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Determination Of Coffee Content In Coffee Mixtures

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Abstract : The Malaysian Food Regulations 1985 [1] states that coffee mixtures must contain not less than 50 percent coffee. Caffeine content of a coffee mixture is usually used to indicate its coffee level. In this study, coffee content of coffee mixtures was determined based on caffeine concentrations using High Performance Liquid Chromatography (HPLC). Caffeine recoveries from spiked standard coffee mixtures ranged from 90.9 to 98.1% while CVs were from 4.8 to 6.6%. Coffee content were determined by using the MS 1360 [2] method and calibration graphs of *robusta*, *liberica* and *arabica*. Accuracy depended on the type of calibration graph referred to and coffee species in the respective samples. All four methods consistently overestimated or underestimated coffee levels, especially when coffee species in the samples were different from that of the calibration graphs. Based on the results obtained, it was concluded that caffeine content may not an accurate coffee content indicator.

Abstrak : Menurut Peraturan Makanan 1985 [1], kopi campuran hendaklah mengandungi tidak kurang daripada 50 peratus kopi. Kandungan biji kopi dalam kopi campuran biasanya ditentukan berdasarkan kepekatan kafeinanya. Dalam kajian ini, kromatografi cecair prestasi tinggi digunakan untuk menentukan kepekatan kafeina dalam kopi campuran dan seterusnya, kandungan biji kopi dalam kopi campuran tersebut ditentu berdasarkan kepekatan kafeinanya. Perolehan semula kafeina dalam kopi campuran yang dikaji berada dalam julat 90.9 hingga 98.1% manakala CVnya ialah 4.8 hingga 6.6%. Kandungan biji kopi ditentukan dengan menggunakan kaedah MS 1360 [2] serta berdasarkan lengkuk piawai *robusta*, *liberica* dan *arabica*. Ketepatan penentuan kandungan biji kopi dalam kopi campuran didapati bergantung pada lengkuk piawai jenis kopi yang dirujukkan serta spesies biji kopi dalam sampel berkenaan. Keempat-empat kaedah yang digunakan telah menghasilkan ketepatan yang kurang memuaskan, terutama sekali jika spesies kopi dalam sampel yang dikaji adalah berbeza daripada spesies kopi dalam lengkuk piawai yang digunakan. Berdasarkan keputusan yang diperolehi, adalah didapati bahawa kepekatan kafeina tidak merupakan penunjuk kandungan biji kopi yang tepat dalam setiap kes.

Key words : caffeine, coffee, coffee mixtures

Introduction

Almost all coffees produced in Malaysia are coffee mixtures comprising of roasted coffee, sugar, margerine and wheat. Food Regulations 1985 [1] states that coffee mixtures must contain not less than 50 percent coffee beans. The caffeine content of a coffee mixture is usually used to indicate its coffee level. Stringent labelling regulations exist but cases of coffee manufacturers not complying with the requirements still arise. Therefore, it is necessary from a technico-legal point of view to detect and quantify the coffee bean content as well as to determine if labelling laws are adhered to. Malaysian Standard MS 1360 [2] assumes that all coffee species contain 0.9% caffeine, although this is certainly not the case. This difference in caffeine content may result in inaccuracy of coffee level determinations as well as poses complications in enforcement practices.

The objectives of this study were: (1) to compare the suitability of spectrophotometric, gas chromatographic (GC) and high performance liquid chromatographic (HPLC) methods in caffeine determination, (2) to evaluate the effect of using different coffee species on the accuracy of coffee determination in coffee mixtures based on caffeine content, and (3) to determine parameters that can improve the accuracy of coffee content determination in coffee mixtures.

Experimental

Apparatus

Spectrophotometry - An ATI Unicam uv/vis Spectrometer Model uv 2 was used with Pye Unicam Vision 3.1 data module for spectrophotometric analysis.

