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Chemical Composition, Caffeine, Antioxidant Activity and Antibacterial Activity of Two Coffee Beans

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ABSTRACT

Roasting is a crucial step in the manufacture of coffee because it allows flavour and aroma to emerge. The goal of this study was to find the amounts of copper (Cu), iron (Fe), zinc (Zn) and lead (Pb) in coffee that was roasted by machine and coffee that was roasted by hand. The ability of these samples to kill bacteria and fight free radicals was also determined. The concentrations of Cu, Zn, Fe, Pb and caffeine were investigated in machine-roasted and hand-roasted coffee samples using a quantitative method. The antibacterial activity was evaluated using the disc diffusion method and the micro-dilution method. The antioxidant potential was determined using DPPH and ABTS assays. The findings revealed that there were no significant differences in the Cu, Zn, Fe and Pb content between the machine-roasted coffee and the hand-roasted coffee samples. In addition, the solid form of machine-roasted coffee had considerably more caffeine than the liquid form of the same sample. In contrast, the liquid form of the hand-roasted sample contained more caffeine than the solid version. Weak antibacterial activity was observed for both samples tested against *Staphylococcus aureus, Streptococcus epidermidis, Escherichia coli* and *Pseudomonas aeruginosa* since the maximum inhibition zone observed was 8.5 mm and the lowest minimum inhibitory concentration (MIC) was 50 mg/ml. Interestingly, the antioxidant activity of the hand-roasted coffee sample (57.05 ppm) was significantly higher than that of the machine-roasted coffee sample (48.5 ppm). These findings recommend that hand-roasted coffee be considered an antioxidant agent. To generalize the results, additional research with larger sample size is needed.

Key words: Coffee, roasting, antibacterial, antioxidant, microelement

INTRODUCTION

Coffee is among the most widely known drinks around the world, with over 2.5 billion cups spent per day. Internationally, the industry in coffee products generates about $ 60 billion annually and is the second most valuable resource after indigestible crude oil. People's coffee consumption varies according to their age, heritage and geographical location. Furthermore, coffee is devoured for its flavour and texture. In accordance with the International Coffee Organization, a maximum intake of 12.0 kg per capita per year has been confirmed in Finland. In Jordan, coffee consumption is lower (about 3.3 kg per capita per year). In 2019, 3 billion cups of coffee were consumed globally, according to the International Coffee Organization and International Coffee Day (Al-Hasan *et al.,* 2019; Phrommarat, 2019; Al-Dalain *et al.,* 2020; Czarniecka-Skubina *et al.*, 2021).

The impact of coffee drinking on people's health has been examined extensively. Multiple ailments, such as Alzheimer's disease, type 2 diabetes and Parkinson's disease were found to benefit from coffee drinking. On the contrary, coffee drinking is thought to be a risk

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for cancer and heart disease patients (Yenisetti, 2016; Guercio, 2022).

Coffee beans include a variety of nutritious constituents, such as proteins, carbs, vitamins and some components that are considered to be the most significant. Several of these components, including zinc (Zn), copper (Cu), cobalt (Co), chromium (Cr), nickel (Ni), and manganese (Mn) are needed for organisms at small doses. However, mercury (Hg), cadmium (Cd), arsenic (As), lead (Pb), uranium (U) and titanium (Ti) are highly toxic and ought to be prevented. Reportedly, certain hazardous substances promote oxidative damage, damage DNA and cause cancer (Albals *et al.*, 2021).

Concerning coffee's effect on bacterial infections, coffee extracts demonstrated potent antibacterial action (Akhlaghi *et al.*, 2019). However, the antioxidant and antibacterial properties of coffee are dependent on its origin, level of roasting, processing method and decaffeination (Tasew *et al.*, 2020). According to their constituents, coffee varietals from various origins vary greatly. Various agro-geographic factors of the coffee plant, such as altitude, soil type, and harvest period, as well as pre-harvest and post-harvest management measures, influence the biological activity of coffee (Velásquez and Banchón, 2022).

