PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 14 (8): 447-452, 2015
ISSN 1680-5194

Caffeine Content in Beverages Commonly Consumed in Jordan

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Abstract: This study aimed to determine caffeine levels in beverages commonly consumed in Jordan. A total sample of 167 caffeinated products were collected from the market in Amman city (the capital of Jordan) and prepared according to the label instructions. Caffeine content was determined using high-performance liquid chromatography (HPLC). Samples were analyzed in triplicate. Caffeine concentration ranged from 12.37 to 194.61 mg/100 ml in coffee samples, 2.57 to 16.11 mg/100 ml in tea, 3.46 to 19.06 mg/100 ml in cocoa and 10.15 to 32.56 mg/100 ml in energy and soft drinks. Expresso coffee and Turkish coffee had the highest content of caffeine among all beverages studied (194.6 and 146.6 mg/100 ml, respectively). This is the first study to investigate caffeine contents in wide types of beverages in the Arab world. The findings of this study are useful for nutrition education, both to control caffeine intake and to establish safe limits of caffeine in beverages consumed in Jordan.

Key words: Caffeine content, beverages, food composition, Jordan

INTRODUCTION

Caffeine (1, 3, 7-trimethylxanthine) is a water-soluble purine alkaloid compound which occurs naturally in the leaves, seeds and fruits of more than 60 plants (Lamina and Musa, 2009; Valek *et al*., 2004). Common sources of caffeine include coffee, tea, cola nuts, cocoa beans, mate and guarana (Babu *et al*., 2008; Nehlig and Boyet, 2000). Therefore, caffeine is present in any food or beverage that contains at least one of these sources, such as soft drinks, energy drinks and chocolate products (Bode and Dong, 2007; Smit and Rogers, 2002). Furthermore, caffeine is found as a complementary chemical substance for specific and distinctive purposes, for example providing a unique flavour (Valek *et al*., 2004) or pharmacological effect (Roehrsa and Rotha, 2008). Given the pleasurable stimulant properties, extensive distribution and rapid accessibility of caffeine-containing products, their consumption is rising all over the world (Bode and Dong, 2007; Lara, 2010).

Caffeine consumers may exhibit dependence-like behaviours and experience unsuccessful attempts to stop caffeine use (Bernstein *et al*., 2002; Nehlig, 1999). Caffeine intake may result in serious health hazards and in rare cases-death if used to excess. Specific symptoms that emerge as a direct result of high caffeine consumption are defined as caffeine toxicity or caffeine intoxication (Reissig *et al*., 2009).

Nevertheless, caffeine is recognized as safe by the Food and Drug Administration (Temple, 2009) and there are currently no recognized health-based guidance values for caffeine, such as acceptable daily intake. Therefore, the least harmful amount of caffeine is not yet well defined (Food Standards Australia New Zealand, 2011). According to Canadian recommendations for daily caffeine intake, children aged 10 to 12 years are not recommended to consume more than 85 mg and adults should not consume more than 400 to 450 mg, with the exception of women of child bearing age who should not consume more than 300 mg (Babu *et al*., 2008). Unfortunately, consumers have little awareness of the amount of caffeine that is present in caffeinated products (McCusker *et al*., 2003) and it is therefore difficult for them to estimate their daily caffeine consumption. The total daily consumption of caffeine is not only important on an individual basis, but it is also vital to consider it in epidemiological studies and in investigations of the physiological and psychological effects of caffeine (Stavric *et al*., 1988).

The caffeine content of different products has been determined in many studies (Chin *et al*., 2008; De Camargo and Toledo, 1999; McCusker *et al*., 2003). However, the amount of caffeine in food and beverages is influenced by the type of caffeinated food or beverage, geographical source, method of preparation (Bell *et al*., 1996; McCusker *et al*., 2003), number of grounds and ground size, grounds-to-water ratio (Bell *et al*., 1996; Mandel, 2002; McCusker *et al*., 2003), degree of roasting (Mandel, 2002; McCusker *et al*., 2003) and the added level of caffeine (Bell *et al*., 1996; Temple, 2009). Coffees from different parts of the world have different genetic properties which might influence their caffeine

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content (Mandel, 2002). The preparation methods of coffee are numerous and each method will extract different amounts of caffeine per one gram of coffee bean; therefore, the caffeine content will be different for each type (Mandel, 2002).
As the preparation and consumption of caffeinated products varies from region to region, their caffeine content also varies. This fluctuation in the caffeine content means that there is a need for regionally-specific determination of caffeine levels in such products. To our knowledge, no study has focused on the levels of caffeine in beverages consumed in Arab countries. However, previous studies in these countries have reported high consumption of caffeinated beverages by both children and adults (Al-Faris, 2009; Al-Hazzaa and Musaiger, 2011; Al-Hazzaa *et al*., 2011). Therefore, this study aims to determine the actual caffeine content in beverages available in the market and those prepared by common coffee shops in Amman City, the capital of Jordan.

