Scientific Papers of the University of Pardubice,
Series A; Faculty of Chemical Technology
24 (2018) 31–38.

Determination of caffeine and glucose in selected energy drinks

Kateřina Pravcová, Milan Sýs, Karel Vytřas, and Lenka Česlová*

Department of Analytical Chemistry, The University of Pardubice,
CZ−532 10 Pardubice, Czech Republic

Received: June 20, 2018; Accepted: July 30, 2018

The aim of this study was to monitor the caffeine and glucose content in several energy drinks. The caffeine was determined by (direct) differential pulse voltammetry at a glassy carbon paste electrode in 0.5 mol L^{-1} sulfuric acid at a potential step of 5 mV, potential amplitude of 40 mV, and the scan rate of 50 mV s^{-1}. The content of glucose was determined using amperometric glucose biosensor in 0.1 mol L^{-1} phosphate (pH 7.0) buffer solution at a constant voltage of +0.4 V and stirring speed of 400 min^{-1}. The selected samples were analysed and the results obtained compared to those declared by manufactures. Except to three samples, namely "Monster", "Red Bull" (without sugar) and "Spark", the individual results were in good agreement with the declared amounts. Moreover, based on the analyses performed, it can be stated that the energy drinks "Big Shock!" and "Red Bull" are the best source of energy when considering the found total sugar via the glucose content.

Keywords: Caffeine; Glucose; Energy drinks; Voltammetry; Amperometry

Introduction

Energy drinks have become a popular beverage for athletes and young population. They are known for their stimulating effects on central nervous system (CNS [1]). The marketing of these drinks mainly relies on the claims that the natural ingredients in energy drinks supply increase energy, reduced symptoms of a

* Corresponding author, ✉ Lenka.Ceslova@upce.cz

hangover, improve alertness, and athletic performance [2,3]. They are usually searched for their good taste and the source of energy being commercially distributed in volumes of 250 or 500 mL, respectively. In most countries, energy drinks are not prohibited for minors. It means that anybody from such a population can consume them uncontrollably [4]. Caffeine, taurine, carbohydrates, glucuronolactone, inositol, niacin, pantenol, and B-complex vitamins belong among the main components of energy drinks [5]. Generally, these beverages derive their energy-boosting properties chiefly from the sugar and caffeine content. The combination of caffeine and glucose can ameliorate deficits in cognitive performance and subjective fatigue during the extended periods of demand [1]. On the other hand, energy drinks may give rise to dangerous side effects. These drinks have been associated with health risks, such as an increased rate of injury —especially, when their usage is combined with alcohol—and excessive or regular consumption can initiate some cardiac and psychotic problems [4]. Finally, the present caffeine (CA) can cause a dehydration of the body [6].

CA is a most common ingredient of energy drinks. It is a xanthine-derived alkaloid being used as a diuretic and a stimulant of CNS. Besides these effects, it may induce a variety of unpleasant side effects at over-consumption which include: nausea, vomiting, restlessness, anxiety, depression, tremors, and difficulty with sleeping [7]. The most common sources of caffeine are coffee, cocoa beans, cola nuts, and tea leaves. Once ingested, caffeine is rapidly absorbed from the gastrointestinal tract. This substance undergoes demethylations resulting in formation of paraxanthine (84 %), theobromine (12 %), and theophylline (4 %). Theobromine and theophylline have very similar chemical structures like caffeine [8]. CA is also added as a flavouring agent to make drinks addictive [7,9]. Its content in energy drinks usually ranges about 30 mg in 100 mL. It is considered that intake more than 200 mg CA delay the need to sleep [10]. Thus, CA reliably enhances vigilance and psychomotor performance. It has been suggested that the beneficial effects of CA on cognitive performance are actually due to the reversal of caffeine withdrawal [11] or the reversal of environmental-induced cognitive impairments, such as sleep deprivation, physical fatigue, and psychological stress [12].

