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Roasted date and barley beans as an alternative's coffee drink: micronutrient and caffeine composition, antibacterial and antioxidant activities

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ABSTRACT

Excessive consumption of coffee and caffeine containing products may increase the likelihood of adverse effects. Therefore, this study designed to evaluate the roasted date and roasted barley beans as an alternative's coffee beverages. The micronutrient (Fe, Zn and Cu) and caffeine contents were measured atomic absorption spectrophotometer. The antibacterial activity was examined using disc diffusion method and the antioxidant activity was evaluated using ABTS and DPPH assays. The results showed that there were no significant differences between all samples tested regarding the concentrations of Fe, Zn and Cu. Besides, the coffee samples exhibited broad spectrum antibacterial activity (inhibition zone ranged from 8.5 to 11.5mm) while roasted date and barley beans showed narrow spectrum antibacterial activity against gram-positive bacteria (8.5-10.5mm). Using ABTS and DPPH assays, roasted coffee (Saudi Habshi) exhibited the strongest antioxidant activity followed by roasted coffee (Colombian), roasted barley, green coffee (Colombian) and roasted dates. The presence of these elements at comparable concentrations and the remarkable antibacterial and antioxidant activities of date and barley beans supports the hypothesis of using them as a coffee substitute.

Keywords: Coffee, date, barley, micronutrient, caffeine, antibacterial, antioxidant.

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INTRODUCTION

Coffee drinks and their products are one of the most used drinks in the world with more than 2.5 billion cups consumed daily. Coffee factories earn more than 60 billion US dollars annually, making these trading one of the most profitable trading after that of oil. Although it has a distinctive taste, coffee consumers diversified according to age, culture and geographical distribution. According to the International Coffee Organization, about 12.0 kg coffee per capita per year consumed in Finland making it the most coffee-consuming country. Even though the rate of coffee consumption in Jordan is less (about 3.3 kg per capita per year), coffee remains one of the most preferred beverages among Jordanians [1].

However, coffee is zero calories beverages; it contains a mixture of components. Coffee contains micronutrient such as copper (Cu), chromium (Cr), cobalt (Co), manganese (Mn), nickel (Ni), and zinc (Zn). Elements such as arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), titanium (Ti), and uranium (U) also found in coffee. It does not contain macronutrients nor vitamins. In addition, coffee is rich with caffeine, which is the most effective ingredient in coffee, as it affects the nervous system. Caffeine is a type of legal psychoactive substance that may lead to addiction. In fact, one cup (240-ml) of coffee contains 100 mg of caffeine and the daily recommended dose for adults is 400 mg (equivalent to four cups of coffee) [1,2].

The effect of drinking coffee on human health was previously studied. Studies have shown that drinking coffee is useful in preventing many diseases, such as type 2 diabetes, liver disease and neurological disorders. Other studies showed that coffee extracts possess antibacterial and antioxidant activities. On the other hand, excessive drinking of coffee may increase the risk of heart disease and cancer. In addition, it was found that too much coffee may lead to caffeine intoxication and addiction in which these in turn leads to symptoms related to the nervous system such as restlessness, nervousness, difficulty sleeping, agitation, muscle twitching, rambling flow of thoughts and speech, flushed face, increased heart rate, stomach upset and increased urination [3,4].

Excessive consumption of coffee and caffeine containing products may increase the likelihood of adverse effects [5]. Some people avoid the harmful effect of excessive coffee drinking by using ground-roasted dates and roasted barley drinks as a substitute. Therefore, the aim of this study was to evaluate the concentrations of caffeine and some micronutrients in selected coffee samples from Jordanian markets. These elements also measured in non-caffeinated drinks including barley and dates in order to stimulate their use as an alternative coffee drink.

MATERIALS AND METHODS

Samples

Coffee samples, date and barley seeds purchased from Jordanian markets. The samples were grinded to fine powder and stored at room temperature for further use.