MATERIALS AND METHODS

Chemicals and reagents: Ultra-pure methanol, water, acetic acid, 0.1M hydrochloric acid, acetonitrile, saturated basic acetate solution and $NaHCO_3$ powder were purchased from Al Faraby Est. (Amman, Jordan). The chemicals were treated in an ultrasonic bath for at least 15 min before being used in the mobile phase preparation. A highly pure (analytical grade) standard of caffeine was supplied by the LABCHEM Company (USA). The external standard calibration method was used. A set of six caffeine standard solutions were prepared and filtered using a 0.45 µm, 33 mm diameter nylon syringe microfilter (Agela Technology, USA).The concentration range of the standard solutions was 0.1 to 1 mg/ml. A caffeine standard curve was constructed using triple injections of each caffeine standard solution.

Sample collection and preparation: The main supermarkets and coffee shops in Amman City were first located. Different commonly-used brands and batches of soft drinks, energy drinks, green and black tea, cocoa products and coffee products were purchased from these supermarkets. Samples were prepared according to the procedure for a normal serving. Various types of coffee products were purchased from popular coffee shops and analyzed “as is”. Two to three samples were analyzed for each product. The total sample collected was 167 for 40 kinds of caffeinated products.

Chemical analysis

Soft and energy drinks: Caffeine in soft and energy drinks was analyzed according to the methods employed by De Camargo and Toledo (1999) and Srdjenovic *et al*. (2008). Samples were degassed in an ultrasonic bath for 15 min to release the CO_2. After the decarbonization process, the samples were filtered through a 0.45-µm nylon filter (Agela Technology, USA) and injected into a high-performance liquid chromatograph (HPLC).

Tea: In order to determine the caffeine content of green and black tea, caffeine was extracted from these products based on the procedure described by De Camargo and Toledo (1999). Tea samples (bags and leaves) were extracted with 160 ml of boiling water for 2 min. After reaching room temperature, 20 ml of each sample was mixed with 10 ml of 0.1M hydrochloric acid and 8 ml saturated basic acetate solution, then centrifuged at 1057.5 x g for 5 min. The supernatant was transferred to another test tube to which $NaHCO_3$ (a ratio of 0.1 g $NaHCO_3$ to 10 ml of solution) was added; this solution was centrifuged again at 1057.5 x g for 5 min. The solution was filtered through a 0.45-µm nylon filter (Agela Technology, USA) and injected into the HPLC.

Cocoa products: The extraction of caffeine from cocoa products was performed according to the procedure described by De Camargo and Toledo (1999). A clean-up step was added before injection into the chromatograph. Samples were weighed into test tubes equipped with Teflon-lined screw caps. The fat was extracted by shaking the samples twice with a 30 ml portion of petroleum ether, centrifuged at 470.0 x g for 10 min and the supernatant was discarded. This step was repeated at least twice to ensure that all fat was removed. The residual solvent was evaporated by placing the test tubes in a warm water bath. To the residue, 30 ml of water and 5 ml of saturated basic acetate solution were added and this solution was centrifuged at 1057.5×g for 5 min. $NaHCO_3$ was added to the supernatant and centrifuged again at 1057.5 x g for 5 min. HCl (0.1M) was added to the supernatant. The solution was then filtered through a 0.45-µm nylon filter (Agela Technology, USA) and injected into the HPLC.

Coffee products: The coffee samples in this study were either purchased as ready-to-drink products or as grounds to be prepared. The ready-to-drink samples (Mr. Brown products and products purchased from coffee shops) were filtered through a 0.22-µm nylon filter (Agela Technology, USA) and injected into the HPLC. Arabic coffee (95% coffee and 5% cardamom, coarse grained), Turkish coffee (95% coffee and 5% cardamom, finely grained), instant coffee products (Nescafe) were prepared according to the manufacturers’ or sellers’ instructions. For the preparation of Turkish coffee, 6 g was added to 60 ml of boiled water, stirred and re-boiled for 1 min. Forty grams of Arabic coffee were added to 500 ml of boiled water then re-boiled for 30 min. Instant coffee products were prepared according to the manufacturers' directions. The beverage samples were filtered through a 0.22 µm nylon microfilter (Agela Technology, USA) to remove any insoluble material.

