



# Caffeine in Guarana-Containing Foods

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# 1 Introduction

Caffeine occurs naturally in plants such as coffee, tea, cocoa and guarana (*Paullinia cupana*), and has a long history of safe use as a mild stimulant. Food products are also available with added caffeine, including alcoholic beverages, formulated caffeinated beverages (energy drinks), energy shots, kola-type soft drinks, sports foods, and weight loss products. Dietary exposure to caffeine is a topical issue given global developments in caffeinated products.

Guarana powders, or extracts, prepared from guarana seeds contain between two and 15% caffeine (dry weight basis) and <0.2% of the related methylxanthines theobromine and theophylline (Bempong *et al.*; 1993, Carlson and Thompson 1998; Weckerle *et al.*, 2003). Guarana seeds contain about twice the concentration of caffeine as coffee beans (Bempong *et al.*, 1993).

A number of data gaps have been identified in both the Australia and New Zealand food composition databases which are used to underpin dietary exposure assessments. Analytical data is available on the caffeine content of a range of foods such as tea, coffee, kola beverages, some energy drinks and chocolate-containing products (FSANZ, 2011; S Sivakumaran *et al.*, 2012). However there is very limited data on foods which contain guarana specifically as a source of caffeine. In order to conduct accurate dietary exposure assessments of caffeine in the diet, robust food composition data on key sources of caffeine in the diet is required.

## 1.1 PROJECT AIM

The aim of this project was to provide analytical and label information on the caffeine, theobromine and theophylline concentrations in guarana-containing foods and beverages available for sale in Australia and New Zealand between October and November 2012.

In New Zealand, dietary supplements including pills, capsules and tablets are regulated under the Dietary Supplements Regulations (DSR) 1985. Supplemented foods, including bars, powders and liquids are regulated under the New Zealand Food (Supplemented Food) Standard (SFS) 2010. The DSR 1985 is administered by Medsafe and the SFS is administered by the Ministry for Primary Industries. Supplemented foods consumed by specific population groups were included within the scope of this survey; however guarana-containing dietary supplements were considered to be outside the scope of this work.

## 2 Methodology

### 2.1 SAMPLING PROTOCOL

Food products available in Australia and/or New Zealand that contain guarana were identified from retail outlets including supermarkets, service stations, convenience stores, liquor outlets and through an ad hoc internet search. Market scanning was carried out over the period August through November 2012.

Identified products were assigned a priority of A (highest priority), B or C based on perceived frequency of consumption, special purpose and price. Sampling was biased to priority A and priority B samples. None of the priority C products were included in the sampling plan.

In general, triplicate batches of each product were purchased (where possible) and sub-samples were composited to a single sample for analysis. A single sample of several products, including one alcoholic beverage, two guarana powders, two teabag products and five sports/weight loss supplements, was included in the sampling plan, as per the prioritisation process, for the purpose of providing preliminary data.

Where there were multiple flavours/colours of a product, a single batch of each was purchased and sub-samples were composited to a single sample for analysis.

### 2.2 LABEL INFORMATION

Product name, brand, batch details, ingredients (with respect to caffeine and guarana) and claimed caffeine content of each product purchased was recorded from the label, where available.

### 2.3 CHEMICAL ANALYSIS

#### 2.3.1 Analytical method

For liquid samples, a portion of each sample was degassed in a sonicator for 15 minutes prior to analysis. Powdered samples and teabags were prepared as per label instructions and diluted if necessary to achieve concentrations within the standard curve for each analyte.

Caffeine, theobromine and theophylline were analysed by high pressure liquid chromatography using direct injection, and chromatographic conditions based on Alltech HPLC Applications. Separation was achieved on a C8 column and solvent programme of mobile phase A (0.05M phosphate buffer pH 3.0) and mobile phase B (40% Acetonitrile: 60% H<sub>2</sub>O) over 20 minutes (for caffeine) or 12 minutes (for theobromine and theophylline). Detection was by photodiode array at 272nm.

