Occurrence of caffeine in carbonated drinks cola type from Romanian market

MIRCEA OROIAN
Food Technologies, Food Production and Environment Safety Department
Faculty of Food Engineering, Stefan cel Mare University of Suceava
Universitatii Street, No. 13, Suceava
ROMANIA
m.oroi@fia.usv.ro

Abstract: - Caffeine is a natural substance present in leaves and seeds of many plants. The aim of this study is to evaluate the caffeine level in the carbonated drinks from the Romanian market. For this survey were taken 30 samples of different carbonated drinks. The caffeine was determined and quantified using a HPLC with diode array detector at 206 nm. The caffeine retention time was at 17.785 minutes. The calibration curve was build using 3 stock solution of caffeine (25, 50 and 100 mg/L). The equation of the calibration curve was y=176460x, with a correlation coefficient R²=0.999. The caffeine content in carbonated drinks from the Romanian market ranged between 25.20 – 109.68 mg/L.

Key-Words: - caffeine, carbonated drinks, HPLC, UV detection, risk assessment, hazard quotient

1 Introduction

Caffeine, the common name for the chemical compound 1, 3, 7 – trimethylxanthine, is the most widely consumed methylxanthine alkaloid group [1] and is the most widely used pharmacologically active substance in the world [2]. It occurs naturally in many plant species but is mainly derived from coffee beans (Coffea Arabica and Coffea robusta) and cocoa beans (Theobroma cacao). In food and drink products is used a synthetic form of caffeine [3] as well as medication and dietary supplements [4]. A recent review of the functionality of caffeine suggests it is one of the most widely consumed food ingredients worldwide with the tea and coffee being the most prominent sources in the diet [5], of which coffee is the second most commonly consumed beverage worldwide after water [6,7].

Although caffeine is found in a number of foods, it is most frequently consumed in coffee, tea and cola beverages [8]. It is recognized that beverages with caffeine as a natural component will have a wide range of caffeine levels. These variances are probably the result of many factors which include the species of plant origin [9], growing environment, effects of commercial processing and storage along with variances at the retail level such as amount of coffee, tea or cola used, the extraction method (e.g. percolated, drip, etc.) and the temperature and amount of water used in beverage preparation.

To assess likely consumer exposure to caffeine from popular commercial beverages from the Romanian market we initially quantified the content and range of caffeine found in commercial beverages (n=30), most consumed by Romanian people. According to the National Institute of Statistics of Romania, the average consume of carbonated drinks in Romania it is around 70 L/inhabitant.

The European Union regulation regarding the labeling of the products which contains caffeine, in concentration higher than 150 mg/L, and intended for consumption without modification or after reconstitution, must be labeled as “High caffeine content” [10]. This directive covers most caffeinated energy drinks, however, tea and coffee based beverages are exempt so long as the product is clearly labeled as containing tea or coffee.

High doses of caffeine may cause many undesired symptoms and even adverse effect on the human body health, especially for infants and children, such as: chills, agitation, loss of appetite, irritability, insomnia, weakness, gastrointestinal problems, hypertension, delusions, fever, evendeath and tachycardia [11-13].

The daily intake of caffeine is not regulated at national level, however is thought that the daily intake should not over pass 3-4 mg/kg b.w./day. The aim of this study is to analyze the caffeine content of carbonated drinks from the Romanian market and to establish the health risk assessment of caffeine.
2 Materials and methods

2.1 Materials

30 samples of carbonated drinks from the supermarkets from the Suceava county from the North part of Romania. The samples have been de-carbonated and filtered through a 0.45 μm filter.

2.2 Reagents

KH₂PO₄, H₃PO₄ and acetonitrile were HPLC purity grade. The solutions were prepared using de-ionized water (18.2 Ω resistivity). The water was de-ionized using a Thermo Fisher system.

2.3 Method

Caffeine has been detected and quantified with a HPLC Schimadzu system equipped with a LC-20 AD liquid chromatograph, SIL-20A auto samples, CTO-20AC auto sampler and a SPD-M-20A diode. The λ has been set at 276 nm.

Data collection and subsequent processing were performed using the LC solution software. A stainless steel analytical column (250 x 4.6 nm, 4.5 μm) was used. A buffered mobile phase (KH₂PO₄ 0.02 M/ACN (90:10)), at 1 mL/min, which pH was rigorously controlled at 4.2, was used.

The calibration curve was constructed using 3 stock solutions: 25, 50 and 100 mg/L caffeine. The calibration curve is presented in figure 1. The calibration curve is y = 176460 x, and the correlation coefficient is R² = 0.9999.