Energy drinks can be sweetened with artificial sweeteners or sugars, such as sucrose, fructose, and glucose (here, via glucose-fructose syrup). It is known that above mentioned monosaccharide glucose (Glc) is an immediate source of energy for human organism. Many energy drinks contain 5–6 g glucose per 100 mL [14] and, for that reason, energy drinks should be used in high physical exertion rather than normally consumed item. It can be stated that energy drinks with a higher glucose content ratio with other sugars are considered to be of higher quality. Glc can be found in fruits and honey. It is absorbed directly into the bloodstream during digestion because of its importance for cell function. High concetration level of Glc in the capillary blood (higher 3.3–6.6 mmol L^{-1}) is called hyperglycaemia also known as *Diabetes mellitus*. In healthy individuals, any Glc content does not have to be proven in the human urine.

Materials and methods

Chemicals and Reagents

Analytical standards of caffeine and glucose, lyophilized powder of glucose oxidase from *Aspergillus niger* (EC 1.1.3.4) with catalytic activity 100000–250000 U g^{-1}, and ruthenium dioxide, RuO_2, were purchased from Sigma-Aldrich. Graphite powder (type "CR-2" with particles size >2 µm; Graphite, Týn nad Vltavou, Czech Republic) and paraffin oil (Lučební závody, Kolín; Czech Republic) were used for preparation of the bare (unmodified) carbon paste electrode used for further modification and forming the electrode proper of a Glc-biosensor. Glassy carbon powder (type "Sigradur-G"; particle size of about 5–20 µm; HTW Maintingen; Germany) and silicone oil (type "MV 8000"; Lučební závody, see above) were chosen for preparation of a special glassy carbon paste electrode (GCPE) proposed for the determination of CA. Aqueous solution of 0.5 mol L^{-1} H_2SO_4 and 0.1 mol L^{-1} phosphate buffer (pH 7.0) were selected as optimum supporting electrolytes for determination of CA and Glc, respectively. Both electrolytes were prepared from ultrapure water (ρ = 18.3 MΩ cm) passed through a Milli-Q purification system (Millipore; Burlington, MA, USA).

Working electrode

Preparation of glassy carbon paste electrode

The GCPE was prepared by hand-mixing of 0.3 g glassy carbon powder and 0.05 g silicone oil in a ceramic mortal for twenty min. After that, the resultant paste was pushed into the cavity (with diameter of 3 mm) of a Teflon® piston-like electrode holder [15]. It was necessary to remember that the height of paste column could not be higher than 20 mm to avoid a difficult extrusion. The working electrode surface did not need to be renewed after each analysis due to satisfactory precision of the individual repetitions.

Preparation of glucose biosensor

Modified carbon paste was prepared in the same (cleaned) mortar by homo-genizing 0.5 g graphite powder with 0.15 mL paraffin oil, redox mediator RuO_2, and glucose oxidase (GOx) for twenty min. So prepared paste was then filled into the Teflon® piston-like electrode holder of the same construction like above. In this case, the electrode surface had to be renewed after each analysis, by squeezing out of small portion of the carbon paste filling and polishing against a dry filter paper to achieve the spilling of the present GOx.

Instrumentation

All the electrochemical measurements were carried out in standard glass vessel (capacity: 50 mL) at 25 °C. Conventional three-electrode system consisted of the GCPE or glucose biosensor (working), a Ag/AgCl with 3.5 mol L^{-1} KCl as salt bridge (reference), and a platinum wire (auxiliary) electrode; all being connected to a potentiostat Autolab ("PGSTAT101"; Metrohm, Prague, Czech Republic), controlled by software Nova version 1.11 (the same manufacturer). All potentials are referred to the above mentioned reference electrode.

Methods

All eight samples of energy drinks (namely: Big Shock!, Hell, Monster, Red Bull, Red Bull without sugar, Spark, Spark without sugar and Tiger) were deaerated in an ultrasonic bath for minimally 5 min. The standard addition method was used for determination of both CA and Glc. Each analysis was at least three times repeated.