GC - A Hewlett Packard 5890 Series II Plus gas chromatograph with a flame-ionization detector was used. Peak integration was measured with Hewlett Packard Chem-Station Data System. A Carbowax 30m x 0.320mm analytical column was used. The helium flowrate was 1.7ml/min. Oven, injector and detector temperatures were maintained at 190°C, 193°C and 250°C, respectively.

HPLC - A Jasco PU 980 HPLC pump with a Jasco uv 975 uv/vis detector set at 280 nm was used. Methanol : water : glacial acetic acid (20:79:1) was pumped

through a Waters Nova-Pak® C18 (3.9 x 300 mm) analytical column at 1 ml/min. Separation was carried out at ambient temperature.

Samples

Coffee beans - Raw *robusta, liberica* and *arabica* beans were obtained from Federal Agricultural and Marketing Authority, Banting, Selangor. The beans were roasted in a boiling flask immersed in silicon oil heated at 190°C in an IKAMAG® Thermostatic Bath for 20 minutes while being stirred with a L-shaped stirring rod connected to a Citenco FHP Motor Circulator set at Speed No. 2. The types of beans were roasted separately as well as in blends. After roasting, the beans were removed from the apparatus, cooled and kept in airtight containers until further use.

Coffee mixtures - Coffee mixtures were prepared as described by FAMA, Banting, Selangor [3] with modifications. Ingredients used were sugar, wheat, margarine, ghee, salt and sesame seeds in the ratio of 133:50:7.5:7.5:1:1. Sugar, margarine and ghee were heated in a wok before the roasted coffee beans were added. Pre-roasted wheat was added, followed by sesame seeds and salt. The mixture was stirred until the sugar caramelized. The coffee mixture was then poured out and left to cool before it was ground up in a Waring Blender for 2 minutes.

Commercial coffee samples - Twenty four brands of commercial coffee mixtures were bought from supermarkets, mini markets and grocery shops in Kuala Lumpur, Kajang and Bandar Baru Bangi.

Procedure

Caffeine extraction

Caffeine was extracted from the samples as described by Terada & Sakabe [4].

Comparison of three methods of caffeine determination

A caffeine solution (concentration 0.700g/100 ml) was supplied by the Food Quality Research Unit, Universiti Kebangsaan Malaysia and its caffeine content was determined by the methods below.

(i) Caffeine determination by spectrophotometric method

Caffeine content was determined by the Association of Official Analytical Chemists (AOAC) [5] method.

(ii) Caffeine determination by GC method

Caffeine concentration was determined by the method of Strahl *et al.* [6]. Injection volume was 2 µl. Caffeine retention time was observed to be 18.8 minutes. Caffeine concentration was determined using linear regression analysis of the caffeine standard solutions data points.

(iii) Caffeine determination by HPLC method

Caffeine concentration was determined by the methods of the AOAC [5] and Terada & Sakabe [4]. The caffeine solution to be tested (1 ml) was defatted as described by AOAC before its caffeine was extracted while another 1-ml of the same solution was extracted without defatting. Caffeine was extracted as described by Terada & Sakabe. Caffeine concentrations were determined using linear regression analysis of the caffeine standard solutions data points. Caffeine retention time was 8.8 minutes.

Determination of caffeine content in robusta, liberica and arabica coffees

Roasted *robusta* coffee was added with water as a substitute for ingredients normally used in coffee mixtures to form a series of standard coffee concentrations (45, 60, 75 and 100 g coffee/100 g coffee and water mixture). A calibration graph was formed with the Y-axis as caffeine content (w/w) and the X-axis as coffee content series. The steps above were repeated using *liberica* and *arabica* coffees to form separate calibration graphs for *liberica* and *arabica* coffees.

Determination of coffee content

Caffeine content of samples were referred to the *robusta*, *liberica* and *arabica* calibration graphs to obtain the corresponding coffee content. The calculation method of Malaysian Standards MS 1360 [2] was also used to obtain coffee contents of the samples.