Digestion procedure and microelements composition

The elements composition of the tested samples was digested using wet digestion method. Briefly, one gram of each samples was transferred to the pre-cleaned Teflon digestion vessels, where 10 ml of nitric acid (HNO3, 65%) and 2 ml of perchloric acid or HClO4 (70%) where added to each vessel. Subsequently, vessels were incubated at 70 C°. for 24hrs incubation, then cooled and disassembled and then the extracted samples were diluted to a final volume of 50 mL using deionized water,

which later was clarified using filter syringe (0.7 um) and stored at 4C [1].

The microelements composition of the tested samples was measured using a double beam AA-6200 atomic absorption spectrophotometer (Thermo Jarrell Ash MODEL 757, Franklin, MA, USA).

Electric Conductivity and pH of Coffee Solutions

A mixture of 1 gm of each sample was prepared using 50 mL HPLC-grade water for the measurement of pH and conductivity using electrode of an LF 537 conductivity meter (WTW, Weilheim, Germany). The conductivity is measured in milliS/cm units [1].

Caffeine content

Five grams from each grinded sample were added to 100 ml distilled water containing 3 gm of sodium carbonate ($NaCO_3$), which then boiled for 15 min. The collected cool solutions were mixed with 20 ml chloroform in a separatory funnel. The mixture was mixed gently and allowed to stand for some time in order to separate the colorless chloroform layer to which calcium sulfate as added to remove excess water. The different samples were filtered to remove calcium sulfate and then samples were kept at rotary evaporator to evaporate the whole solvent and then the final collected caffeine was weighted using analytical balance. The caffeine content (mg/gm of sample) of each sample was calculated using equation: Mass of caffeine / mass of coffee.

Antibacterial activity

Extraction

The fine prepared powder sample was soaked in 98% methanol for 24hrs. The crude extracts of each sample collected, filtered and the solvent removed.

Disc diffusion method

Two-gram negative bacteria (*Escherichia coli* and *Enterobacter aerogenes*) and another two-gram positive bacterium (*Staphylococcus aureus* and *Streptococcus epidermidis*) were used in this study. The antibacterial activity was evaluated using disc diffusion method [6]. Briefly, 100 µL of bacterial suspension (adjusted to 0.5 McFarland's standard (1.5x108 CFU/mL)) was spread on Mueller-Hinton agar plates. Then, 6 mm blank disc containing 100 ug of the tested samples was placed on the surface of inoculated plates. After 24h incubation at 37C, the inhibition zone around each disc was measured as millimeter diameter [6].

Antioxidant activity

ABTS free radical scavenging assay:

The scavenging activity of the tested samples was evaluated using ABTS assay [7]. Seven mM ABTS radical was prepared in 2.15 mM potassium persulfate; the mixture placed in dark at room temperature for 16hrsand then diluted with ethanol to reach an absorbance equal to 0.70±02 at 734 nm. To determine the ABTS radical scavenging ability of the tested samples, 2.0 mL of diluted ABTS radical mixed with 20 µl of the tested samples, positive or negative control (solvent). After 6 min, the absorbance measured at 734 nm and the ABTS scavenging activity calculated using the following formula.

ABTS radical scavenging activity (%) = [(A0-A1)/A0) x100]

Where:

A0: absorbance for control, A1: absorbance for sample

A standard curve of Trolox in concentrations ranging from 50 to 600 µg was prepared. The ABTS radical-scavenging activity expressed in mg Trolox equivalents (TE)/gm extracts.

DPPH radical scavenging activity

The antioxidant activity of the tested samples was evaluated using DPPH assay [8]. This was performed by mixing 1.9 ml of 0.1mM of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) methanolic with 0.1 ml of the tested samples. The mixtures shaken vigorously. After 30 min incubation at room temperature, the absorbance for each mixture measured at 517 nm. Gallic acid used as positive control. The DPPH radical scavenging activity calculated using the following formula.

