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Nutrition, Metabolism and Cardiovascular Diseases xxx (xxxx) xxx

Contents lists available at ScienceDirect

Nutrition, Metabolism and Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Research Paper

# Cafestol and kahweol concentrations in workplace machine coffee compared with conventional brewing methods

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ARTICLE INFO

Handling Editor: Dr. A. Siani

ABSTRACT

*Background and aims:* Unfiltered coffee contains high concentrations of cholesterol-raising diterpenes. We aimed to measure the levels of diterpenes in machine coffee.
*Methods and results:* Coffee samples were collected from Swedish workplaces and compared with home-made coffee brews. Concentrations of cafestol and kahweol were measured by liquid chromatography-mass spectrometry.
The median (range) cafestol and kahweol concentrations were 176 (24–444) mg/L and 142 (18–434) mg/L for brewing machines (n = 11), 8 (2–343) mg/L and 7 (2–288) mg/L for liquid-model machines (n = 3), and 12 (4–24) mg/L and 8 (3–19) mg/l for home-brewed, paper-filtered coffees (n = 5). Boiled coffee had high concentrations of cafestol and kahweol, 939 mg/L and 678 mg/L, but having it poured through a fabric filter reduced the concentrations to 28 and 21 mg/L. Other coffee brews (percolator, French press) contained intermediate levels of cafestol (~90 mg/L) and kahweol (~70 mg/L), with the exception of some espresso samples with high levels (up to 2447 mg/L cafestol).
*Conclusion:* Most coffees from workplace brewing machines contain higher diterpene concentrations than paper-filtered coffee, but lower than unfiltered coffee. Intake of insufficiently filtered coffee during working hours could be an overlooked factor for cardiovascular health due to its effect on plasma cholesterol concentrations.

## 1. Introduction

The Nordic countries have high coffee intakes [1] and workplaces usually provide their staff free coffee during working hours as a tax-deductible benefit. Thus, self-serve coffee machines are common in rest areas. Little is known how such coffee consumption impacts long-term health.

Although coffee consumption has an overall association with health rather than harm [2], unfiltered coffee was demonstrated in the 1980s to increase low-density lipoprotein (LDL) cholesterol [3–6], unlike paper-filtered coffee, instant coffee [7], or from coffee pads [8]. In the 90s, the diterpenes cafestol and kahweol were discovered as the culprits [9], as were their retention by common paper filters [10]. One review has estimated LDL cholesterol to increase 0.0104 mmol/L per mg cafestol, and 0.0016 mmol/L per mg kahweol daily ingested [11].

Unfiltered coffee brews have been reported to contain high cafestol in concentrations whereas brews using metal filters have reported intermediate concentrations, e.g., espresso and moka, or plungers, e.g., French press (also called cafetière) [12,13]. Surprisingly few diterpene measurements have been performed and reported in the literature on machine-brewed coffee, and seemingly only on espresso [14–16].

From randomised controlled trials of five years duration, each mmol/L reduction in LDL cholesterol translates to a 22 % relative risk reduction of atherosclerotic cardiovascular disease (ASCVD) [15]. However, the cumulative burden of prolonged LDL cholesterol exposure causes further atherosclerosis formation, and additionally increases disease risk. Thus, the expected relative risk reduction over a full working life (40 years) is substantially greater (54 %) [17].

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https://doi.org/10.1016/j.numecd.2025.103933
Received 6 November 2024; Received in revised form 1 February 2025; Accepted 19 February 2025
Available online 20 February 2025

It has recently been confirmed that habitual consumption of unfiltered coffee is associated with higher total and ASCVD mortality over 20 years in a large Norwegian cohort [18]. Taken together, filtered coffee appears to be the safer choice regarding cardiovascular health. In line with this reasoning, the Nordic Nutrition Recommendations from 2023 recommend filtered in place of unfiltered coffee [19].

Since substantial amounts of machine coffee is consumed at work, because of the potential role of diterpenes in modulating ASCVD risk, and due to the apparent gap in knowledge on the levels of diterpenes in such coffee, we aimed to determine the concentrations of diterpenes in samples from machine coffees from real-life Swedish hospitals and primary care facilities.

## 2. Methods

### 2.1. Categories of coffee machines

After a systematic Scopus search including the search terms "coffee machine*", "coffee pod", and "coffee brew*" and following contacts with coffee machine distributors regarding technical details, three main machine types could be identified; brewing machines, liquid-model machines, and instant machines. Brewing machines produce coffee from whole or ground beans in approximately 10–30 s, as the hot water mixes with the coffee and passes a metal filter. Liquid-models can provide a cup within seconds and do not use a filter, but instead mix a liquid coffee concentrate with hot water. Instant machines mixes use instant, freeze-dried (usually paper-filtered) coffee with hot water.

