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Organic and Conventional Coffee (*Coffea arabica* L.): Differences in the Content of Minerals and Studies in Healthy and Induced Cancer Rats

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Abstract

Coffee is one of the most important agricultural products in international trade. The agricultural management system may influence the chemical composition of the beans in addition to altering the bioavailability of nutrients essential for humans. Therefore, the concentrations of Cu, Fe, Zn and the proximate composition of powder and coffee infusions from beans grown under organic or conventional agricultural systems were evaluated. In addition, the effect of these products on hepatic mineral content *in vivo* was investigated, in healthy and induced cancer rats. Our results showed that the levels of Cu, Fe and Zn were higher in conventional coffee powder than in organic powder. However, despite these differences, the organic coffee had higher extraction yield for all infuses, and its infusion at 20% (w/v) had higher level of Zn than conventional infusion. These results were associated with the agricultural system used and the extraction process employed during the preparation of infusions. The conventional coffee provides more adsorbent compounds, decreasing the availability of this mineral in the beverage. In terms of the mineral content *in vivo*, the ingestion of diets prepared with infusions or coffee powder did not influence the hepatic content of Cu, Fe and Zn.

Keywords: *Coffee Arabica* L.; Organic; Conventional; Micro minerals; Hepatic composition

Introduction

Coffee is one of the most important agricultural products in international trade. In 2009-2010, the amount of *Coffea arabica* exported throughout the world was approximately 62 million bags [1]. The area of production of conventionally grown coffee has increased [2], and this growth causes environmental impacts. The excessive application of chemical fertilizers and agricultural defensives (quantity and frequency), which usually exceeds the retention capacity of the soil, causes an imbalance in the ecosystem. To minimize these impacts, there is a consumption incentive for organically managed products, which have high prices compared to conventional products, despite the designation of quality and certification [3].

The International Federation of Organic Agricultural Movements (IFOAM) defines organic agriculture as "*all agricultural systems that promote environmental, socially and economically safe production of food and fibers*"[4]. However, the use of organic matter for the fertilization of coffee can interfere in the equilibrium of metals in the soil because organic acids act as ligands for many metals and the soil composition of these elements is reflected in the food [5,6]. Some studies have demonstrated differences between organic and conventionally produced coffee in terms of nutrients [7,8]. Among the nutrients, Cu, Zn and other toxic elements, such as Cd and Cr contained in some inorganic and organic fertilizers, can be increased in concentration in the soil and consequently be taken up by the plants [5].

Coffee consumption occurs mainly in the form of an infusion, resulting in one of the most appreciated and consumed beverages in the world [1]. The chemical composition of the drink is quite variable and is largely dependent on the species used [9-11]. and the system of management [2,5,7]. The concentrations of Fe, Cu and Zn, which are of acknowledged nutritional importance, in the infusion are the consequence of their levels in roasted beans, their physical-chemical characteristics (sorption) and the preparation conditions (filter paper and concentration).

Coffee is often consumed for its stimulatory effects owing to its phytochemistry, such as caffeine (the most prominent) [12], chlorogenic acid, lignans and some minerals components, which possess therapeutic potential, providing protection against cardiovascular diseases [13], diabetes mellitus [14], Parkinson's disease [15], Alzeheimer's disease [16], carcinogenesis [17] and antioxidants [7], as also observed in some drug treatments [18,19]. These aspects are highlighted since the coffee holds second position in consumption among all beverages after water [20].

However, some compounds in coffee like caffeine interact with some xenobiotics especially in women taking hormones to cure postmenopausal problems, in addition to other such as melanoidins and phenolic polymers, which can interact with the minerals present in the diet, interfering with their bioavailability [21,22].

Cu, Fe and Zn are essential micro minerals, whose absorption is relatively low and bioavailability subject to dietary, physiological and pathological factors, e.g. gastrointestinal tract cancers [23]. Besides, the neoplasms are also related to Zn and Fe deficiencies in animals and humans [24-26]. However, other studies have shown that higher

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levels these micro minerals in diet also can affects the tissues, such as liver [27,28]. Therefore, the aim of this work was to evaluate the concentrations of Cu, Fe, and Zn in coffee (powdered and infusions) from two different management systems (organic and conventional), and to investigate the hepatic mineral content in healthy and induced cancer rats, fed with these coffees.