Results and discussion

Caffeine determination by spectrophotometric, GC and HPLC methods

HPLC without the defatting step was the most accurate with an accuracy value of 98.6% (Table 1). Spectrophotometric and HPLC with the defatting step methods showed satisfactory accuracy and precision as well. Comparison of the two HPLC methods showed that HPLC without the defatting step was better. This result is consistent with the results of Terada & Sakabe [4], whereby they observed that the defatting step had a detrimental effect on caffeine recovery. Thus, the following caffeine determinations in this study were conducted by HPLC without the defatting step.

Recovery and precision of HPLC without the defatting step method was studied using 3 coffee mixture samples which were spiked with caffeine so that their final concentrations were double of their original. Satisfactory recoveries were observed, ranging from 90.9 to 98.1% while CVs ranged from 4.8 – 6.6%. This shows that the method used had a high caffeine extraction efficiency.

Table 1 : Caffeine determination by spectrophotometric, GC and HPLC methods

Method	Calculated value (g/100 ml)	Precision		Accuracy (%)
		Measured value (g/100 ml)[1]	CV (%)[2]	
Spectrophotometry	0.7	0.64 ± 0.02	3.1	91.4 ± 3.4
GC	0.7	0.53 ± 0.11	20.8	75.7 ± 16.0
HPLC with defatting step	0.7	0.65 ± 0.04	6.2	92.9 ± 5.4
HPLC without defatting step	0.7	0.69 ± 0.01	1.4	98.6 ± 1.2

[1] Average ± standard deviation, n = 4

[2] $\frac{\text{Standard deviation}}{\text{Average}} \times 100$

Coffee content determination

Table 2 shows the relationships between the coffee and caffeine content of coffee-water mixtures for *robusta*, *liberica* and *arabica*. As each species of coffee may contain different amounts of caffeine, it was necessary to prepare three separate calibration graphs. The slope of each graph was different due to the different caffeine content of the coffee species. *Robusta* had the highest caffeine concentration, 2.26 g/100 g, followed by *arabica* which had a caffeine concentration of 1.61 g/100g. *Liberica* had the lowest caffeine concentration at 1.23 g/100 g. As the amount of coffee in a mixture increased, so did its caffeine content. Therefore, by referring to the calibration graphs of the coffee-water extracts, it was possible to determine the coffee level of a sample based on its caffeine content.

Table 2: Relationships between caffeine (g/100g) with coffee content in coffee -water extracts

Type of coffee	Relationship (p< 0.05)	
Robusta	Coefficient of determination, $r^2 = 0.9892$	Y = 0.022X + 0.089
Liberica	Coefficient of determination, $r^2 = 0.9899$	Y = 0.012X + 0.013
Arabica	Coefficient of determination, $r^2 = 0.9735$	Y = 0.015X + 0.085

Table 3 shows the coffee recoveries obtained when the three calibration graphs and the Malaysian Standard MS 1360 [2] method were used to determine coffee content of coffee mixtures containing only one species of coffee. When samples containing only *robusta* were analysed using the *robusta* calibration graph, good recoveries (108.2 – 113.4%) were obtained but not with the *liberica* or *arabica* calibration graph. The *liberica* calibration graph gave good coffee recoveries for samples containing only *liberica* but not *robusta* or *arabica*. Likewise, the *arabica* calibration graph gave good recoveries for samples containing *arabica* only. The MS 1360 method did not give good recovery with any of the samples. These results showed that coffee content determination was accurate only when the calibration graph used was from the same species of coffee in the sample analyzed. Using the calibration graph of another type of coffee may result in overestimation or underestimation of the coffee content. The MS 1360 method also did not yield satisfactory results because it assumes all coffee species to contain 0.9% caffeine, which was clearly not the case.