Due to the diversity of essential components, coffee is beneficial to human health. However, in order to characterize the quality of the coffee, colour, aroma and flavour must emerge during roasting, which is a vital step in the manufacture of coffee. To maximize the development of aroma and flavour, the temperature and time conditions of the roasting step must be optimized and controlled. Depending on the type of roaster being used and the desired coffee grade, several roasting conditions are used in practice (Anisa *et al.*, 2017; Fikry *et al.*, 2019). Consequently, the purpose of this research was to determine the concentration of elements such as Cu, Zn, Fe and Pb in machine-roasted and hand-roasted coffee. The antibacterial and antioxidant activities of these samples were also investigated.

MATERIALS AND METHODS

Two types of coffee samples were used in this study: machine-roasted coffee and hand-roasted coffee. Both coffee samples were purchased from a local market in Jordan belonging to the same Brazilian trade brand. The hand-roasted coffee sample was prepared from a raw coffee bean that was roasted using a traditional method. This was performed by roasting coffee beans in aluminum dishes at a temperature between 170 to 190°C (Jeszka-Skowron *et al.*, 2020). The roasting process continued for 8 min, or until the beans became brown to black in colour. Both samples; the machine-roasted coffee sample, and the hand-roasted coffee sample, were ground to a fine powder.

Ions components were extracted from samples of coffee powder using a wet digestion technique. Exactly, 1.00 g of each sample was suspended in 10 ml of 65% nitric acid and 2 ml of perchloric acid in a vessel. The vessels were then sealed and placed in the oven for the night at 70°C. The vessels were cooled and the extracted samples were then transferred to a volumetric flask and diluted with deionized water to a final volume of 50 ml. The diluted samples were filtrated using micro-filter (0.7 µm) and stored at 4°C.

Using a double beam AA-6200 atomic absorption spectrophotometer, the ions' constituents were quantitatively analyzed based on internal calibration curves for a series of suitable standard solutions (Thermo Jarrell Ash MODEL 757, Franklin, MA, USA). For each ion, five standard solutions were made. To determine the ions concentrations in each sample, 1.00 g of water digest was mixed with 50 ml water (HPLC grade) and boiled for 15 min. After allowing the solution to cool, the solution was filtrated using microfilter (0.7 µm). The collected filtrates were diluted using water of HPLC grade. Subsequently, the ions contents of the prepared samples and standard solutions were analyzed using ion chromatography (Dionex model DX-I00, USA). Calibration curves for each ion were drawn and the ion concentration was calculated.

The caffeine content in each tested sample was quantitatively determined according to Tarawneh *et al.* (2021). An exact amount of 5 g from each sample was mixed with distilled water (100 ml) containing 3 g of sodium carbonate. The mixture was boiled for 15 min and combined with chloroform (20 ml). After 24 h, the chloroform-containing calcium sulfate layer was removed, and the caffeine-containing layer was concentrated using a

rotary evaporator. The collected powder of caffeine was collected and weighed using an analytical balance. The content of caffeine (mg/g) was calculated by dividing the mass of caffeine by the mass of the coffee.

An exact amount of 100 g of the finely produced powder sample was soaked in 250 ml of 96% ethanol. After 24 h, the solvent was collected by filtration, and it was removed using a rotary evaporator. The crude extract was collected and stored at 4°C.

Four bacterial species were used including *Staphylococcus aureus, Streptococcus epidermidis, Escherichia coli* and *Pseudomonas aeruginosa*. These bacteria were obtained from Karak Government Hospital (Al-Karak-Jordan). They were isolated from patients who had symptoms of a urinary tract infection. BIOMÉRIEUX VITEK® 2 SYSTEM was used to classify the isolates.

The antibacterial activity of the machine-roasted coffee sample (MRC) and the hand-roasted coffee sample (HRC) was evaluated using disc diffusion method (Qaralleh, 2018; Khleifat *et al.*, 2019). using sterile swap, 100 µl of bacterial suspension containing 1.5 x 10^8 CFU/ml was cultured on Mueller Hinton Agar plate. Then, 20 µl (from a stock solution of 50 mg/mL of the tested sample in DMSO) was pipetted onto a sterile disc (6 mm). After 15 min, the prepared disc was transferred to the surface of the agar plates under aseptic condition. The plates were incubated at 37°C for 24 h, and the inhibition zone formed was measured using a ruler in mm. Each sample was tested in triplicates.