Voltammetric detection

A volume of 2 mL of the sample was added into 18 mL of 0.5 mol L^{-1} H_2SO_4. After short stirring, differential pulse voltammetry (DPV) was performed under the following conditions: potential window from +0.5 to +1.7 V vs. ref.; potential step, 5 mV; pulse amplitude, 40 mV, and scan rate, 50 mV s^{-1}. Then, minimally three successive additions were applied by adding 100 µL caffeine standard solution (c = 2 mg mL^{-1}).

Amperometric detection

Amperometry in the batch configuration was performed always in 10 mL phosphate buffer at a constant voltage of +0.4 V, interval time 1.0 s, and stirring rate of 400 min^{-1}. Volume of 10 µL sample was added into continuously stirred supporting electrolyte. After the baseline stabilization, again, three successive standard additions of 10 µL were applied in 120s time periods, when using the glucose stock solution (c = 20 mg mL^{-1}).

Results and discussion

Working conditions of electroanalytical methods used in the determination of CA and Glc in selected real samples of energy drinks obtained from Czech stores were taken from the appropriate literature [16,17]. At the beginning, it was important to verify declared linear ranges of used electroanalytical methods so that standard addition methods can be applied within these linear ranges described by equation $I_p = 0.626\,c - 0.177$ with coefficient of determination (R^2) 0.9965 for 10–60 µg mL^{-1} CA and with equation $I_p = 0.008\,c - 0.048$ and $R^2 = 0.9989$ for 20–120 mg mL^{-1} Glc, where I_p and c mean peak current response and the concentration of analyte, respectively. From these equations, the limits of quantification (LOQ) and limits of detection (LOD) could be estimated. LOQs corresponded to the lowest concentration values of the linear ranges (10 µg mL^{-1} for CA and 20 mg mL^{-1} for Glc). LODs were calculated as 3.2 µg mL^{-1} for CA and 6.7 mg mL^{-1} for Glc, respectively. The recovery rate was determined using analysis of model sample containing 0.32 mg mL^{-1} CA and 0.1 g mL^{-1} Glc. Values of 102 % for CA and 97 % for Glc were found.

Typical voltammograms from the analysis of Red Bull sample focused on caffeine and amperograms for Big Shock! sample focusing on glucose determination are shown in Figs. 1 and 2, respectively.

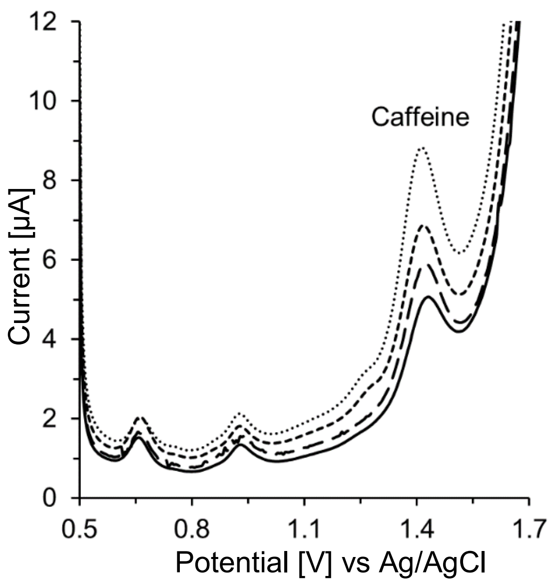

Fig. 1 *Voltammetric analysis of the Red Bull sample*

Legend: sample solution (solid line), after 1st (dashed), 2nd (intermittent), and 3rd (dotted) standard addition(s). Experimental conditions: DPV with GCPE; potential step, 5 mV; pulse amplitude, 40 mV; scan rate, 50 mV s^{-1}.