Determination of caffeine levels by HPLC: The analysis of caffeine levels was performed using a reversed-phase high-performance liquid chromatography (Varian prostar) method. The separation was carried out on a C18 column (150 mm long, 4.6 mm internal diameter, 0.45 µm particle diameter), ultraviolet absorbance was monitored at 254 nm wavelength and the injection volume of samples and standards was 20 µl.

Caffeine was eluted isocratically at a flow rate of 1.0 ml/min using the mobile phase. The mobile phase used was methanol-water (25:75, v/v) for soft drinks, methanol-water (30:70, v/v) for tea, acetonitrile-water (10:90, v/v) for cocoa and methanol-water-acetic acid (20:75:5, v/v/v) for coffee products. The peak of caffeine levels in the samples was identified by comparing the retention time with that of a standard.

Data presentation: The concentration of caffeine in the tea, soft drinks, energy drinks, cocoa beverages and home-prepared coffee and coffee shop samples was calculated based on linear regression (r>0.99). The results are presented in two ways: the first is caffeine concentration per 100 ml for ease of comparison between products; the second is caffeine content per commonly used serving to establish a dataset for estimating daily intake.

RESULTS AND DISCUSSION

The caffeine concentrations of coffee samples are presented in Tables 1 and 2. The inter- and intra-variation among coffee types reflects the effects of preparation and source disparity. Espresso had the highest concentration (194.61±52.73 mg/100 ml) and Mr. Brown Café Americano (packaged products) had the highest content per serving (431.10±13.19 mg/330 ml). This high content of caffeine in one serving draws attention to the importance of serving size. As pointed out by McCusker *et al.* (2003), a larger volume of coffee increases the amount of caffeine that may be consumed at one time. On the whole, larger portions have also been documented to encourage people to consume more (Young and Nestle, 2002). The variation between the caffeine content of home-prepared Turkish coffee and that purchased from coffee shops can partly be explained by the degree of roasting of the coffee beans. In the home-prepared samples, green and intensified roasted coffee were prepared and analyzed separately, whereas the coffee shop sellers indicated the use of a mixture of 50:50 in weight. Caffeine levels were found to be lower in the intensified roasted coffee beans than in green beans, a result congruent with that of Hecimovic *et al.* (2011) and may explain the lower concentration of Turkish coffee mixture in the coffee purchased from coffee shops.

Coffee is the most abundantly-consumed stimulant worldwide (Corti *et al.*, 2002); however, the consumption of a single amount of as little as 32 mg to 50 mg of caffeine has been observed to stimulate improvements in alertness and in concentration significantly for 20 minutes and to induce enhancements in performance and mood (Glade, 2010; Smith, 2009).

The solubility of caffeine in water varies widely with temperature (Ramalakshmi and Raghavan, 1999); it is moderately soluble in water at room temperature, but is very soluble in boiling water (Graham, 1978). This property of caffeine may explain the lower levels in chilled beverages than in hot. This was observed in both the coffee and tea samples.

The results of the tea analysis in this study are shown in Table 3. The Twinings English Breakfast tea samples were found to have the highest concentration of caffeine compared to other tea samples and iced tea had the lowest. Many studies have investigated caffeine content in tea; the results vary widely in relation to the source, water-to-tea ratio and steeping time (Chin *et al.*, 2008; Stavric *et al.*, 1988). This study, as did that of Hicks *et al.* (1996), found that green tea contains more caffeine than black tea. Green tea is made from fresh tea leaves by blanching, twisting and drying, whereas black tea manufacture includes a fermentation step (Wang *et al.*, 1994), which effectively reduces the caffeine level in black tea leaves (Cloughley, 1983). The differences between the brands of black tea analyzed could be due to the variation in time and temperature of fermentation (Cloughley, 1983).