Using the micro-dilution method, the lowest concentration of the coffee extract that suppresses the observable growth of the test microorganisms was determined (Altarawneh *et al.*, 2022; Dmour *et al.*, 2022). A stock solution of 200 mg/ml of the tested substances in DMSO was prepared. Two-fold dilution was performed using 96-well plates to produce a concentration equal to 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 mg/ml. Then, 10 µl of a bacterial suspension containing 10^8 CFU/ml was applied to each well. The plates were incubated at 37°C for 24 h. The concentration at which no observable growth was detected using a spectrophotometer was reported as the minimal inhibitory concentration (MIC). Each test was conducted in triplicate.

Using the ABTS assay, the examined samples' scavenging capacity was assessed (Hajleh *et al.*, 2022). The mixture of potassium persulfate (2.15 mM) and ABTS radical (7 mM) was made; it was then let to stand at room temperature in the dark for 16 h before being diluted with ethanol to achieve an 0.70±0.2 absorbance at 734 nm.

Then, 20 µl of a stock solution of 100 mg/ml of the tested samples in DMSO was combined with 2.0 ml of diluted ABTS radical to assess the tested samples' capacity to scavenge ABTS radicals. After incubation at room temperature for 6 min, the OD734 nm was measured, and the ABTS scavenging activity was determined using the formula:

ABTS radical scavenging activity (%) = [{(control OD734 – sample OD734)/ control OD734}] × 100

Trolox standard curves in concentrations from 50 to 600 ppm were created. The amount of mg Trolox equivalents (TE)/g extracts represent the ABTS radical scavenging activity.

Using the DPPH assay, the antioxidant activity of the investigated substances was assessed (Qaralleh *et al.*, 2020, 2021). This was done by combining 0.1 ml of a stock solution of 100 mg/ml of the tested samples in DMSO with 1.9 ml of 0.1 mM 1,1-diphenyl-2-picryl-hydrazyl (DPPH) methanolic. The liquids were quickly shaken. At 517 nm, the absorbance of each mixture was measured following a 30-min incubation period at room temperature. A positive control was utilized, which was gallic acid. Using the formula below, the DPPH radical scavenging activity was determined.

DPPH radical scavenging activity (%) = [{(control OD517 – sample OD517)/ control OD517}] × 100

The findings were presented as means±standard deviation (SD) of three independent experiments. Statistical differences between the means of machine-roasted coffee and the hand-roasted coffee samples were determined using GraphPad Prism T-test. For all statistical analyses, a p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The concentrations of Zn, Cu, Fe and Pb in the machine-roasted coffee and the hand-roasted coffee samples were determined using spectrophotometric assay. There was no significant difference ($P < 0.05$) in the Cu, Zn, Fe and Pb contents in between the machine-roasted coffee and the hand-roasted coffee samples (Table 1). However, these elements occurred at higher concentrations in machine-roasted coffee samples compared to those in the hand-roasted coffee samples. In both the samples, the highest concentration observed was for Fe, followed by Cu, Zn, and Pb. The Cu, Zn, Fe and Pb contents of the machine-roasted coffee were 0.31, 0.284, 1.186, and 0.095 ppm, respectively. These elements' concentrations in the hand-roasted coffee sample were 0.293, 0.279, 1.175, and 0.11 ppm, respectively.

Table 1. Cu, Zn, Fe and Pb contents in the machine-roasted coffee sample and the hand-roasted coffee

Elements	MRC (ppm)	HRC (ppm)
Cu	0.310±	0.293±
Zn	0.284±	0.279±
Fe	1.186±	1.175±
Pb	0.095±	0.110±

MRC – Machine-roasted coffee and HRC – Hand-roasted coffee.

The caffeine content of the machine-roasted coffee and the hand-roasted coffee samples was determined (Table 2). The results showed that the content of caffeine in the machine-roasted coffee was significantly higher ($P < 0.05$) than the hand-roasted sample.

