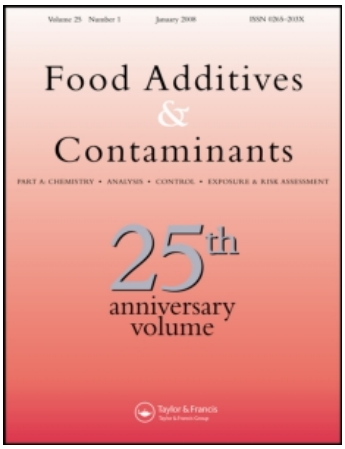

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Survey of caffeine levels in retail beverages in Portugal

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Survey of caffeine levels in retail beverages in Portugal

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Abstract
The caffeine content of 85 retail beverage samples purchased from local supermarkets between 1995 and 2004 was determined. The potential intake of caffeine through the consumption of these beverages (but excluding coffee) was estimated for students of the University of Coimbra, Portugal. The caffeine content of the beverages ranged from 47.5 to 282.5 mg l^{-1} for teas, from 20.1 to 47.2 mg l^{-1} for tea extracts samples, and from 80.7 to 168.7 mg l^{-1} for cola soft drinks. Caffeine was not completely absent from caffeine-free colas, and energy drinks had a far greater caffeine content than regular drinks, ranging from 21 to 2175 mg l^{-1}. Soft drinks were consumed by 72% of the individuals, although 14% of the survey participants did not drink any of the different types of the beverages studied. Contrary to expectations for this age group, no consumptions of energy drinks was reported. Daily caffeine intake was estimated to range from 4.7 to 200 mg day^{-1}, but with only 5% reporting a daily intake around 200 mg caffeine. Cola-type beverages were an important dietary source of caffeine for the population studied. Statistical differences in the caffeine intake between the male and female populations were found, with $p = 0.014$, being higher for the male population. Of the beverages studied, cola-type drinks showed statistical differences for the male population, $p = 0.03$, and tea showed statistical differences for female population $p = 0.013$, respectively.

Keywords: *Caffeine, beverages, daily intake high-performance liquid chromatography*

Introduction

Caffeine, a trimethylated xanthine, is almost certainly the most widely consumed psychoactive substance in the world (Kendler and Prescott 1999). It is a stimulant that exhibits biological activity, and although moderate amounts of caffeine are not harmful to human health, the possibility that caffeine consumption can have adverse effects on human health was assessed based on results of published human studies (Nawrot et al. 2003).

Caffeine-containing beverages are popular in part due to effects of decreasing fatigue, increasing mental activity and improving cognitive functioning following the intake of moderate doses (Tanda and Goldberg 2000). Note that the caffeine content of food and beverages can vary significantly (Christian and Brent 2001), which can interfere with obtaining valid interpretations from many epidemiological studies.

Although coffee drinking is sometimes considered a marker for caffeine intake, it is also important to evaluate the caffeine content of other caffeinated drinks in order to estimate the correct caffeine intake (Brown et al. 2001). Caffeine is found in common beverages, coffee, tea, soft and energy drinks, which are widely consumed at different levels by most of segments of the population, and also in some medications.

Caffeine is the most important purine base present in tea leaves (*Camelia sinensis*) (3–5%) and in guaraná seeds (*Paulinia cupana kunt*) in amounts of 3–5% that sometimes can exceed 6%. The seeds of Cola plants, mainly *nitida* and *accuminata* spp. have caffeine content of 1.5–2.5 and 0.8–1.3%, respectively (Costa 1994). It plays an integral role in the flavour profile of cola soft drinks and consequently it is added as an ingredient to approximately 70% of soft drinks in the USA (Griffiths and Vernotica 2000).

The popularity of energy drinks has risen considerably over the past few years, especially for younger adults, and thus there is interest in monitoring levels in beverages and in ensuring intakes do not exceed recommended levels. It is thus imperative for consumers to be knowledgeable about the caffeine content of these beverages. The present study arose out of the concern that in Portugal there is limited information concerning caffeine consumption data,

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from these types of beverages. For this reason, the caffeine content of several commercial beverages of Portugal (teas, beverages with tea extracts, colas, guaraná and energy drinks) purchased from local supermarkets in 1995–2004 were determined. Furthermore, a survey of the potential daily average intake of caffeine through the consumption of these beverages, among a group of students of the University of Coimbra, Portugal, was conducted, based on the caffeine contents of the beverages analysed in this study.