DPPH radical scavenging activity (%) = [(A0-A1)/A0) x100]

Where:

A0: absorbance for control

A1: absorbance for sample

Statistical analysis

To investigate any significant differences between groups, one-way analysis of variance (ANOVA) was used followed by Dunnett's post hoc test. The data analyzed by SPSS© 22 (SPSS, Inc., USA). The rest of results were presented as means± standard deviation (SD) of 3-4 independent experiments. For all statistical analysis, a p-value of less than 0.05 was considered statistically significant. p values of less than 0.001 were considered of a highly significant statistical difference.

RESULTS AND DISCUSSION

With the aim of obtaining a coffee substitute drink, the mineral contents of coffee beans, dates beans, and barley beans analyzed. The concentrations of Fe, Zn, Cu and Pb measured using atomic absorption spectrophotometer and the Caffeine content measured too.

Perhaps the beneficial elements in coffee are many. In this study, the concentrations of iron, zinc and copper measured in samples of coffee and compared with the concentrations of these elements in samples of dates and barley seeds. The concentration of lead element as a toxic element measured in these samples. With reference to table 1, all samples tested contain Fe, Zn and Cu. All samples contain these elements. There were no significant differences between all samples tested regarding the concentration of Fe, Zn and Cu, with the exception of this is the concentration of Zn in Roasted Barley and Green coffee (Colombian). The presence of these elements at comparable concentrations in date and barley seeds may make them as a good source that can provide the body with these elements and hence support the hypothesis of using them as a coffee substitute.

Table 1: Concentrations of transition elements (Fe, Zn, Cu and Pb)

Samples	Conc (ppm)			
	Fe	Zn	Cu	Pb
Roasted dates	0.8706±0.0047	0.3713±0.0017	0.2147±0.0005	0.1098±0.0009
Roasted barley	1.0130±0.0101	0.5325±0.0006	0.1391±.0006	0.1448±0.0002
Roasted coffee (Saudi Habshi)	1.1009±0.0003	0.4456±0.0009	0.2592±.0003	0.1317±0.0006
Roasted coffee (Colombian)	1.1865±0.0003	0.2741±.0026	0.3349±.0009	0.1535±0.0016
Green coffee (Colombian)	1.0486±0.0186	0.1624±0.0014	0.1964±.0002	0.1535±0.0016

However, the lowest concentrations of Fe and Pb found in roasted dates with no significant differences in these elements' concentrations among all tested samples (P≤ 0.05). Regarding Zn concentration, there was no significant differences seen in roasted dates, roasted barley, roasted coffee (Saudi Habshi), these samples were the richest with Zn. Statistically, roasted coffee (Colombian) was the richest sample with Cu. There were no significant differences in Cu concentrations between roasted dates, roasted coffee (Saudi Habshi) and green coffee (Colombian).

Accordingly, to provide the daily recommended dose for each of these elements by using date and barley seeds, the dose should be calculated based on their contents of Fe, Zn and Cu and other microelements and macroelements.

Coffee contains beneficial elements for human's health. In this study, all samples tested contain Fe, Zn and Cu with variable concentrations. Fe is an essential element for all living organisms, as it is involved in many metabolism processes in the cell. It is component of some proteins and enzymes that possess a vital role in electron transport system and synthesis of DNA. It is involved in oxygen transfer from lungs to all body parts. The recommended daily iron intake ranged from 11.5 to 19.0 mg/day and this average is variable based on age and gender. Moreover, iron deficiency may lead to serious health problems such as anemia, immunodeficiency, shortness of breath and fatigue. On the other hand, overdose of Fe may cause systemic toxicity including metabolic acidosis, liver failure, shock and multi-organ failure [9].

Zn is vital for human health; it is essential for body growth and development. Zn play a significant role in the development of immune system, wound healing, and metabolism of carbohydrates. The recommended daily intake of zinc should not be less than 8 milligrams (mg) for women and 11 mg for adult men. Zn deficiency may lead to serious health problems in many body organs and systems such as skin, gastrointestinal tract, central nervous system, immune system, skeleton, and reproductive system. On the other hand, zinc overdose may suppress copper and iron absorption and it may cause broad symptoms such as nausea, vomiting, diarrhea, flu-like symptoms, decrease HDL Cholesterol and suppress immune system [10].