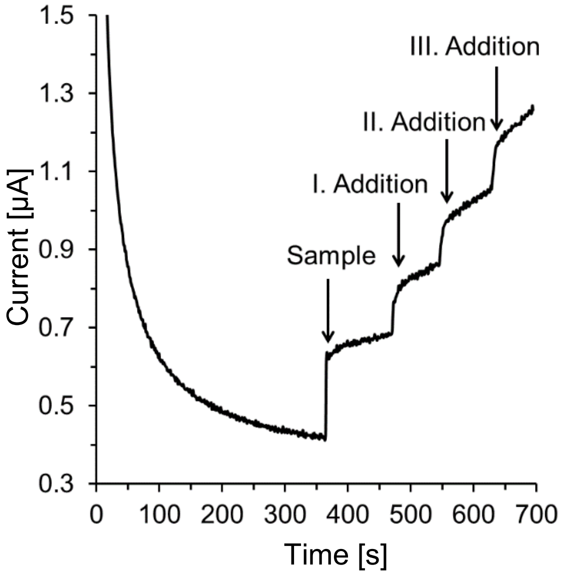

Fig. 2 *Amperometric analysis of the Big Shock! sample in the batch configuration*

Experimental conditions: (constant) potential, +0.4 V vs. ref.; interval time, 1.0 s; stirring rate: 400 min^{-1}.

Final results of electrochemical analysis of selected energy drinks together with declared amounts are presented in Table 1. The surveyed values are presented as arithmetic means ($\bar{x}$) with corresponding standard deviation (s) for minimally three repetitions (n). Based on the results obtained, it was possible to make a decision if these tested samples had contained the declared amounts and whether or not a consumer might be deceived. Significantly (considering 5% error of measurement) lower CA content was found in three samples of energy drinks; namely, in Monster, Spark, and Red Bull without sugar (with more than 10 % less content).

Table 1 *Results of energy drinks analyses*

Sample / Energy drinks	Measured values		Declared values	
	Caffeine	Glucose	Caffeine	Sugars
Big Shock!	27.8 ± 0.6	4.87 ± 0.09	32	12.5
Hell	28.2 ± 0.4	0.55 ± 0.01	32	10.9
Monster	25.7 ± 0.5	3.06 ± 0.10	32	17.0
Red Bull	32.2 ± 0.9	3.21 ± 0.18	32	11.0
Red Bull without sugar	20.4 ± 0.3	—	32	—
Spark	25.3 ± 0.4	0.88 ± 0.03	30	5.0
Spark without sugar	31.6 ± 0.8	—	30	—
Tiger	31.7 ± 0.7	1.55 ± 0.14	32	12.7

Values given as mg per 100 mL for caffeine and glucose with sugars as g per 100 mL.

Concerning the sugar content, producers have declared that the source of sugars is economically advantageous glucose-fructose syrup being frequently used in food technology. However, the amount of glucose in the analysed samples was very small (see Table 1). Identification of other sugars and determination of their concentration levels were not target of our study because we had assumed that the glucose would be a major constituent of the energy drinks as optimum source of energy. Nevertheless, it will be useful to determine a total content of sugars to make a comparison with the next research, where sucrose and fructose, for example, can represent model accompanying sugars.

Conclusions

In this study, the contents of caffeine and glucose have been determined in eight energy drinks available in local markets in the Czech Republic. For their quantification, no standard reference analytical method was used. Thus, the results obtained could only be compared with the data declared by the manufacturer. From the difference between the total sugar content and glucose, it can be concluded that energy drinks Big Shock!, Monster, and Red Bull samples are the best sources of energy, because they contain the highest amount of glucose. However, the glucose represents just a small part of the total sugar in the samples analyzed. (It was probably replaced by other cheaper sugars as sucrose). In terms of the caffeine content, good agreement between the results and declared data was found for some samples only: Red Bull, Sparks, and Tiger. Other real samples can be included in a lower quality category; especially, Red Bull sample without sugar for which the difference between the measured and declared data was about 36 %.

Acknowledgement

A support from the Faculty of Chemical Technology, University of Pardubice (project No. SGS-2018-001) is gratefully acknowledged.

References

[1] Malinauskas B.M., Aeby V.G., Overton R.F., Carpenter-Aeby T., Barber-Heidal K.: A survey of energy drink consumption patterns among college students. *Nutrition Journal* **6** (2007) 1–7.