![Calibration curve of caffeine](image1.png)

Fig.1. Calibration curve of caffeine

The chromatogram view of the 100 mg/l caffeine standard solution is presented in figure 2. The caffeine retention time is at 17.865 minutes.

![Chromatogram view of 100 mg/l caffeine standard](image2.png)

Fig.2. Chromatogram view of 100 mg/l caffeine standard

2.4 Health risk assessment

In this study we assessed the human health risks posed by the exposure to the caffeine contained in carbonated drinks. The risk posed by caffeine via carbonated drinks consumption was estimated computing the hazard quotient (HQ) by the following equations [14,15]:

\[
HQ = \frac{ADD}{RfD}
\]

\[
ADD = \frac{C \cdot IR}{BW}
\]

where ADD is the average daily caffeine intake (μg/kg-day), RfD is the daily intake reference dose (μg/kg-day) suggested by the literature (3-4 mg caffeine/kg b.w./day), C is the mean caffeine concentration in apple juice (μg/l), IR is the carbonated drinks rate (kg/person-day), and BW is the average body weight (kg).

2.4 Statistical analysis

Statistical analysis was performed using the SPSS trial version. One-way ANOVA was used to determine the significant differences among the caffeine content in carbonated drinks cola type. The probability level of p < 0.05 was considered statistically significant.

3 Results and discussion
Caffeine has been found in different foods and beverages (tea, coffee, milk chocolate, dark chocolate, type cola beverage, etc) that are commonly consumed worldwide.

Recovery values were between 83.2 % for caffeine at fortification levels of 100 mg/L, and 87.4% for levels of 50 mg/L. Intra-day repeatability oscillated between 2.9% for caffeine at 50 mg/L, and 3.3% for caffeine at 100 mg/L.

3.1 Caffeine occurrence

The level of caffeine in the samples analyzed ranged between 25.20 - 109.68 mg/L in agreement with the literature. The level of caffeine in cola type drinks from United Kingdom is around 108 mg/L [7], Portugal 97 mg/L [16]. In figure 3 is presented a typical chromatogram of caffeine in cola type carbonated drinks. As in the case of the stock solution, the caffeine retention time was at 17.865 minutes. The average level of caffeine in the 30 samples is 77.63 mg/L.

![Fig.2. Chromatogram view of a carbonated drink (caffeine content 78.61 mg/l)](image)

### Table 1. Caffeine content in carbonated drinks type cola from the Romanian market

<table>
<thead>
<tr>
<th>Sample</th>
<th>Caffeine (mg/l)</th>
<th>Sample</th>
<th>Caffeine (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>109.68</td>
<td>S16</td>
<td>65.80</td>
</tr>
<tr>
<td>S2</td>
<td>81.80</td>
<td>S17</td>
<td>75.01</td>
</tr>
<tr>
<td>S3</td>
<td>76.10</td>
<td>S18</td>
<td>78.61</td>
</tr>
<tr>
<td>S4</td>
<td>50.20</td>
<td>S19</td>
<td>115.72</td>
</tr>
<tr>
<td>S5</td>
<td>62.20</td>
<td>S20</td>
<td>30.07</td>
</tr>
<tr>
<td>S6</td>
<td>54.00</td>
<td>S21</td>
<td>88.19</td>
</tr>
<tr>
<td>S7</td>
<td>88.07</td>
<td>S22</td>
<td>102.56</td>
</tr>
</tbody>
</table>

3.2 Caffeine risk assessment

According to the national institute of statistics from Romania, the average consumption level of carbonated drinks in Romania is around 70 L / inhabitant, however the consumption of cola type drinks is not known. For this study it was assumed like consumption of cola type drinks a level of 35 L/inhabitant, around of 200 mL/day. The average caffeine content of the 30 beverages from the Romanian market was established as 77.63 mg/L. The average daily intake of caffeine for an adult, with 70 kg body weight is 0.221 mg/kg b.w/day. The hazard quotient has not over passed the 1 value for representing a major concern (HQ=0.055).

4 Conclusion

The caffeine is a natural product present in the cola type carbonate drinks. It presents a maximum of absorption at 206 nm and a retention time of 17.865 min. The level of caffeine in cola type from Romania ranged between 25.20 - 109.68 mg/L. However the average daily intake do not over pass the 4 mg/kg b.w./day caffeine.

References:


[16] Lino, C.M., Pena, A., Occurrence of caffeine, saccharin, benzoic acid and sorbic acid in soft drinks and nectars in Portugal and subsequent exposure assessment, *Food Chemistry*, 121(2), 2010, 503-508