Materials and Methods

Coffee samples and chemicals

To minimize the influence of environmental factors in the analysis, all samples of coffee were produced in the same geographic region and were harvested during the 2008-2009 season. So, any differences found can be attributed predominantly to the genotypic characteristics of the coffee and to the agricultural management style [7]. The samples of ground roasted coffee were from the 2008-2009 crop, Catuai red variety, and medium-roasted (200 - 215°C) for 15 min. The organic and conventionally grown samples were provided by the Association of Small Producers of Poco Fundo, Minas Gerais, Brazil, and the organic coffee was certified (BCS - OKO Garantie Master Certificates No POCO-7569/07.08/14291-BR). Fertilization of the organic coffee was with castor cake, and the conventional coffee was fertilized with nitrogen, phosphorus and potassium (20:5:20).

All solutions were prepared with analytical grade reagents and deionized water (Milli-Q, Millipore, Bedford, MA, USA). For the preparation of the samples and standards, the following chemicals were used: Zn (1000 mg L^{-1}), H_2O_2 30% (v/v), ferric chloride hexahydrate as the Fe stock solution (1000 mg L^{-1}) and cupric sulfate prepared as the Cu stock solution (1000 mg L^{-1}), which were obtained from Merck (San Diego, CA, USA); nitric acid (65% v/v) (Sigma-Aldrich, Steinheim, Germany). Laboratory glassware was kept overnight in a 10% (v/v) nitric acid solution. This glassware was rinsed with deionized water and freshly dried in a dust-free environment before use.

Proximate composition of coffee samples

A compositional analysis of all the samples (moisture, fat, protein, ash and carbohydrate) was performed in triplicate. Moisture was determined by loss while drying with an Infrared IV200 Moisture Analyzer (Gehaka) for 8 min at 120°C [29]. The total nitrogen content was analyzed by the Kjeldahl procedure (the conversion factor was 6.25 to protein), and carbohydrates were calculated to the remainder (the difference using the fresh weight-derived). The ash content was determined by the incineration of samples at 550°C in a muffle furnace. Fat content was measured in a Soxhlet system by extraction with solvent [30].

Sample preparation and Cu, Fe, Zn determination

The infusions were prepared in amounts equivalent to the average coffee consumption of the population (10% w/v), its half (5% w/v) and its double (20% w/v). Thus, 5, 10 and 20 g of coffee were added to 100 mL of water at 90°C and followed by vacuum filtration using qualitative paper filters (Framex, model 389/3) [7]. The infusions were reduced to1/4 of the total volume at 60°C.

The rats liver and experimental diets were homogenized and dried at 105°C, until reaching constant weight [30]. For the destruction of organic matter, the microwave-assisted acid digestion was used (Millestone® Ethos Plus microwave digestion system, Sorisole, Bergamo, Italy). Before digestion, all the samples were conditioned with HNO_3 and H_2O_2 for 30 min at room temperature. The heating program utilized (sample masses (n=4), the volumes of HNO_3 and H_2O_2) is described in Table 1. The samples digested were transferred to glass volumetric flasks, and the volume was brought to 25 mL with deionized water (Table 1).

The analytes Cu, Fe and Zn were determined using a flame atomic absorption spectrometer (FAAS) Shimadzu® AA-6800 (Chiyoda-ku, Tokyo, Japan) equipped with a deuterium background corrector. The hollow cathode lamps (Perkin-Elmer) for Cu (λ=324.8 nm), Fe (λ=248.3 nm) and Zn (λ=213.9 nm) were used. The operation conditions for FAAS were those described in the apparatus manual. The air-acetylene gas mixture was 2.0 - 1.8 mL min^{-1} for all analytes. For the standard preparations, the salts were dried at 105°C for 12 h and then cooled in a desiccator. For analytical curves, the standard concentrations for Cu and Zn were 0.1 - 2.5 mg L^{-1} and 0.5 - 6.0 mg L^{-1} for Fe. The limits of detection (LOD) and the limits of quantification (LOQ) were calculated according to the procedures of the *International Union Pure and Applied Chemistry* [31], and the accuracy was verified using a recovery test in all samples by adding an amount of standard equal to 1.0 mg L^{-1} [32].