Table 2. The caffeine content of the machine-roasted coffee and the hand-roasted coffee samples

	MRC (mg)	HRC (mg)
Caffeine	32±**	24±

MRC – Machine-roasted coffee and HRC – Hand-roasted coffee.

The antibacterial activity of the machine-roasted coffee and the hand-roasted coffee samples was evaluated using qualitative and quantitative assays (Table 3). Generally, coffee samples evaluated in this report showed weak antibacterial activity against gram-negative and gram-positive bacteria. There were no significant differences between the antibacterial activity of the machine-roasted coffee and the antibacterial activity of the hand-roasted coffee. However, the MIC values were 50 mg/ml against all bacterial species, the maximum inhibition zones of 8.5 and 8 mm were observed for the machine-roasted coffee and the hand-roasted coffee against *S. epidermidis*. *P. aeruginosa* was the most resistant strain to the tested samples.

Table 3. Antibacterial activity of the machine-roasted coffee and the hand-roasted coffee samples using disc diffusion method and micro-dilution method

Bacterial	MRC (mg)		HRC (mg)	
	mm	MIC	mm	MIC
S. aureus	7.0±0.0	50	7.5±0.5	50
S. epidermidis	8.5±0.3	50	8.0±0.0	50
E. coli	7.5±0.5	50	7.0±0.0	50
P. aeruginosa	0.0±0.0	50	0.0±0.0	50

MRC – Machine-roasted coffee and HRC – Hand-roasted coffee.

The antioxidant activity of the machine-roasted coffee and the hand-roasted coffee samples was evaluated using DPPH and ABTS assays (Table 4). The results of the antioxidant activity using DPPH and ABTS were expressed in equivalent to gallic acid and trolox, respectively. The ability of the hand roasted coffee sample (80.27 ppm) to scavenge the ABTS radicals was significantly ($P < 0.001$) higher than the ability of machine roasted coffee sample (59.23). This significant ($P < 0.01$) antioxidant activity was also observed using DPPH assay. The antioxidant activity of the hand roasted coffee sample (57.05 ppm) was significantly higher than that of the machine roasted coffee sample (48.5 ppm).

Table 4. Antioxidant activity of the machine-roasted coffee and the hand-roasted coffee samples using ABTS and DPPH assays

	MRC (ppm)	HRC (ppm)
ABTS	59.23±0.99	80.27±3.74***
DPPH	48.50±1.33	57.05±2.57**

MRC – Machine-roasted coffee and HRC – Hand-roasted coffee.

In general, roasted coffee includes various concentrations of specific components. There may be a conflict between the chemical components of coffees and factors that affect the plantation and cultivation of coffee trees. It has been discovered that the varieties of soil and fertilizer used in coffee crops influence their chemical components (Salamanca-

Jimenez *et al.*, 2017). Other variables that may alter the concentration of particular components comprise storage and shipping circumstances and the method of preparing roasted coffee.

According to reports, coffee powder includes a diverse array of microelements. The following elements were present in the roasted coffee beans: potassium, magnesium, calcium, sodium, manganese, zinc and copper (Albals *et al.*, 2021). Moreover, it was found that roasted coffee comprised no or only traces amounts of hazardous metals such as lead and cadmium. There are reports of rather high quantities of Mn, Cu and Zn in roasted coffee and the Zn contents might reach a high concentration of 19 mg/kg (Gebretsadik *et al.*, 2015).

Several of the discovered components should be addressed for human health from a nutritional standpoint. As an illustration, the daily copper intakes recommended for females and males are 1.0-1.1 and 1.2-1.6 mg/day, respectively (Altarelli *et al.*, 2019). These findings may be significant when discussing detected intakes from supplements in United States, demonstrating a strong resemblance between recommended intakes and actual copper supplement intakes. In this domain, minimal data are available; however, some pertinent information may be presented for nickel, which is associated with the consumption of numerous foods, including coffee (Tajik *et al.*, 2020). However, it is challenging to collect accurate presumptions and theoretical data in this setting due to two factors: (1) the availability of some macro-elements and micro-elements varying significantly among coffee products and (2) there are various extraction procedures, resulting in a wide range of element-specific results (Gebretsadik *et al.*, 2015).