Due to its vital role in the body, 1-3 mg of Cu daily is required. Cu is important for the development and functioning of the nervous system and cardiovascular system. It is also important for the skin and immune system. Cu deficiency may lead to serious health problems such as anemia, leukopenia, neurological malfunction, and problems related to connective tissues and muscles. On the other hand, Cu overdose may lead to Kidney failure and death [11].

Regarding the concentration of the toxic element, copper, in these samples, found that all samples contain this element (Table 1). Pb is a toxic element that may cause critical effect on the developing of the central nervous system, cardiovascular and systolic blood pressure and kidney. It was estimated that the average exposure of lead from coffee corresponded to 40% of the lead intake from beverages and thus to almost 20% of the total dietary intake of lead. In this study it was found that the lowest sample containing copper is date seeds, while the samples of coffee and barley were the richest with this element [12].

As seen in table 2, the pH of all tested samples was between 4.65 and 5.42. The conductance ranged from 457 to 1677. The pH and conductance of roasted dates, roasted coffee (Colombian) and roasted barley were the lowest comparing with the other samples.

Table 2: Conductance and pH measurements for tested samples solutions.

Samples	pH (Log_{10})	Conductance (milli S/cm)
Roasted Dates	4.65	457
Roasted Barley	5.10	853
Roasted Coffee (Saudi Habshi)	5.40	1135
Roasted Coffee (Colombian)	4.96	1172
Green coffee (Colombian)	5.42	1677

Table 3: Caffeine concentrations in tested samples.

Samples	Caffeine (mg/5g coffee)
Roasted Dates	0.0±0.0
Roasted Barley	0.0±0.0
Roasted Coffee (Saudi Habshi)	7.3±0.0416
Roasted Coffee (Colombian)	6.4±.0451
Green coffee (Colombian)	5.8±.0493

The caffeine concentrations of the tested samples presented in table 3. Caffeine found in all coffee samples whereas dates and barley contain no caffeine. The highest concentration of caffeine was found in roasted coffee (Saudi habshi) with 7.3 mg/5g coffee followed by roasted coffee (Colombian) (6.4 mg/5g coffee) and green coffee (Colombian) (5.8 mg/5g coffee). There is variation in the level of caffeine between coffee samples. This is due to

several factors such as the methods of roasting and brewing. The concentration of caffeine in green coffee infusion is ranged from 113 to 293 mg/L [13].

Antibacterial activity

Table 4: Bacterial zone inhibition using disc diffusion method.

Samples	Zone of inhibition (mm)			
	S. aureus	*S. epidermidis*	*E. coli*	*E. aerogenes*
Roasted Dates	8.5±0.5	10.5±0.5	0.0±0.0	0.0±0.0
Roasted Barley	8.5±0.5	9.0±0.0	0.0±0.0	0.0±0.0
Roasted Coffee (Saudi Habshi)	10.5±0.5	11.5±0.75	9.5±0.5	9.0±0.0
Roasted Coffee (Colombian)	11.0±0.0	11.0±0.0	10.0±0.0	9.5±0.5
Green coffee (Colombian)	9.0±0.75	7.5±0.5	9.0±0.0	8.5±0.5

The antibacterial activity of tested samples seen in table 4. The inhibitory activity of roasted dates, roasted barley, roasted coffee (Saudi Habshi), roasted coffee (Colombian) and green coffee (Colombian) was evaluated using disc diffusion method. The coffee samples exhibited broad-spectrum antibacterial activity against both gram-positive and gram-negative bacteria. However, green coffee appears to possess weak antibacterial activity (inhibition zone ranged from 7.5-9.5 mm). The roasted coffee (Colombian) extract was the most effective extract against gram-positive bacteria. *S. aureus* and *S. epidermidis* was sensitive to roasted coffee (Colombian), roasted coffee (Saudi Habshi) with inhibition zone ranged from 10.5-11.5 mm. on the other hand, roasted dates, and roasted barley extracts exhibited weak antibacterial activity against gram-positive bacteria. The gram-negative bacteria appear to be resistant to the free caffeine extracts (dates and barley) since no inhibition zone observed.