Material and methods

Reagents and solvents

All solvents were high-performance liquid chromatography (HPLC) grade (Carlo Erba, Milan, Italy). Water HPLC degree (conductivity = 18 mΩ cm^{-1}) was obtained by purifying distilled water in a Milli-Q filtration system (Millipore, Bedford, MA, USA). All solutions were filtered through a 0.2 μm filter (Schleicher & Schuell, Dassel Germany) and degassed in ultrasonic bath.

Caffeine standard was purchased from Sigma (Darmstadt, Germany).

Stock standard solution (0.5 mg ml^{-1}) was prepared in water HPLC degree and stored at 4°C. The final working standard solutions were prepared dissolved in deionized water ranging from 0.0025 to 2.0 mg ml^{-1}.

Apparatus and chromatographic conditions

The liquid chromatographic system consisted in a pump model 305, injector with a 20 μl fixed loop, a ultraviolet light detector model 116 (all Gilson, Villiers-le-Bel France) set a 254 nm. The analysis was performed on a reversed-phase μBondapack C_{18} column (390 × 300 mm i.d., 10 μm particle size; Waters, Milford Ireland). The chromatograms were recorded on a SP 4270 integrator (Spectra Physics, San Jose, CA, USA). The injection volume used was 5 μl.

The mobile phase consisted of a 20% reagent-grade glacial acetic acid in water, buffered to pH 3.0 with saturated reagent grade sodium acetate solution. This solution was filtered through a 0.2 μm pore size filter (Schleicher & Schuell), degassed in ultrasonic treatment before use and is stable for 2–3 days. The flow rate applied was 2 ml min^{-1}. All chromatographic separations were carried out at ambient temperature.

Sample collection

All the 85 samples studied were purchased from local markets in the central zone of Portugal between 1995 and 2004, except for energy drinks, which were obtained in 1998, 2001, 2002, 2003 and 2004. The sample were divided into five categories: teas ($n = 18$), beverages with tea extracts (12), herbal infusions (3), cola soft drinks (30) and energy drinks (20), and beverages with guaraná extracts (2). Each sample was analysed in triplicate (3).

Statistical analysis

Database management and statistical analysis were performed with the SPSS 10.0 Microsoft version. The female and male populations were compared by the Mann–Whitney *U*-test. An assymptation value ≤ 0.05 (two-tailed) was considered to be statistically significant.

Sample preparation

With the exception of teas, all the other samples were liquid and ready to submit to sample preparation. The teas were prepared according to package prescription.

Sample preparation was made according the reference method of Food and Drug Administration (1983). All the samples in study were degassed by ultrasonic treatment and filtered through a 0.45 μm pore size filter. The first 1 ml filtrate was discarded. The filtered solution was injected directly into the HPLC column.

Recovery studies

Three different fortification levels, 40, 80 and 120 mg l^{-1}, for each sample studied were performed using the analytical procedure described above.

Consumption study

The population studied comprised presumably of healthy volunteers who were students of the University of Coimbra, chosen randomly. The study involved 140 participants, 63.6% ($n = 89$) were female and 36.4% (51) were male. The students were aged between 19 and 28 years old (22 ± 0.6 years). The women and men were aged between 18 and 25 (22 ± 0.82 years) and from 21 to 28 (23.6 ± 0.71 years), respectively.

The survey, which was based on data on the frequency of beverage consumption, took place in the spring of 2003. All volunteers were asked to complete a rapid questionnaire about sex, age, weight and specific habits related to the consumption of teas, beverages with tea extracts, herbal infusions, cola soft drinks, energy drinks and beverages with

guaraná extracts. Consumption was related to six time periods during the day: breakfast, morning, lunch, afternoon, dinner and evening. The quantity and frequency of consumption of these beverages were recorded a daily basis. The quantity ingested was recorded in units of volume.

Assessment of caffeine intake

Individual total daily intake of caffeine was estimated from the consumption data of the beverages generated by the survey and the caffeine levels determined in the identified products. Total daily intake of caffeine (mg day^{-1}) was calculated by multiplying the ingested volume of each beverage, by its median caffeine content obtaining in this study, and then summing for all sources.

Results and discussion

Chromatographic analysis and detection

The FDA methodology was optimized and validated under our experimental conditions. The composition of the mobile phase was slightly modified and 2% isopropanol was not added. The chromatogram of a caffeine standard solution clearly showed the adequate resolution of caffeine peak (Figure 1a). The mean retention time for caffeine was 5.7 min, and based on eight parallel determinations, the standard deviation (SD) of the retention time was 0.039.