Animal experiments and the isolation of liver samples

One hundred and forty animals were randomly allocated into

Samples	Quantities of samples	HNO_3 (mL)	H_2O_2 (mL)	Digestion program				References
				Steps	Time (min)	Power (W)	Temperature (°C)	
Coffee powder	300 (mg)	7	1	1	5	750	120	45
	(n=4)			2	3	750	120	
				3	10	750	210	
				4	15	750	210	
Coffee infusion*	2 (mL)	5	1	1	5	400	80	46
	(n=4)			2	5	400	120	
				3	5	400	210	
Rat livers and diets	300 (mg)	6	2	1	3	250	130	46
	(n=4)			2	5	630	130	
				3	22	500	130	
				4	15	0	130	

*All infusion volumes were reduced to 1/4 of the initial volume.

Table 1: Conditions of digestion program used in coffee samples, rat livers and diets.

Group/Treatment	nº of rats	chemicals	form of coffee applied	diet
G1/commercial	10	DMH	-	CD
G2/organic	10	DMH	5% infusion	ORC 5%*
G3/organic	10	DMH	10% infusion	ORC 10%
G4/organic	10	DMH	20% infusion	ORC 20%
G5/organic	10	DMH	powder	ORC 4%
G6/conventional	10	DMH	5% infusion	COC 5%
G7/conventional	10	DMH	10% infusion	COC 10%
G8/conventional	10	DMH	20% infusion	COC 20%
G9/conventional	10	DMH	powder	COC 4%
G10/commercial	10	EDTA	-	CD
G11/organic	10	EDTA	20% infusion	ORC 20%
G12/organic	10	EDTA	powder	ORC 4%
G13/conventional	10	EDTA	20% infusion	RCC 20%
G14/conventional	10	EDTA	powder	RCC 4%

DMH=1,2-dimetilhidrazine (40mg. Kg^{-1} body weight), a promoter of pre-neoplastic lesions in the colon. EDTA 1,5% (w/v) in NaCl 0,9% (w/v) (vehicle). CD=commercial diet; COC=diet with organic coffee; COC=diet with conventional coffee.*Considering the infusion embedded the feed (incorporated 100 mL infusion/kg commercial diet).

Table 2: Experimental design for the 12-weeks experiment to investigate the hepatic mineral content in healthy and induced cancer animals (DMH), fed with these coffees.

fourteen groups (*n*=10) (Table 2). The University Ethical Committee for Animal Research approved the protocols used in this study (protocol no. 235/09). We used 4-week-old male *Wistar* rats obtained from Centro Multidisciplinar para Investigacao Biologica (CEMIB) (UNICAMP Campinas, SP, Brazil). The animals were kept in polypropylene cages (5 rats/cage) covered with metallic grids and maintained at 22 ± 2°C, 55 ± 10% humidity and with 12 h light-dark cycles. They were fed commercial Nuvilab CR-1® diets (Nuvital Nutriente S/A, Colombo, PR, Brazil). For the preparation of modified diets, organic and conventional coffees in powder or infusion form were used. The infusions (5, 10 and 20% w/v) were prepared as described above. To incorporate the infusions in the commercial diet, a proportion of 100 mL of infusion per kg of commercial diet was used. This proportion allowed an appropriate pelleted diet. The significance of fiber as modulator of physiological and pathological processes prompted us to explore new potential applications. This way, the powdered coffee was incorporated in the proportion of 40 g powder per kg of diet [7]. After pelleting and drying, the conventional (COC) and the organic (ORC) coffee diets containing infusions of 5, 10 or 20% (w/v), or 4% (w/w) powder were obtained. These diets were fed to the animals for 12 weeks (Table 2).