In this study, roasted coffee showed moderate antibacterial activity against both gram-positive bacteria and gram-negative bacteria. Daglia et al.[14] found that the roasted coffee possesses broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. However, this antibacterial activity correlated with degree of roasting and the coffee species. Coffee extracts and extracted compounds including trigonelline, chlorogenic acid, caffeic acid, and protocatechuic acid were reported with bacterial inhibition activity against *Streptococcus mutans* [15,16]. Several factors might affect the antibacterial activity of coffee beans extract including brewing procedure, degree of roasting and the coffee species [17].

In this study, the roasted date seeds showed moderate antibacterial activity against gram-positive bacteria. The antibacterial activity of date seeds been reported previously. Aqueous, ethanol and chloroform extracts of date seed extract possesses potential antibacterial activity [18]. Metoui et al.,[19] reported that acetone and methanol extracts of date seed possess significant antibacterial activity against *Escherichia coli, staphylococcus aureus, staphylococcus epidermis,* and *Salmonella Typhinurium*.

The antibacterial activity of barley was previously described. Jebor et al., [20] showed that the methanol-water extract of barley exhibited antibacterial activity against *E coli, Staphylococcus aureus, Salmonella typhi* and *Klebsiella pneumonia*. Thaumatin like protein that has been isolated form barley showed inhibition activity against *Candida albicans, Bacillus subtilis, E. coli* and *Saccharomyces cerevisiae* [21].

Antioxidant activity

The antioxidant activity of roasted dates, roasted barley, roasted coffee (Saudi Habshi), roasted coffee (Colombian) and green coffee (Colombian) was evaluated using ABTS and DPPH assays. The antioxidant activity to scavenge ABTS radical was determined in equivalent to Trolox. As shown in table 5, the ability of the tested samples to scavenge the radical ABTS was in the range "between" 140.19 to 354.22. Roasted coffee (Saudi Habshi) exhibited the strongest scavenging activity followed by roasted coffee (Colombian), roasted barley, green coffee (Colombian) and roasted dates.

The total antioxidant activity using DPPH assay (table 5) was parallel to those obtained by ABTS assay. The highest value observed for roasted coffee (Saudi Habshi) (120.29 mg/g) and the lowest for roasted dates (41.41 mg/g).

Table 5: Antioxidant activity of tested samples using ABTS and DPPH assays

Samples	ABTS (mg/g)	DPPH (mg/g)
Roasted Dates	140.19±7.1	41.41±5.5
Roasted Barley	289.11±4.6	109.05±0.2
Roasted Coffee (Saudi Habshi)	354.22±0.3	120.29±0.2
Roasted Coffee (Colombian)	296.71±10.0	112.19±0.7
Green coffee (Colombian)	280.97±3.0	100.83±4.4

Coffee extracts possess an antioxidant activity, Bobková et al., [22] also showed that aqueous coffee extracts possess variable antioxidant activity based on roasting process and the activity reach maximum in light roasted coffees (DPPH inhibition ranging from 69.08 ± 1.33% to 78.55 ± 0.89%). Compounds such as phenylalanine and heterocyclic compounds are formed during the roasting process in which these compounds are believed to be related to the antioxidant activity of roasted coffee [23,24].

CONCLUSION

The results indicate that roasted barley beans and roasted date beans contain micronutrients such as Fe, Zn and Cu in concentrations close to those found in coffee beans. In addition, roasted barley beans and roasted date

beans contain no caffeine; it has antibacterial and antioxidant effects. Based on these results, we can say that roasted barley beans and roasted date beans as an alternative to coffee. Further investigations about toxicity using animal experimental models the taste and are highly recommended.

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