Validation results

The limit of quantification (LOQ) was estimated in accordance to the baseline noise, considering a signal-to-noise ratio (based on peak height) of 10 : 1, and the value obtained for this method was 1 mg l^{-1}. The calibration curves, obtained by plotting the ratio of the peak areas of caffeine against their concentration ratio, were linear from 1.0 to 2000 mg l^{-1}, with a mean-squared correlation coefficient of 0.9995. Concerning the analysis of real samples, the matrices did not cause any specific difficulty in the analysis. Hence, a straightforward determination of caffeine in different types of beverages was feasible. No interfering peaks from the matrix were observed at the retention times of caffeine, as can be observed in the HPLC chromatogram of the blank assay of an energetic drink, containing caffeine (Figure 1b).

In general for all samples in the survey, experiments with spiked samples showed that mean recoveries ranged from 88 to 115% at the three fortification levels of 40, 80 and 120 mg l^{-1} (Table I). For the three fortification levels, the recovery mean ranged from 83.7 to 97.5% for teas, from 89.0 to 97.6% for teas beverages, from 96.2 to 100.3% for cola soft drinks, and from 85.8 to 115% for energy drinks. The standard deviation oscillated from 1.3 to 10.4. These values accord with the maximum acceptable values for accuracy and precision established by the Report of the AOAC/FAO/IAEA/IUPAC Expert (1999).

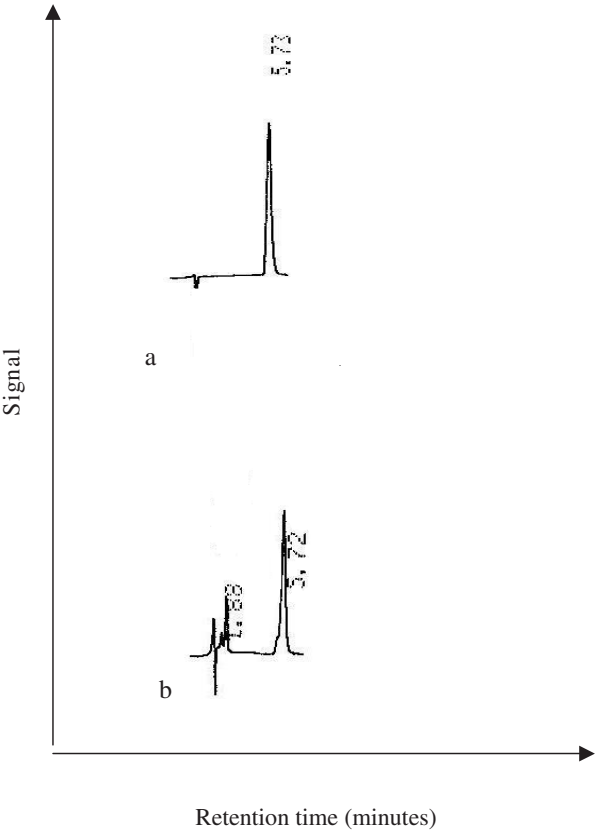

Figure 1. (a) HPLC chromatogram of a standard solution of caffeine; and (b) HPLC chromatogram of blank assay of an energetic drink, containing caffeine.

Table I. Accuracy and precision data for caffeine in different samples.

Samples	Fortification level (g l^{-1})	Number of assays (*n*)	Recovery mean (% ± SD)
Teas	40 mg l^{-1}	5	83.7 ± 3.9
Tea beverages		5	89.0 ± 1.63
Cola soft drinks		5	96.2 ± 9.8
Energy drinks		5	85.8 ± 8.4
Teas	80 mg l^{-1}	5	97.5 ± 2.1
Tea beverages		5	95.0 ± 7.5
Cola soft drinks		5	98.8 ± 1.3
Energy drinks		5	103.4 ± 9.9
Teas	120 mg l^{-1}	5	91.0 ± 2.7
Tea beverages		5	97.6 ± 8.4
Cola soft drinks		5	103.0 ± 2.1
Energy drinks		5	115.0 ± 10.4

SD, standard deviation.

Table II. Content of caffeine in retail samples.

Samples	Number of assays (*n*)	Variation of caffeine content ($mg\,l^{-1}$)
Teas	3	12.5–282.5
Beverages with tea extracts	3	20.1–100
Herbal infusions	3	34–104
Cola soft drinks		
Regular colas	3	88–171
Diet colas	3	81.5–124
Caffeine free colas	3	1.2–14.7
Energy drinks	3	21–2175

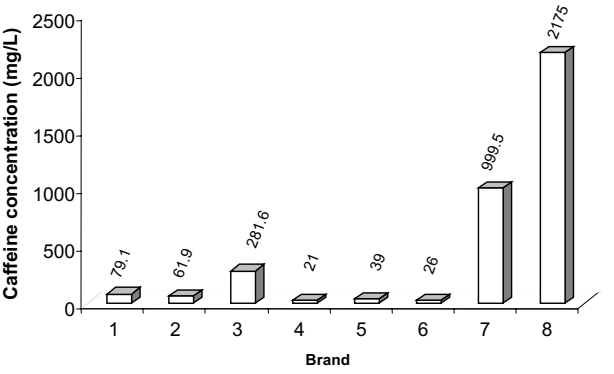

Figure 2. Caffeine content of the eight different brands of energy drinks analysed in 1998.