To assay the effects of coffee in the animals with cancer, groups 1 to 9 received four injections of 1,2-dimethylhydrazine (DMH), a carcinogenic drug that induces pre-neoplastic colonic lesions. The doses administered were 40 mg kg^{-1} body weight, subcutaneous, twice a week, for two weeks (Table 2). In order to study animals without cancer, groups 10 to 14 received similar injections of the control vehicle [etilenediaminetetracetic acid (EDTA) 1.5% w/v] to simulate the stress of injection. At the end of this experimental step, all animals were anesthetized with ketamine and xylazine and euthanized by withdrawing blood from the heart. At necropsy, the livers were removed from all animals and stored at -18ºC until analysis [7] (Table 2).

Statistical analysis

In order to verify the differences between organic and conventional managements, the proximate (samples: coffee infusions, powders, diets) and micronutrient analysis (samples: coffee infusions, powders, diets and liver of rats), were compared using the Student's t test for paired data at 5% confidence level using the software *BioEstat*® 5.0.

The different mineral extractions from the infusions and the nutritional evolution of the animals (body-mass gain, diet consumption and consumption per animal per day of coffee powder) was examined with one-way analysis of variance (ANOVA/Tukey's test, $p<0.05$) using the software *Sisvar*® 5.1.

Results and Discussion

The results of the nutritional composition analysis revealed no significant differences between the powder and the infusions from either management system (Table 3). These results are in agreement with the Brazilian recommendation of National Agency of Sanitary Surveillance [33].

The micromineral contents were determined, and the LOD/LOQ for Cu, Fe and Zn were 0.065/0.215 mg L^{-1}, 0.127/0.424 mg L^{-1} and 0.024/0.081 mg L^{-1}, respectively. To check the accuracy, the recovery varied from 95 to 103% for all samples. The measurement precision, checked from variation coefficients of readings in triplicate, was always lower than 3% [32].

The conventional coffee powder contained higher concentrations of Cu, Fe and Zn than the organic powder (Table 4). This result can be attributed to the common use of chemical fertilizers in the conventional management system, which provide higher levels of N, P, K, S, Fe, Mn, Cu, B, Cd and Zn [5] compared to the natural fertilizer (castor cake) used in organic management systems [2,8]. These results demonstrate the influence of agricultural management in the amount and availability of minerals (Table 4).

Mineral composition of the powder and the coffee infusions (mg

Samples[a]	Carbohydrate	Ash	Fat	Protein	Moisture
ORC	58.30 ± 1.50	4.14 ± 0.27	18.76 ± 0.21	17.94 ± 0.56	0.81 ± 0.02
COC	58.36 ± 0.17	4.36 ± 0.01	18.59 ± 0.01	17.80 ± 0.07	0.79 ± 0.03
IORC 5%	0.10 ± 0.01	0.22 ± 0.01	0.10 ± 0.00	1.29 ± 0.33	98.54 ± 0.01
ICOC 5%	0.10 ± 0.01	0.21 ± 0.02	0.09 ± 0.01	1.37 ± 0.08	98.49 ± 0.21
IORC 10%	0.10 ± 0.02	0.41 ± 0.02	0.17 ± 0.02	1.58 ± 0.16	97.40 ± 0.02
ICOC 10%	0.10 ± 0.02	0.41 ± 0.01	0.19 ± 0.01	1.68 ± 0.09	97.42 ± 0.01
IORC 20%	0.11 ± 0.02	0.71 ± 0.06	0.31 ± 0.01	2.89 ± 0.17	94.96 ± 0.04
ICOC 20%	0.11 ± 0.02	0.73 ± 0.02	0.29 ± 0.01	2.68 ± 0.14	94.94 ± 0.01

COC=conventional coffee; ORC=organic coffee; ICOC=infusion conventional coffee; IORC=infusion organic coffee. No statistic difference by Student's t-test ($p<0.05$).