The methodology was validated with quality assurance procedures described below. In addition, the laboratory participated in a caffeine Food Analysis Performance Assessment Scheme (FAPAS) in December 2012.

### 2.3.2 Quality assurance procedures

Samples were analysed in batches of two to 22 samples. Each analytical run included a minimum of one blank, two spiked samples, two samples analysed in duplicate and at least one kola drink (caffeine control sample). No control samples were available for theobromine and theophylline.

Limits of detection (LOD) were calculated from duplicate results of samples at low concentration (Telarc, 1987). A “low” concentration was assumed to be <15 mg/100ml for caffeine and <0.1 mg/100ml for theobromine and theophylline.

## 3 Results

### 3.1 SAMPLES

In the initial market scanning phase, a total of 95 products were identified, including all flavours where multiple flavours of a product were identified. Excluding multiple flavours, there were 81 different products.

At the time of purchasing, some products could not be found and a few different products were identified. A total of 144 food items were purchased representing 65 different products or flavours. After compositing multiple batches or flavours of 40 products, a total of 58 samples were analysed for caffeine, theobromine and theophylline.

The distribution of product type, availability, assigned priority (A, B, C) and the number of products purchased is summarised in Table 1. Food product types were categorised on the basis of perceived intended use. There is an element of subjectivity in the food product categorisations.

**Table 1: Types of guarana-containing foods available for sale in Australia and New Zealand between August and November 2012, prioritisation, and products purchased**

Food Product Category	Number of products identified <sup>1</sup>	Availability		Priority assigned	Number of products purchased <sup>1</sup>
		NZ and/or Australia	NZ and Australia only		
Alcoholic beverage	14	12	2	12 @ B 2 @ C	6
Energy drink	39	39	0	39 @ A	35
Energy shot	4	3	1	4 @ A	4
Fruit juice	1	1	0	A	1
Guarana liquid supplement	1	0	1	C	0
Guarana powder	2	0	2	2 @ A	2*
Iced coffee	1	1	0	A	0
Nutrient water	3	0	3	3 @ A	3
Sports bar	2	0	2	2 @ A	0
Sports drink	1	1	0	A	1
Sports gel	1	0	1	A	1
Sports/weight loss supplement	24	1	23	7 @ A 5 @ B 12 @ C	5
Tea	2	0	2	2 @ A	2
Total	95	58	37	63 @ A 17 @ B 15 @ C	65

<sup>1</sup>=Includes different flavours of some products but not multiple batches of the same product.

\*Both products actually the same, although analysed separately.



## 3.2 QUALITY ASSURANCE

The recoveries from spiked samples ranged from 72 to 98% (Table 2).

Table 2: Recovery of caffeine, theobromine and theophylline from spiked samples

Analyte	Spike level mg/100ml	Number of spikes	% recovery
Caffeine	15	8	91
	32	7	72
Theobromine	0.3	10	83
	1.0	11	98
	2.0	8	97
Theophylline	0.3	10	93
	1.0	11	94
	2.0	12	95

Based on duplicate analyses of samples at low concentrations, LODs were 0.9, 0.01 and 0.02 mg/100ml for caffeine, theobromine and theophylline, respectively.

Analyses of the FAPAS samples were within the acceptable range. For the December 2012 FAPAS sample, a mean concentration of 43.4 mg/L was achieved (n=4, z= 0.1). The assigned concentration was 43.0 mg/L. A z score of  $\pm 2$  is acceptable. Precision, expressed as %CV was 3.5 (n=5) and 2.0 (n=4) for the two kola drink control samples.

## 3.3 LABEL CLAIMS, CAFFEINE, THEOBROMINE AND THEOPHYLLINE CONCENTRATIONS

Information on product type, unit size, ingredient and caffeine label claims, measured concentrations of caffeine, theobromine and theophylline in each of the 58 samples is shown in Table 3.

