocha coffees Sample weight in g : Turkish (5), Espresso and Indian Filter (10), Mocha (20), French press (16), Filer paper (25), Electrical drip (30). % values means g/ 100 g coffee powder

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