### 2.2. Coffee sample collection

We analysed real-world coffee samples from 14 machines located in four health care facilities. After oral consent, we included workplace coffee machines from Uppsala University Hospital and Falu County Hospital (six machines each), and two primary health care centres (one machine each), in the two Swedish regions Uppsala and Dalarna. We selected the standard setting and size (if available) for a brewed coffee cup and took two samples from each machine, 2–3 weeks apart on different weekdays. The machines and coffees represented different commercial brands. The samples were collected in Falcon tubes, frozen in −18 °C, stored in Uppsala, and within four weeks collectively transported to Gothenburg for analysis at Chalmers University of Technology.

### 2.3. Coffee preparation

For comparison, we prepared additional common coffee brews; Scandinavian-style drip-brewed coffee, percolator, French press/cafetière, and boiled coffee. Drip-brewed coffee was prepared with a common household coffee brewer and ground coffee from five common brands (all medium to dark roast Arabica coffees with medium (~700 μm) grind size, using 1 tablespoon (tbsp., ≈7 g) coffee and 1 dL water), with two different paper filters from commercially available brands (with and without micro perforations for increased permeability and aroma). Percolator coffee was prepared with 2 tbsp. (≈13 g) coarse-ground (~1000 μm) coffee beans and 3 dL water. French press was prepared by pouring 1.25 dL boiling water over 7 g coarse-ground (~1000 μm) coffee in the plunger pot, stirring and waiting a few minutes before pressing. Boiled coffee was prepared by mixing 3.5 tbsp. (≈21 g) extra course-ground (~1300 μm) coffee beans with 3.5 dL water in a pot, bringing up to a boil and letting it boil under a lid for about 3 min. After letting the powder sink to the bottom of the pot, the coffee was poured into the tube without using any filter. Finally, the same boiled coffee was poured through a two-layer polyester/acrylic sock (an occasional household recommendation as potential substitution for a paper filter). In addition to these home-made brews, four espresso samples were collected at three cafeterias and one laboratory workplace coffee machine in Gothenburg. All samples were stored (0–4 weeks) at −18 °C before analysis.

### 2.4. Laboratory analyses

Each sample (500 μL) and internal standard (Tranilast, Fischer Scientific, OCROS Organics) was hydrolysed in 2.5 M KOH (Scharlau, reagent grade) in ethanol (Solveco, 99.7 %, Spectro) for 1 h at 80 °C with shaking. Samples were kept cold on ice and 3 mL diethyl ether (Fischer Scientific, 99.5 %, analytical reagent grade) was added and the samples vortexed for 2 min to extract hydrolysed cafestol and kahweol. Five mL $H_2O$ (MilliQ, Merck, LC-Pak Polisher) was added to further improve the extraction and the samples were centrifuged at 3000 rpm for 5 min to separate the phases. The organic (top) phase was collected, and the samples were dried under a stream of nitrogen at room temperature. Finally, the samples were resuspended in 800 μL acetonitrile (ACN, Fischer Scientific, OPTIMA, LCMS grade) before analysis.

The samples were analysed by LC-MS/MS (liquid chromatography-tandem mass spectrometry) using a 6500+ QTRAP triple-quadrupole mass spectrometer (AB Sciex, 11432 Stockholm, Sweden) which was equipped with an APCI source and operated in the positive-ion mode. Chromatographic separations were performed on a Waters BEH 1.7u. 15 cm. RP 186002353 UPLC column. LC-MS grade $H_2O$ (100 % solvent A) and ACN (100 % solvent B) were the mobile phases for gradient elution. The LC flow rate was 0.5 mL/min and the column temperature was 45 °C, the autosampler was kept at 12 °C.

LC starting conditions at 5 % B, held for 30 s, 31 sec 60% B, 48 sec 70% B, 49 sec 52% B, 2.5 min 55 % B, 2.7 min 62 % B, 3 min 65 % B, 4 min 82 % B, 4.1 min 85 % B, 4.6 min 100 % B, and at 5.2 min. Followed by a flush (100 % B) and recondition (5 % B), total runtime 6 min. The MRM transitions were optimized for the analytes one by one by direct infusion containing 25 mM of cafestol (PhytoLab standard 82294, Batch 129555963) and kahweol (PhytoLab standard 82293, Batch 134670312),respectively. The Q1/Q3 pairs were used in the MRM scan mode to optimize the collision energies for each analyte, and the two most sensitive pairs per analyte were used for the subsequent analyses. The retention time window for the scheduled MRM was 1 min for each analyte. The two MRM transitions per analyte, the Q1/Q3 pair that showed the higher sensitivity was selected as the MRM transition for quantitation. The other transition acted as a qualifier for the purpose of verification of the identity of the compound. An 8-point calibration curve was used for both cafestol and kahweol were the lowest point was 0.098 μg/mL and the highest 12.5 μg/mL. Normalization was done based on the internal standard that was included in each sample during the extraction process.