Of the 58 samples, 56 claimed to contain caffeine and/or guarana or *Paullinia cupana*. Of the 58 samples, caffeine was detected (above the LOD) in all but one product. Theobromine was measured in 48/58 and theophylline in 37/58 samples.

Table 3: Claimed and measured concentrations of caffeine, theobromine and theophylline in guarana-containing foods

Product Type	Unit size	Included on ingredient list	Claimed caffeine <sup>a</sup>	Measured caffeine	Measured /claimed caffeine	Measured Theobromine	Measured Theophylline
			mg/100g or mg/100ml	mg/100g or mg/100ml	%	mg/100g or mg/100ml	mg/100g or mg/100ml
Alcoholic beverage 1	700 ml	NA	No claim	ND <sup>d</sup>	NA	ND	ND
Alcoholic beverage 2	250 ml	guarana	NA <sup>b</sup>	10.2 <sup>d</sup>	NA	0.11	0.36
Alcoholic beverage 3	300 ml	caffeine, guarana	9.3	10.5	113	0.95	1.31
Alcoholic beverage 4	250 ml	NA	32	32.3 <sup>d</sup>	101	ND	ND
Alcoholic beverage 5	250 ml	caffeine, guarana	15	23.0	153	ND	ND
Alcoholic beverage 6	250 ml	guarana	NA <sup>b</sup>	12.4 <sup>e</sup>	NA	ND	ND
Energy drink 1 <sup>c</sup>	250 ml	caffeine, guarana	32	33.8 <sup>e</sup>	106	ND	ND
Energy drink 2 <sup>c</sup>	250 ml	caffeine, guarana	32	36.4 <sup>e</sup>	113	1.00	0.15
Energy drink 3	500 ml, 250 ml, 1L	caffeine, guarana	32	36.1	113	0.02	0.04
Energy drink 4	500 ml	caffeine, guarana	32	32.4	101	0.74	ND
Energy drink 5	500 ml/568 ml	caffeine, guarana	32	34.8	109	ND	ND
Energy drink 6	568 ml	caffeine, guarana	32	33.6	105	0.15	0.03
Energy drink 7	250 ml, 355 ml, 440 ml	caffeine, guarana	14.5	15.8	109	0.95	ND
Energy drink 8	250 ml and 355 ml	caffeine, guarana	32	33.1	103	1.31	0.10
Energy drink 9	500 ml	caffeine, guarana	32	34.0 <sup>d</sup>	106	0.09	0.03
Energy drink 10	500 ml	caffeine, guarana	32	32.6	102	0.30	ND
Energy drink 11	500 ml	caffeine, guarana	29	31.2	108	0.19	ND
Energy drink 12	340ml	caffeine, guarana	32	31.8	99	0.25	0.02
Energy drink 13	340 ml	caffeine, guarana	32	32.7	102	0.25	0.01
Energy drink 14	500 ml	caffeine, guarana	32	33.9	106	ND	ND
Energy drink 15	500 ml	caffeine, guarana	32	34.4	107	1.18	0.91
Energy drink 16	500 ml	caffeine, guarana	32	33.5	105	0.76	0.03