Tables 4 and 5 show the caffeine content of cocoa products and soft and energy drinks, respectively. Per 100 ml of cocoa, the highest level of caffeine was 19.06±0.53, which indicates the very low caffeine content of cocoa beans. In general, only small quantities of caffeine are found in cocoa beans (Matissek, 1997). As Table 5 shows, the caffeine content of soft drinks ranged from 33.80±0.84 mg to 54.07±1.09 mg per serving. Although the single servings did not contain very high amounts of caffeine, these beverages have become very popular nowadays. Frary *et al.* (2005) found that soft drinks were the second highest source of caffeine intake in the diet of young American adults, while it is the primary source of caffeine among children and adolescents in the same population. According to the food balance sheet for Jordan, the per capita supply of soft drinks available per year increased from 39.7 kg in 2002 to 74.2 kg in 2010 (Department of Statistics-Jordan, 2002, 2010).

Energy drinks had higher caffeine content per serving than soft drinks (Table 5). Concern is growing about the consumption of energy drinks, not only because of their high caffeine content but also the extent and volume of advertising, the wide range of available products on the market and ease of accessibility. Energy drinks have been advertised as enhancing performance and promoting stimulatory functions (Higgins *et al.*, 2010).

Table 1: Caffeine content (mg) in coffee beverages

		Per 100 ml	------------------ Per serving -----------------	
Coffee products	N	Mean±SD	Mean±SD	Serving size
Mr. Brown cafe latte (Bottle)	2	44.78±1.05	149.13±3.51	330 ml
Mr. Brown iced coffee (Bottle)	2	48.13±0.49	115.52±1.17	240 ml
Mr. Brown cappuccino iced coffee (Bottle)	3	39.05±0.97	93.72±2.32	240 ml
Mr. Brown cafe americano (Bottle)	3	129.46±3.96	431.10±13.19	330 ml
Turkish coffee green (g)	3	146.63±1.58	73.32±0.80	50 ml
Turkish coffee black (g)	3	120.17±6.31	60.09±3.16	50 ml
Arabic coffee (g)	3	58.14±3.70	14.53±0.93	25 ml
Nescafe red mug (g)	3	50.29±2.62	80.46±4.20	160 ml
Nescafe matinal (g)	3	48.77±2.21	78.04±3.54	160 ml
Nescafe gold (g)	3	40.04±1.16	64.06±1.85	160 ml
Nescafe three in one (Sachet)	3	54.24±5.93	86.78±9.49	160 ml
Nescafe two in one (Sachet)	3	43.76±1.73	70.01±2.77	160 ml
Nescafe strong (Sachet)	3	55.85±2.17	89.36±3.46	160 ml
Nescafe cappuccino (Sachet)	3	33.69±2.55	53.91±4.07	160 ml
Nescafe latte (Sachet)	3	36.16±3.06	57.85±4.90	160 ml
Nescafe mocha (Sachet)	3	37.08±2.22	59.32±3.54	160 ml
Nescafe vanilla (Sachet)	3	30.88±1.77	49.41±2.83	160 ml
French coffee (g)	3	85.76±8.93	42.88±4.47	50 ml

Table 2: Caffeine content (mg) in selected coffee shop beverages

		Per 100 ml	----------------------- Per serving -----------------------	
Coffee products	N	Mean±SD	Mean±SD	Serving size
Turkish coffee	3	102.19±15.92	51.10±7.96	50 ml
Cappuccino	3	56.44±19.13	187.93±63.72	330 ml
American coffee	3	39.05±18.92	130.04±63.00	330 ml
Espresso	3	194.61±52.73	48.65±13.18	25 ml
Latte	3	46.06±1.03	153.37±3.44	330 ml
French coffee	2	55.86±3.08	27.93±1.54	50 ml
Mochachino	2	30.73±2.55	102.34±8.50	330 ml
Mocha	3	53.89±3.88	179.46±12.93	330 ml
Caramel coffee	2	68.15±1.73	226.93±5.77	330 ml
Greek coffee	2	12.37±1.14	41.20±3.80	330 ml
Nescafe	3	62.84±3.49	209.24±11.63	330 ml
Iced latte	2	32.91±12.62	109.58±42.02	330 ml
Iced mocha	2	50.24±1.17	167.31±3.89	330 ml
Iced cappuccino	2	30.59±6.16	101.86±20.51	330 ml
Frappuccino	2	14.87±0.90	49.51±3.01	330 ml
Iced mochachino	2	21.65±1.75	72.09±5.82	330 ml
Iced coffee	2	13.64±1.57	45.43±5.23	330 ml