### 2.5. Extrapolation to plasma LDL cholesterol and ASCVD risk

We assumed that a healthcare worker could consume three cups of coffee per workday, five days per week. The difference in cafestol and kahweol concentrations in mmol/L between a typical (median diterpene concentration and volume) cup of machine-brewed vs. paper filter coffee was combined with their known effects on LDL cholesterol, as established by Urgert and Katan [9]. For comparisons with known effects on LDL cholesterol by oat milk and cream, established reductions after 3g of daily beta-glucan intake [20] and equations for the effects of saturated fatty acids on serum lipoproteins by Mensink were used [21].

The estimated achieved difference in LDL cholesterol in mmol/L (ΔLDL-C) was then combined with known effects on ASCVD risk, as estimated by Ference et al. [17]. The corresponding five-year ASCVD risk per LDL cholesterol was calculated from formula $[1–0.78^{\Delta LDL-C}]$. The risk reduction over 40 years was estimated from Mendelian randomization studies as $[1–0.46^{\Delta LDL-C}]$ [17].

### 2.6. Statistical analyses and ethical considerations

The mean difference between ordinary and perforated paper filters

were estimated by t-tests, considering $P < 0.05$ as statistically significant, providing sufficient power to detect a difference of approximately $10 \pm 7$ vs 23 mg/L cafestol at $\beta = 0.20$. No ethical approval was necessary, as no human participants were involved.

## 3. Results

Samples were successfully analysed in one batch. The LC-MS method was tested for accuracy and reproducibility using quality control (QC) samples at low, medium and high cafestol and kahweol concentrations. The QC samples were analysed in triplicate, with CVs of 1.1 %, 6.8 %, and 4.4 % for low, medium and high cafestol levels and 3.5 %, 2.5 % and 0.9 % for low, medium and high kahweol levels in one batch containing 116 injections.

### 3.1. Diterpene content of different coffee brews

Out of the 14 workplace coffee machines investigated, 11 were brewing machines, 3 were liquid-model, and none were instant machines. The diterpene concentration in these and control brews are presented in Table 1.

Coffees from brewing machines contained higher diterpene concentrations than paper-filtered coffee, but lower than boiled coffee. Only one brewing machine coffee sample had a cafestol concentration below 100 mg/L. For liquid-model machines, there was one outlier sample occasion with unusually high concentrations (cafestol 344.2 and kahweol 288.2 mg/L). When omitting this outlier, the mean and range was 5.9 mg/L (2.4–11.7) cafestol and 4.8 mg/L (1.8–9.3) kahweol for liquid-model machine coffees (on par with paper-filtered variants).

Having boiled coffee (939.2 mg/L cafestol) poured through a sock of polyester/acrylic fabric considerably reduced its diterpene levels (to 28.0 mg/L cafestol). Other coffee variants had intermediate concentrations (68.7–91.2 mg/L cafestol), with the exception of three espresso samples having levels up to 2446.7 mg/L cafestol. An overview of the cafestol content per *cup* for all investigated coffee brews is presented in Fig. 1.

There were no significant differences ($P = 0.43$, 0.52) between the means of ordinary (9.7 mg/L cafestol, 7.4 mg/L kahweol) compared with perforated/aroma paper filters (13.2 mg/L cafestol, 9.7 mg/L kahweol).