Product Type	Unit size	Included on ingredient list	Claimed caffeine <sup>a</sup>	Measured caffeine	Measured /claimed caffeine	Measured Theobromine	Measured Theophylline
			mg/100g or mg/100ml	mg/100g or mg/100ml	%	mg/100g or mg/100ml	mg/100g or mg/100ml
Energy drink 17	500 ml	caffeine, guarana	32	33.2	104	ND	ND
Energy drink 18	568 ml	caffeine, guarana	32	33.1	104	0.01	0.03
Energy drink 19	568 ml	caffeine, guarana	32	33.0	103	0.05	ND
Energy drink 20	568 ml	caffeine, guarana	32	35.0	110	0.06	ND
Energy drink 21	500 ml	caffeine, guarana	32	32.6	102	0.05	ND
Energy drink 22	500 ml	caffeine, guarana	32	32.3	101	0.43	0.04
Energy drink 23	250ml	caffeine, guarana	31	31.8	103	0.09	0.07
Energy drink 24	250ml	caffeine, guarana	31	34.2	110	0.10	0.15
Energy drink 25	500 ml	caffeine, guarana	31	32.4	105	0.27	0.34
Energy drink 26	250ml and 350ml	caffeine, guarana	31	30.9	100	0.29	0.31
Energy drink 27	500 ml	caffeine, guarana	31	32.7	106	0.08	0.10
Energy drink 28	500 ml	caffeine, guarana	31	31.6	102	0.08	0.04
Energy drink 29	500 ml	caffeine, guarana	31	30.9 <sup>d</sup>	100	0.12	0.08
Energy drink 30	340 ml	caffeine, guarana	32.0	30.7	96	0.12	0.07
Energy drink 31	440 ml	caffeine, guarana	32	32.1	100	0.01	0.04
Energy drink 32	500 ml	caffeine, guarana	32	29.6 <sup>d</sup>	93	0.08	0.04
Energy drink 33	500 ml	caffeine, guarana	32	31.8	99	0.03	0.04
Energy drink 34	440 ml	caffeine, guarana	32.0	31.8	99	0.20	0.06
Energy drink 35	440 ml	caffeine, guarana	32.0	31.9 <sup>e</sup>	100	0.05	0.03
Energy shot 1	60 ml	caffeine, guarana	333	337	101	54.2	5.11
Energy shot 2	60 ml	caffeine, guarana	200	205	102	3.24	0.64
Fruit juice	350 ml	caffeine, guarana	14	13.1	93	0.61	1.02
Guarana powder 1 <sup>c</sup>	100 g	guarana	3500	2640 <sup>d</sup>	75	194	115
Guarana powder 2 <sup>c</sup>	100 g	guarana	3500	2370 <sup>d</sup>	68	174	113

Product Type	Unit size	Included on ingredient list	Claimed caffeine <sup>a</sup>	Measured caffeine	Measured /claimed caffeine	Measured Theobromine	Measured Theophylline
			mg/100g or mg/100ml	mg/100g or mg/100ml	%	mg/100g or mg/100ml	mg/100g or mg/100ml
Nutrient water 1	575 ml	caffeine, guarana	14.5	14.5 <sup>d</sup>	100	1.49	ND
Nutrient water 2	500 ml	caffeine, guarana	16	15.9	100	6.59	ND
Nutrient water 3	500 ml	guarana	NA <sup>b</sup>	ND <sup>d</sup>	NA	0.03	0.02
Sports drink	1000 ml	caffeine, guarana	16	16.6	104	ND	ND
Sports gel	41 g	caffeine, guarana	122	77.7	64	3.8	0.53
Sports/weight loss supplement 1	21 g	caffeine, guarana	1620	1690 <sup>d</sup>	105	ND	ND
Sports/weight loss supplement 2	750 g	guarana	13	14.2 <sup>d</sup>	109	1520	ND
Sports/weight loss supplement 3	400 g	guarana	NA <sup>b</sup>	1.4 <sup>d</sup>	NA	2.54	4.50
Sports/weight loss supplement 4	500 ml	caffeine, guarana	75	30.1 <sup>d</sup>	40	10.1	3.67
Sports/weight loss supplement 5	500 g	Paullinia cupana	80	79.5 <sup>d</sup>	99	72.6	ND
Tea 1	30 teabags	guarana	NA	8.8 <sup>d</sup>	NA	0.55	0.52
Tea 2	16 teabags	caffeine	NA	9.7 <sup>d</sup>	NA	1.95	0.76

NA = not available

ND = not detected. LOD: Caffeine= 0.9 mg/100g , Theobromine = 0.01mg/100g, Theophylline = 0.02 mg/100g

a= As stated on the label

b= Guarana extract listed but not caffeine concentration

c= Products were duplicates of the same product, purchased in different countries or jurisdictions.

d= A single batch was analysed, all other samples were a composite of 2-3 batches

e= A composite of flavours

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