The availability of Cu and Fe in trace amounts does not diminish their physiological activity. Copper is necessary for the development of melanin, whereas iron is the most important element for the formation and function of red blood cells. Coffee includes components that are healthy to human health. In this study, all investigated materials had varied quantities of Cu, Zn and Fe. Fe is vital to all living beings because it is engaged in numerous metabolic activities within the cell. It is an essential structural component of proteins and enzymes involved in the ETC (electron transport chain) and DNA synthesis. It facilitates oxygen transport from the lungs to the rest of the body. The recommended daily allowance for iron consumption ranged from 11.5 to 19.0 mg per day, with this average varying by gender and age. In addition, iron deficiency can result in major health issues including fatigue, immune-deficiency, anemia and shortness of breath. In contrast, an excess of Fe intake can result in systemic side effects, such as liver failure, metabolic acidosis, shock and systems and organ malfunction (Quintaes and Diez-Garcia, 2015; Cappellini *et al.*, 2020). Znic is crucial for a human being; it is required for the development and growth of the organism. Zn has an important part in immune system maturation, wound healing, and glucose metabolism. Zinc consumption must not be below 8 milligrams (mg) per day for females and 11 mg per day for adult males. Numerous human organs and systems, including the skin, immune system, central nervous system, gastrointestinal tract and reproductive system, and skeleton may experience severe health issues due to zinc deficiency. On the other side, zinc overload can inhibit other elements' absorption such as copper and iron, and induce a variety of symptoms, including flu-like symptoms, nausea, diarrhea, vomiting, immunological suppression and a reduction in the level of HDL cholesterol (Quintaes and Diez-Garcia, 2015).

Due to its essential role in human health, a dose of 1 to 3 mg of Cu must be consumed daily. Cu is essential for the formation and operation of the cardiovascular and neurological systems. Additionally, it benefits the skin and immunological system. Cu deficiency can cause major health difficulties including connective tissue and muscle disorders, anemia, neurological dysfunction, and leukopenia. Excess intake of Cu, however, may result in kidney failure and death (Mohammadifard *et al.*, 2019; Silva *et al.*, 2019). Pertaining to the concentration of copper, as the toxic element, in the tested samples, found that the machine-roasted coffee and the hand-roasted coffee samples have this element. Pb is a poisonous substance that may have a significant physiological and pathological impact on the heart, kidney, and blood pressure. According to estimates, the mean exposure to lead from coffee accounted for 40%

of the lead consumed through beverages, or nearly 20% of the total amount of lead consumed through food (Khanam *et al.*, 2020). In this investigation, the inhibitory effect of roasted coffee samples against both gram-positive bacteria and gram-negative bacteria was weak. Several variables may influence the antimicrobial property of coffee extract, including brewing method, roasting intensity and coffee plant species (Tasew *et al.*, 2020). Díaz-Hernández *et al.* (2022) reported that roasted coffee wass bactericidal against both Gram-positive and Gram-negative microorganisms. This antibacterial potential was, however, proportional to the level of roasting and the species of the coffee plant. Coffee bean extracts and isolated components, including trigonelline, protocatechuic acid, chlorogenic acid and caffeic acid, had been reported to suppress *Streptococcus mutans* growth (Akhlaghi *et al.*, 2019).

Bobková et al. (2020) also demonstrated that the extract of coffee beans had varying antioxidant capacity based on the roasting procedure, with the activity reaching its peak in lightly roasted coffees. During the roasting procedure, new chemical derivatives were generated such as phenylalanine and heterocyclic compounds, and some of these formed compounds were assumed to be associated with the scavenging capacity of roasted coffee (Rahal-Bouziane and Abdelguerfi, 2018; Asamenew *et al.*, 2019).

CONCLUSION

The results of this study showed that there were no significant differences in the micro-elements content and antibacterial activity between the machine-roasted coffee and the hand-roasted coffee samples. However, the antioxidant activity of the hand-roasted coffee sample was significantly higher than the ability of machine-roasted coffee sample. These data suggest considering the use of hand-roasted coffee as an antioxidant agent. Further investigation in a larger sample size is required to generalize the results.

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