**Table 1**
Concentrations of diterpenes cafestol and kahweol in different coffee brews (mg/L).

| Coffee type | Cafestol median (range) | Kawheol median (range) |
|---|---|---|
| Brewing machines (n = 11) | 175.7 (24.4–444.0) | 141.8 (17.8–434.2) |
| Liquid-model machines (n = 3) | 8.3 (2.4–343.4) | 6.7 (1.8–288.2) |
| All coffee machines (n = 14)[a] | 174.0 (2.4–444.0) | 135.4 (1.8–434.2) |
| Paper-filtered drip-brew (n = 5)[b] | 11.5 (4.2–23.8) | 8.2 (2.8–19.0) |
| Paper-filtered drip-brew, normal filters (n = 5) | 6.1 (4.2–23.8) | 4.5 (2.8–19.0) |
| Paper-filtered drip-brew, perforated (aroma) filters (n = 5) | 15.8 (8.2–17.0) | 11.8 (5.5–12.9) |
| Espresso (n = 4) | 1059.3 (35.6–2446.7) | 620.8 (23.9–1964.9) |
| | **Mean of duplicate analyses** | **Mean of duplicate analyses** |
| French press (cafetière) | 86.8 | 68.7 |
| Percolator | 91.2 | 69.2 |
| Boiled coffee | 939.2 | 677.9 |
| Boiled coffee filtered through fabric | 28.0 | 21.2 |

[a] Data are means of the two sample occasions, with ranges for all (n = 28) samples.
[b] Data are means of both filter types, with ranges for all (n = 10) samples.

For brewing machines, the mean difference between the two sample occasions was 103.3 mg/L for cafestol (58 %) and 88.3 mg/L (65 %) for kahweol. The distribution of cafestol concentrations from both sampling occasions for all coffee machines is presented in Fig. 2, with the only outlier being the previously mentioned liquid model machine.

### 3.2. Estimated effects on plasma LDL cholesterol and ASCVD risk

The average cup volume obtained from the machines was 137.5 ml. Replacing three cups of brewing machine coffee with paper-filtered coffee five days per week was estimated to reduce LDL cholesterol by 0.58 mmol/L. For comparison, this estimated diterpene effect equals to adding 60 mL full fat (40 %) cream per cup of paper-filtered coffee. Conversely, adding 250 ml of oat milk containing 1 g of cholesterol-lowering beta-glucans to each cup would not fully neutralize the effect of the diterpenes. The corresponding reduction in ASCVD relative risk for a 0.58 mmol/L reduction in LDL cholesterol was 13 % over five years; or 36 % over 40 years.

## 4. Discussion

The diterpene concentrations in coffee from brewing machines considerably exceeded paper-filtered coffee, but were lower than in boiled coffee. Concentrations varied considerably between machines and between the 2–3-week sample intervals. Most liquid-model machine coffees contained low diterpene levels, on par with paper-filtered coffee. Most other brewing methods produced intermediate diterpene concentrations. For espresso, there was a considerable and unexplained variation in diterpene concentration between our four samples tested. This needs further study but may be of importance for regular espresso consumers.

One potential explanation for the elevated diterpene levels in brewing machine coffee is the lack of a fine filter, which would allow more diterpenes to pass through, bound to coffee particles. Regarding the high variation between duplicate samples from the same machines, a possible factor could be the impact of cleaning schedules on filter porosity. Paradoxically, cleaning a metal filter could hypothetically result in greater permeability and more diterpenes in the product.

The clear retention of diterpenes by the polyester/acrylic fabric is in line with previous similar experiments [22] and indicates the filtering process as a crucial factor. The diterpene concentrations were higher in our analyses than previously demonstrated, e.g., for boiled coffee [11, 12], potentially overestimating the effects on LDL cholesterol (and thus also on ASCVD risk) from machine coffees, if the present analysis is more sensitive than in the studies from the 90s when the associations between diterpenes and cholesterol levels were established. Our estimated effect on LDL cholesterol by machine coffee intake was greater than has been demonstrated in RCTs (randomized controlled trials) (+0.39 mmol/L after six cups of unfiltered coffee daily) [23]. Machine coffee had lower diterpene levels than boiled coffee in the present study (as expected). Thus, reasonably, the effect on LDL cholesterol from coffee machines should be lower, rather than greater. Thus, our estimated effects on LDL cholesterol and ASCVD risk should be cautiously interpreted. On the other hand, diterpenes also negatively impact other lipoproteins such as triglycerides [11,24], which we have not taken into account. Interestingly, in the present analysis French press coffee had only moderate diterpene concentrations despite its coarse filter and in contrast to earlier studies, in which concentrations resembled those in boiled coffee [11].