[2] Attila S., Cakir B.: Energy-drink consumption in college students and associated factors. *Nutrition* **27** (2011) 316–322.

[3] Clauson K.A., Shields K.M., McQueen C.E., Persad N.: Safety issues associated with commercially available energy drinks. *Pharmacy Today* **14** (2018) 52–64.

[4] Reissig C.J., Strain E.C., Griffiths R.R.: Caffeinated energy drinks – a growing problem. *Drug and Alcohol Dependance* **99** (2009) 1–10.

[5] Oteri A., Salvo F., Caputi A.P., Calapai G.: Intake of energy drinks in association with alcoholic beverages in a cohort of students of the school of medicine of the University of Messina. *Alcoholism: Clinical and Experimental Research* **31** (2007) 1677–1680.

[6] Ferreira S.E., De Mello M.T., Pompéia S., De Souza-Formigoni M.L.O.: Effects of energy drink ingestion on alcohol intoxication. *Alcoholism: Clinical and Experimental Research* **30** (2006) 598–605.

[7] Sather K., Vernig T.: Determination of caffeine and vitamin B6 in energy drinks by high-performance liquid chromatography (HPLC). *The Concordia College Journal of Analytical Chemistry* **2** (2011) 84–91.

[8] Safranow K., Machoy Z.: Methylated purines in urinary stones. *Clinical Chemistry* **51** (2005) 1493–1498.

[9] Andrews K.W., Schweitzer A., Zhao C., Holden J.M., Roseland J.M.,. Brandt M., Dwyer, J.T., Picciano M.F., Saldanha N.G., Fisher K.D., Yetley E., Betz J.M., Douglass L.: The caffeine contents of dietary supplements commonly purchased in the US: analysis of 53 products with caffeine-containing ingredients. *Analytical and Bioanalytical Chemistry* **389** (2007) 231–239.

[10] Frary C.D., Johnson R.K., Wang M.Q.: Food sources and intakes of caffeine in the diets of persons in the United States. *Journal of The American Dietetic Association* **105** (2005) 110–113.

[11] James J.E., Rogers P.J.: Effects of caffeine on performance and mood: withdrawal reversal is the most plausible explanation. *Psychopharmacology* **182** (2005) 1–8.

[12] Koppelstaetter F., Poeppel T.D., Siedenttopf C.M., Ischebeck A., Kolbitsch C., Mottaghy F.M., Felber S.R., Jaschke W.R., Krause B.J.: Caffeine and cognition in functional magnetic resonance imaging. *Journal of Alzheimer's Disease* **20** (2010) 71–84.

[13] EDE – Energy Drinks Europe: Glucose. http://www.energydrinkseurope.org/glucose/ (accessed June 13, 2018)

[14] Dzoyem, J.P., Kuete, V., Eloff N.J.: Biochemical parameters in toxicological studies in Africa: significance, principle of methods, data interpretation, and use in plant screenings, in: Kuete V. (Ed.): *Toxicological Survey of African Medicinal Plants*. Elsevier, London 2014, pp. 659–715.

[15] Švancara I., Metelka R., Vytřas K.: Piston-driven carbon paste electrode holders for electrochemical measurements; in: *Sensing in Electroanalysis, Volume 1* (Vytřas K., Kalcher K., Eds.), pp. 7-18. Press Centre of the University of Pardubice, Pardubice, 2005.

[16] Valassi L., Tsimpliaras D., Katseli V., Economou A., Švancara I., Stočes M., Mikysek T., Prodromidis M.: Disposable nafion-modified screen printed graphite electrodes for the rapid voltammetric assay of caffeine. *Insights in Analytical Chemistry* **1** (2015) 1–8.

[17] Miscoria S.A., Barrera G.D., Rivas G.A.: Analytical performance of a glucose biosensor prepared by immobilization of glucose oxidase and different metals into a carbon paste electrode. *Electroanalysis* **14** (2002) 981–987.