Determination in real samples

The caffeine content obtained for the different samples studied is reported in Table II. Caffeine levels ranged from 12.5 (in a decaffeinated tea sample) to 282.5 $mg\,l^{-1}$ for teas, 20.1 to 100 $mg\,l^{-1}$ for beverages with tea extracts, and from 34 to 104 $mg\,l^{-1}$ for herbal infusion beverages. The amounts found in some of the tea samples analysed are comparable with those found in the literature. Stavric et al. (1988) reported an average of 23.9 mg/150 ml cup (a concentration of 159 $mg\,l^{-1}$) for regular home-brewed tea. Industry-based data provided by S. Wheeler (Thomas J. Lipton, Inc., personal communication, 1989) indicated caffeine contents of approximately 30 mg/150 ml (200 $mg\,l^{-1}$) for brewed leaf or bag tea and 20 mg/150 ml cup (133.3 $mg\,l^{-1}$) for instant tea. Also, the values used by Barone and Roberts (1996) as standard values for caffeine content of tea leaf and instant tea, are 30 and 20 mg/150 ml, respectively. Some tea samples contained caffeine concentrations higher than 200 $mg\,l^{-1}$, although the label instructions were strictly followed in respect to the time the tea was allowed to infuse. Galasko et al. (1989) observed an increase of the caffeine content of Ceylon tea blends, from about 8 mg/30 ml after 1 min to about 12 mg/30 ml after 20 min. Tea that had been allowed to infuse for 20 min had a caffeine content similar to that of coffee. Chen et al. (1998) found higher caffeine levels for tea drinks (45–158 $mg\,l^{-1}$) than the reported in this study for beverages with tea extracts (20.1–100 $mg\,l^{-1}$).

The caffeine content of soft drinks varies by brand, and different countries have established maximum caffeine levels for these beverages. Portugal, for cola-type beverages fixed as maximum amount 150 $mg\,l^{-1}$ (Portaria 1989), and the US Food and Drug Administration (FDA), for carbonated beverages, limits the maximum amount to 6 mg/oz. Therefore, the caffeine content allowed in a 355 ml (12 oz) can of soft drinks is 72 mg. Hence, soft drinks may contain caffeine in the range 84.5–200.8 $mg\,l^{-1}$ (National Soft Drink Association 1999).

As can be seen in Table II, caffeine concentrations found in cola soft drinks analysed between 1995 and 2004 ranged from 40 to 171 $mg\,l^{-1}$, reflecting the differences observed in caffeine content for different brands. These values are comparable with those reported by the National Soft Drink Association (1999), levels of 89.2–100 $mg\,l^{-1}$ reported by Chen et al. (1998) and levels of 100 $mg\,l^{-1}$ reported by Barone and Roberts (1996). In general, only regular and diet cola soft drinks contain caffeine, but we observed that a few caffeine-free products also contained caffeine. In six samples of caffeine-free cola-type beverages, caffeine levels were found between 1.2 and 14.7 $mg\,l^{-1}$. The two only brands of guaraná drinks available in the Portuguese market, analysed in this study, contained a low mean level of caffeine of 14 $mg\,l^{-1}$.

The caffeine levels of the energy drinks were determined during 1998 and 2001–04. The samples studied were those available in the market in each year. The eight samples analysed in 1998 (Figure 2) contained caffeine concentrations ranging from 21 to 2175 $mg\,l^{-1}$, and two of them had concentrations of 999.5 and 2175 $mg\,l^{-1}$, which were the highest levels of caffeine found during this study.

During the last four years, 2001–04, the caffeine levels of the energy drinks available in the market were lower, although in 2001, one brand contained 613 $mg\,l^{-1}$ (Figure 3). The mean concentration obtained for all samples analysed was 421.4 $mg\,l^{-1}$ (Table II). Chen et al. (1998) reported 184 $mg\,l^{-1}$ for sports drinks.

An interesting case was observed with one brand. Since it was the only energy drink brand available during the whole study, a determination of its caffeine content was carried out. From 1998 to 2004, the caffeine content decreased from 999.5 to 289 $mg\,l^{-1}$. In fact, it seems that might reflect a concern of the producers. Perhaps the above reported facts have made it essential for manufacturers to monitor and assess the concentration of caffeine in their respective products.