The study's major limitations are its small sample size and lack of details regarding the designs of each coffee machine, primarily regarding filter characteristics but also water pressure and temperature, contact time with water, degree of grinding and roasting of the beans. Also, the suggested associations between machine coffee intake and plasma cholesterol levels and increased ASCVD risk remain to be directly established in RCTs and prospective observational studies. The

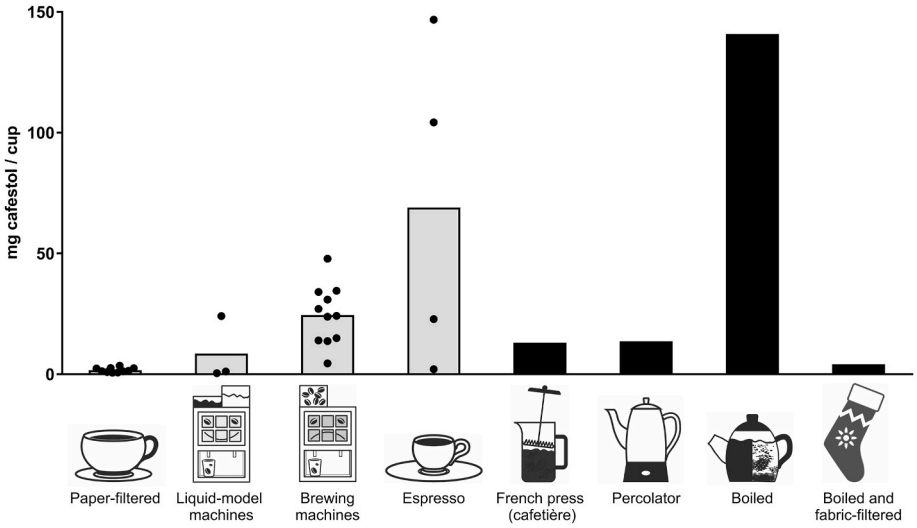


**Fig. 1.** Cafestol content of investigated coffee brews
Bars indicate medians (first four coffee brews) or means of duplicate samples (all others) as mg cafestol per cup for the volumes 60 ml (espresso), 137.5 ml (coffee machines), and 150 ml (all others).

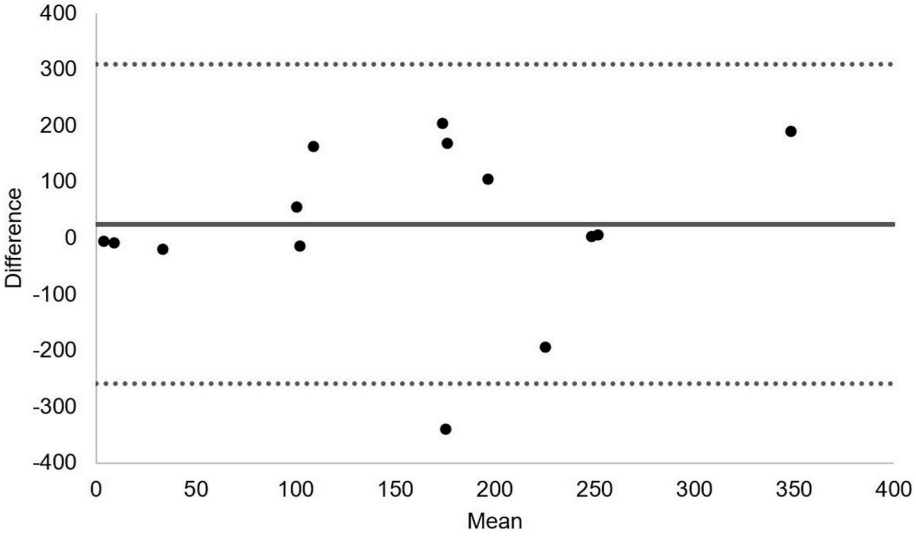


**Fig. 2.** Bland Altman plot for the two sampling occasions for all coffee machines (n = 14, mg/L).

LC-MS method showed high repeatability and accuracy. The usage of internal standard also allows us to account for losses during extraction and for matrix effects in the mass spectrometer ion source. Together this suggest that the variation observed between replicated brews was due to differences inherent to the preparation in the coffee machines rather than in the analysis.

Future studies might further investigate how different machine factors including filters influence diterpene levels. Blinded short-term crossover interventions with coffee from different machine types are warranted in order to confirm their effects on plasma lipids directly. Also, observational studies comparing LDL cholesterol levels of employees at workplaces with coffee machines and those with paper-filtered coffee and long-term prospective studies on cardiovascular outcomes could further confirm the causality of the suggested associations.

## 5. Conclusion

Based on the concentrations of cafestol and kahweol in investigated machine coffees, thoroughly filtered coffee seems like the preferable choice for cardiovascular health. Accordingly, filtered coffee should be preferred, also in workplace settings.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Acknowledgements

We thank (with permission) Matti Marklund and Ulf Holmbäck for valuable contributions, and Katarina Eriksson for creating the illustrations.

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