Coffee content of coffee mixtures containing two different coffee species were determined using the calibration graphs and the MS 1360 method [2]. Table 4 shows the coffee content determined by the MS 1360 method. Duncan's Multiple Range Test showed significant differences in coffee content of samples containing equal amounts of coffee but different species of coffee. This shows that the type and ratio of coffee species in a coffee mixture have a significant effect on its caffeine level and subsequently, its coffee level analyzed. It was

Table 3 : Determination of coffee content in coffee mixtures containing 1 type of coffee species

Formulation	Caffeine (g/100 g)	Recovery [1](%)			
		Robusta	*Liberica*	*Arabica*	MS 1360
***Robusta* :** ingredients[2]					
60 : 40	1.55 ± 0.05	108.2 ± 3.5	207.5 ± 6.1	167.9 ± 5.3	287.4 ± 8.4
50 : 50	1.37 ± 0.01	112.8 ± 0.6	219.0 ± 1.0	175.5 ± 0.9	303.7 ± 1.4
40 : 60	1.13 ± 0.01	113.4 ± 0.6	225.3 ± 1.2	177.5 ± 1.0	313.0 ± 1.6
30 : 70	0.91 ± 0.01	113.1 ± 2.1	233.5 ± 3.7	178.8 ± 3.1	325.5 ± 5.0

Liberica **:** ingredients[2]					
60 : 40	0.86 ±0.01	55.2 ± 0.3	114.3 ± 0.6	87.3 ±0.5	159.4 ± 0.8
50 : 50	0.69 ±0.01	50.1 ± 0.6	108.9 ± 1.1	80.3 ±0.9	152.5 ± 1.4
40 : 60	0.54 ±0.03	46.3 ± 3.7	107.4 ± 6.5	75.6 ±5.6	151.2 ± 9.0
30 : 70	0.40 ±0.01	39.6 ± 0.3	104.3 ± 0.6	67.2 ±0.5	148.0 ± 0.8
Arabica **:** ingredients					
60 : 40	1.06 ± 0.03	70.7 ± 2.5	143.1 ± 6.6	110.8 ± 3.8	196.8 ± 6.0
50 : 50	0.89 ± 0.01	68.8 ± 0.4	141.6 ± 0.6	108.6 ± 0.5	197.4 ± 0.9
40 : 60	0.83 ± 0.01	74.7 ± 1.6	157.2 ± 2.7	118.7 ± 2.4	219.6 ± 3.8
30 : 70	0.62 ± 0.01	70.1 ± 2.4	157.9 ± 4.2	113.5 ± 3.6	221.7 ± 5.8

1 Coffee recovery based on the calibration graph of *robusta*, *liberica*, *arabica* and Malaysian Standard MS 1360 [2] method (n = 4)

2 Ingredients used are sugar, wheat, margarine, ghee, salt and sesame seeds in the ratios (133 : 50 : 7.5 : 7.5 : 1 : 1)

Table 4 : Coffee content of coffee mixtures determined by MS 1360 method (n = 2)

Coffee species and ratio[1]	***Coffee mixtures (Coffee : ingredient)***							
	60 : 40		**50** : 50		**40** : 60		**30** : 70	
	Caffeine[2]	Coffee content[3]	Caffeine[2]	Coffee content[3]	Caffeine[2]	Coffee content[3]	Caffeine[2]	Coffee content[3]
R:L(80:20)	1.37	151.95^{a}	1.22	135.52^{a}	0.98	108.95^{b}	0.78	86.22^{a}
R:L(60:40)	1.33	148.10^{a}	1.09	121.04^{c}	0.87	96.27^{d}	0.79	87.35^{a}
R:L(40:60)	1.17	129.81^{b,c}	1.00	111.15^{d}	0.88	97.45^{c,d}	0.69	76.53^{b}
R:L(20:80)	1.09	120.81^{d}	0.99	109.92^{e,d}	0.79	87.49^{e}	0.62	69.13^{d,c}
R:A (80:20)	1.39	154.12^{a}	1.15	127.93^{b}	1.02	112.86^{a}	0.76	83.91^{a}
R:A (60:40)	1.26	134.79^{b}	1.08	120.32^{c}	0.89	99.39^{c}	0.71	78.17^{b}
R:A (40:60)	1.13	125.59^{c,d}	1.00	110.67^{d}	0.79	87.67^{e}	0.64	70.84^{c}
R:A (20:80)	1.15	128.03^{b,c}	0.95	105.35^{e,f}	0.78	86.30^{e}	0.68	71.47^{c}
L:A (80:20)	0.82	89.64^{f}	0.70	78.02^{h}	0.58	63.96^{g}	0.44	48.38^{e}
L:A (60:40)	0.86	95.79^{f}	0.72	79.93^{h}	0.59	65.60^{g}	0.45	50.21^{e}
L:A (40:60)	1.00	105.65^{e}	0.88	97.66^{g}	0.70	78.28^{f}	0.59	65.90^{d}
L:A (20:80)	1.01	111.72^{e}	0.92	101.85^{f,g}	0.79	87.99^{e}	0.64	70.81^{c}