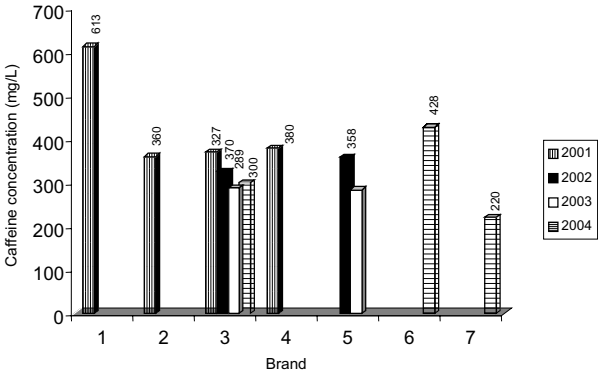

Figure 3. Caffeine content of the energy drinks analysed in 2001–04.

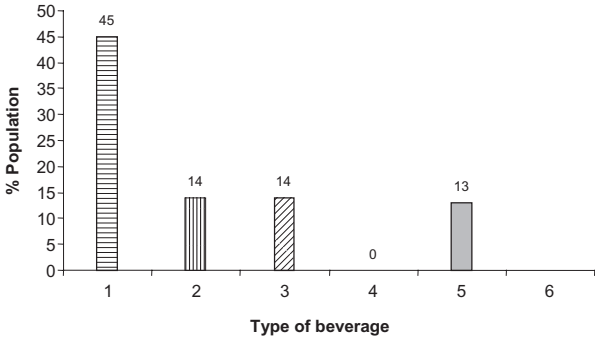

Figure 4. Consumption pattern of the beverages studied.

Caffeine consumption data of the studied population

Among the students interviewed, 14% did not drink the beverages studied and the soft drink consumption pattern obtained in this study is shown in Figure 4. Cola soft drinks, consumed by 45% of the population studied, were the major source of caffeine intake, followed by tea beverages (14%), whilst 13% of the individuals drunk both soft drinks during the day, and 14% drunk tea. The soft drink consumption pattern observed by Camargo et al. (1999) also showed that cola soft drinks were the most frequently consumed (74%). Data on coffee consumption were not included in this study. Note that the studied population reported no energy drink intake.

According to relative frequency of consumption data, 40.7, 40.0 and 36.8% of those interviewed consumed one can of cola type, one can of tea beverage and one cup of tea, respectively. Consumption levels higher than three cans of cola-type or tea beverage or three cups of tea was reported by 20.9, 25.0 and 21% of the population analysed, respectively. Tea consumption at 11% was higher for female students in comparison with 2.9% for male students. The same pattern of 1.4 and 12.8% consumption for tea beverages was found for male and female students, respectively. For cola-type drinks, the consumption was 23.6 and 19.4% by male and female, respectively. The pattern of consumption observed when the participants consumed both soft drinks was 4.3 and 8.7% for male and female, respectively.

Based on the caffeine levels found in this study and on the consumption of these beverages by the student population (excluding coffee), the caffeine intake was estimated to range from 4.7 to 200 mg day^{-1}. A total of 14% of the participants did not drink the beverages studied; 56% of them consumed caffeine from these beverages at levels ranging from 12 to 30 mg day^{-1} and 25% from 31 to 199 mg day^{-1}. Only 5% reported a daily consumption that would equate to an intake around 200 mg caffeine.

Cola-type beverages were an important dietary source of caffeine for the student population, the mean caffeine daily intake was 65.8 ± 1.8 and 42.3 ± 1.1 mg day^{-1} for male and female, respectively. The average consumption of caffeine from tea beverages was 31.0 ± 0.8 and 37.2 ± 0.6 mg day^{-1} for male and female, respectively.

The median individual daily intakes from cola-type, tea beverages and tea were 25.0, 40.0 and 38.0, respectively.

Statistical differences were found in the caffeine intake between the male and female students, with a $p = 0.014$, being higher for the male population. The cola-type beverage showed statistical differences for male population, $p = 0.03$, and tea showed statistical differences for female population, $p = 0.013$, respectively.

In conclusion, this limited study of caffeine in the beverages indicated that with exception of decaffeinated beverages, samples with tea extracts showed the lowest amounts, and the energy drinks contained the highest levels. The caffeine values obtained in this study are comparable with those reported by the National Soft Drink Association. Soft drinks were consumed by most of the surveyed individuals (72%) and cola-type drinks were the most frequently consumed. Contrary to expectations for this age group, no energy drink intake was reported. Cola-type beverages were an important dietary source of caffeine for the student population.

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