Table 3: Caffeine content (mg) in tea products

		Per 100 ml	------------------ Per serving -------------------	
Tea products	N	Mean±SD	Mean±SD	Serving size
Iced tea (Bottle)	3	2.57±0.16	6.44±0.40	250 ml
Black tea lipton (Bag)	3	10.14±0.62	16.22±0.99	160 ml
Black tea rabea (Bag)	3	7.03±0.76	11.24±1.22	160 ml
Black tea rabea (Leaf)	3	7.51±0.12	12.02±0.20	160 ml
Black tea algazaleen (Bag)	3	6.73±1.30	10.77±2.08	160 ml
Black tea algazaleen (Leaf)	3	10.33±2.24	16.53±3.58	160 ml
Green tea lipton (Bag)	3	13.33±1.31	21.33±2.10	160 ml
Green tea rabea (Bag)	3	12.97±1.02	20.75±1.64	160 ml
Twinings earl grey (Bag)	2	8.56±0.08	13.70±0.12	160 ml
Twinings English breakfast (Bag)	3	16.11±0.92	25.77±1.48	160 ml
Twinings green (Bag)	2	13.58±0.47	21.73±0.78	160 ml

Many energy drink brands found on the world market may contain caffeine up to 505 mg/can or bottle (Attila and Cakir, 2011; Higgins *et al.*, 2010; Reissig *et al.*, 2009). Unlike energy drinks, the caffeine content of soda drinks is limited by the USA Food and Drug Administration at approximately 18 mg/100 ml (Babu *et al.*, 2008; Reissig *et al.*, 2009). Unfortunately, no worldwide regulations have been established for the caffeine content of energy drinks or for their sale, use and promotion (Babu *et al.*, 2008; Higgins *et al.*, 2010;

Table 4: Caffeine content (mg) in cocoa products

		Per 10 mg	Per serving	
Cocoa products	N	Mean±SD	Mean±SD	Serving size
Cadbury cocoa	2	12.70±1.31	8.26±0.85	6.5 g
Hershey's cocoa	2	19.06±0.53	38.12±1.07	20 g
Cadbury chocolate	2	4.98±0.62	10.96±1.37	22 g
Nesquik cocoa	2	3.46±0.10	6.23±0.18	18 g
Noon cocoa	2	11.62±0.28	4.88±0.12	4.2 g

Table 5: Caffeine content (mg) in soft and energy drinks

		Per 100 ml	Per serving	
Soft and energy drinks	N	Mean±SD	Mean±SD	Serving size
Soft drinks				
Coca cola	3	10.15±0.25	33.80±0.84	330 ml
Diet coca cola	3	14.13±0.11	47.05±0.36	330 ml
Cola zero	3	10.15±0.30	33.81±1.00	330 ml
Pepsi regular	3	11.33±0.16	37.74±0.55	330 ml
Diet pepsi cola	3	12.41±0.52	41.33±1.75	330 ml
Mountain dew	3	16.24±0.33	54.07±1.09	330 ml
Energy drinks				
Red bull	3	31.42±1.55	78.55±3.88	250 ml
Power horse	3	32.56±2.22	108.42±7.39	330 ml
Power horse with cola	3	29.75±0.99	99.08±3.29	330 ml
Bison	3	24.01±1.41	60.04±3.52	250 ml
Psychik carrefour	3	29.71±0.41	74.26±1.03	250 ml

Reissig *et al*., 2009) and the absence of restrictive regulations has resulted in the misleading marketing of energy drinks (Higgins *et al*., 2010). However, the Jordanian Food and Drug Administration has adopted the Syrian regulations for the caffeine content of energy drinks, limiting the maximum allowed caffeine level to 32 mg/100 ml (Syrian Arab Organization for Standardization and Metrology, 2007) and all of the energy drinks analyzed in this study were at or below this level.

Conclusion: This is the first study to investigate the caffeine levels in beverages that are commonly consumed in the Arab world. The highest caffeine content was generally found in coffee-type beverages, followed by energy drinks. Cocoa products had the lowest caffeine content. This study provides useful information for health and nutrition educators to give the public advice on which beverages are healthier in relation to caffeine content. Also the findings of this study provide useful base-line data to establish caffeine limits for certain beverages. However, inter- and intra-variations in caffeine levels among different brands and products highlight the need to establish actual caffeine consumption in epidemiological, clinical and experimental studies.

Conflict of interest: The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was sponsored by the Arab Center for Nutrition, Bahrain. The Center had no role in the study design, in the collection, analysis or interpretation of data, in the writing of the report, or in the decision to submit the article for publication.

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