Table 3: Proximate analyses of powder and infusion (organic and conventional coffee) samples (mean ± SD) (g 100 g^{-1}) (*n=4*).

Samples	Cu	Fe	Zn
ORC	21.29 ± 0.47[a]	40.93± 0.88[a]	22.22 ± 0.59[a]
COC	25.03 ± 0.22[b]	45.70± 0.51[b]	27.08 ± 0.99[b]
IORC 5%	<LOD	<LOD	2.53 ± 0.12[a]
ICOC 5%	<LOD	<LOD	2.73 ± 0.12[a]
IORC 10%	<LOD	<LOD	2.50 ± 0.20[a]
ICOC 10%	<LOD	<LOD	2.27 ± 0.06[a]
IORC 20%	<LOD	<LOD	2.93 ± 0.03[a]
ICOC 20%	<LOD	<LOD	1.80 ± 0.03[b]

conventional coffee; ORC=organic coffee; ICOC=infusion conventional coffee; IORC=infusion organic coffee; LOD=limit of detection. [a,b] Statistical difference of powders and between each infusions concentration by Student's t-test ($p<0.05$).

Table 4: Mineral composition of the powder and the coffee infusions (mg kg^{-1}) (mean ± SD) (*n=4*).

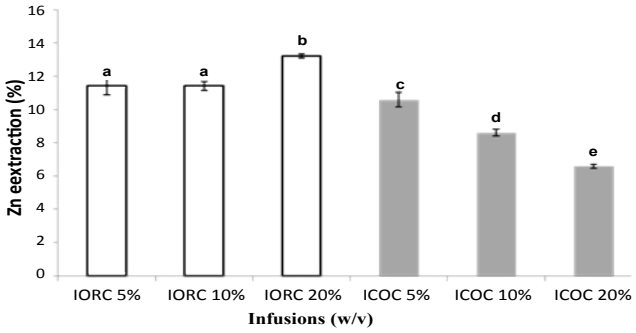

Figure 1: Zn extraction yield in organic and conventional infusions.

ICOC = infusion conventional coffee; IORC = infusion organic coffee; [a,b,c,d,e] Statistical difference among columns. [a,b,c,d,e] Statistical difference between the columns by ANOVA/Tukey's test ($p<0.05$).

kg^{-1}) (mean ± SD) (n=4).

In the infusion studies, we observed that although Cu and Fe were present in higher concentrations than Zn in the powdered coffee, they were poorly extracted by the infusion method and their concentrations were lower than the LOD, independently from the agricultural management system (Table 4). Therefore, they were strongly adsorbed in the powder and/or the paper filter. On the other hand, Zn was detected in all infusions. Furthermore, in the 20% (w/v) infusion prepared from the organic powder, Zn was present at higher levels than in the conventionally managed sample (Table 4).

We observed that although the conventional coffee powder presented higher levels of zinc than organic coffee powder, its extraction yield for all infuses was lower than organic coffee, as showed clearly in Figure 1. This fact can be explained by the relationship between the quantity of water and the powder mass used for each infusion, suggesting that coffee can act as an adsorbent. This may have occurred due to the inherent characteristics of the powder, which contains potential desorption/adsorption properties, mainly due to the roasting process, when the temperature of pyrolysis reaches 220°C. Therefore, the Zn level may be the result of the interaction of Zn with degraded compounds during the roasting of the coffee, *i.e.*, with the formation of insoluble ZnO in an aqueous solution [34] or by the interaction of Zn with hydrosoluble compounds of samples that may have eluted during filtration [35,36] This adsorbent property of coffee was used by Boonamnuayvitaya et al.,[37] who used the residues produced by the instantaneous coffee manufacturing to remove heavy metal ions in the solution (Figure 1).

The different mineral extraction efficiencies from the infusions may also be compared to the affinity of these elements in carbon-based adsorbents, which vary in the order of $Zn^{+2} < Fe^{+3}/Fe^{+2} < Cu^{+2}$, [38] and to their adsorption in filter paper, which varies in the increasing order of Zn (0.06%), Cu (2.11%) and Fe (5.34%) [39] According to Kononova et al.,[38] the ion affinities may be attributed to the different values of the stability constants of the compounds formed with the functional groups on the surface of the adsorbents. These affinities explain the higher extraction of Zn in the infusion compared to Cu and Fe.

In addition to the adsorption/elution properties and the extraction sequence, which are common to the infusions from both management systems, it is also concluded that coffee from the conventional management system provides more adsorbent compounds and decreases the availability of Zn in the infusion.

Comparing the Zn levels to the Recommended Dietary Allowance (RDA) [40], the usual consumption of 300 mL of a coffee infusion can supply the human body with up to 1.61% of the RDA, then the possible food replacement by coffee in improper diets cannot be performed.

Although the influence of elements of coffee complex matrix on interactions with macronutrients from diet such as fibers or protein, in the mouth or gastrointestinal tract, have been clearly demonstrated in many reports [41,42] a better understanding of these interferences in the diet is essential. For this purpose, we evaluated the possible effects of coffee matrix in the liver of healthy rats or cancer induced (treated with DMH) through the concentration of Fe, Cu and Zn. The infuses and ground coffee were incorporated in the diet for mimicking the common ingestion of coffee together other foods and their possible interactions.

The drug DMH used to induce colon cancer is metabolized in the liver to produce methyl free-radicals, generating hydroxyl radicals or hydrogen peroxide that can induce oxidative damage in vulnerable targets, such as DNA bases, resulting in cancer, inflammation and aging [43]. In this *in vivo* study, there were no significant differences in the composition of the diets with and without coffee; and the average concentrations in diets were as follows (g 100 g^{-1}): carbohydrate 45.5 ± 5.5, ash 8.8 ± 2.3, lipid 4.9 ± 0.4, protein 27.8 ± 6.4 and humidity 11.8 ± 3.5; and for minerals were (mg kg^{-1}): Cu 35.02 ± 0.72, Fe 113.72 ± 14.53 and Zn 0.16 ± 0.21. The compound levels in the experimental diets are consistent with those suggested by Ammerman et al. [44] and Oliveira et al. [45] and fulfill the needs of the animals. Besides, to ensure study homogeneity, the nutritional parameters of animals showed that there were no significant daily changes in the consumption of the diets (25.5 ± 3.1 g per rat) or in body-mass gain (26.12 ± 9.23 g per rat), for all groups. Based on the diet consumption, the animals from each respective group ingested coffee daily in the following amounts: 0.14 ± 0.01 g (infusion groups 5% w/v), 0.26 ± 0.03 g (infusion groups 10% w/v), 0.54 ± 0.04 g (infusion groups 20% w/v) and 1.03 ± 0.05 g (powdered coffee groups 4% w/v). The proportional consumption of coffee evidenced that the use the diet as vehicle is adequate for this kind of sample. Furthermore, the results showed that the all diets containing coffee (infuses or ground) did not influence the contents of minerals in the livers of all experimental groups, with the following mean values: 0.68 ± 0.05 mg g^{-1} for Fe and 0.14 ± 0.02 mg g^{-1} for Zn. Cu was not detected. As the changes in mineral concentrations in the liver, heart, plasma, bone and bile, are indicative of their bioavailability [46-48], we believe that the hepatic content of Cu, Fe and Zn did not suffer from the action of chelating substances, such as brown-melanoidin-like polymers derived from chlorogenic acids, sugars, proteins [22], and fibers in the coffee powder (4% w/w) or the neoplastic action of DMH.

Conclusion

Overall, our results support the conclusion that the mineral content of coffee is influenced by the conditions of agricultural management styles and the extraction process. The levels of Cu, Fe and Zn were higher in conventional coffee powder than in the organic powder. However, despite differences in the powder, the organic coffee had higher extraction yield for all infuses, and its infusion at 20% (w/v) had higher level of Zn than other conventional infusion. Regarding the mineral content *in vivo*, the ingestion of diets containing infusions or coffee powder did not influence the hepatic content of Cu, Fe and Zn.

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