1 R *Robusta* L *Liberica* A *Arabica*

2 Caffeine concentration (g/100 g, dry weight)

3 Coffee content determined by MS 1360 [2] method

Note : Means in the same column not followed by a common letter differ significantly (α=0.05)

concluded that the usage of one caffeine level to determine coffee levels in various types of coffee mixtures, as in MS 1360, may result in inaccuracies. Coffee content determination using the calibration graphs also yielded inaccurate results (Table 5) as different calibration graphs gave different coffee contents for the same sample.

Coffee content of 24 brands of commercial coffee mixtures were determined using the calibration graphs of *robusta*, *liberica* and *arabica* as well as the MS 1360 [2] method. Results obtained were unsatisfactory and mostly differed from the label claims. The use of MS 1360 method even resulted in several samples calculated to have coffee content exceeding 100%. Accuracy depended on the type and ratio of coffee species in the samples as well as the type of calibration graph used. When the *robusta* calibration graph was used, coffee content ranging from 13.81 to 50.17 g/100g (dry weight) were obtained and 23 of the brands tested were found to contained less than the 50% coffee required by Food Regulations 1985 [1]. Coffee content values of 34.69 to 98.54 g/100 g were obtained using the *liberica* calibration graph, whereby 8 brands failed to meet the Regulations. When the *arabica* calibration graph was used, coffee content ranging from 23.09 to 78.27 g/100g (dry weight) were obtained and 15 brands did not meet the Regulations. If all types of coffees were assumed to contain 0.9 g caffeine/100 g, as is the current practice for enforcement purposes, only one sample did not meet the Regulations.

Based on the results obtained, it was concluded that caffeine content is not an accurate indicator of coffee content unless the type and ratio of coffee beans in a sample is known so that a suitable calibration graph can be used. For coffee mixtures containing more than one type of coffee species, it is necessary to use a calibration graph of the same types and ratio of coffee beans used in the coffee mixture. If the type/ types of coffee used is not known, several errors may arise due to the differences in caffeine content between different species, and sometimes within a species [7]. Also, it is not recommended to use one caffeine value for all types of coffee mixtures, as is currently practised for enforcement purposes. Malaysian

Table 5: Coffee content determined with the *robusta*, *liberica* and *arabica* calibration graphs (n = 2)

Coffee mixture formulation		Recovery[2] (%)		
coffee : ingredients	Coffee used	*Robusta* graph	*Liberica* graph	*Arabica* graph
60 : 40	R : L	94.1 ± 1.7	182.6 ± 3.0	146.4 ± 2.6
50 : 50	(80 : 20)	99.3 ± 0.2	195.3 ± 0.3	155.0 ± 0.2
40 : 60		96.6 ± 1.3	195.7 ± 2.2	151.8 ± 1.9
30 : 70		97.4 ± 0.7	205.7 ± 1.2	154.9 ± 1.1
60 : 40	R : L	91.4 ± 0.5	178.0 ± 0.9	142.3 ± 0.8
50 : 50	(60 : 40)	87.3 ± 0.1	174.2 ± 0.2	136.8 ± 0.2
40 : 60		83.4 ± 0.5	172.6 ± 0.9	132.0 ± 0.8
30 : 70		98.9 ± 0.9	208.5 ± 1.6	157.2 ± 1.4
60 : 40	R : L	78.8 ± 0.4	155.8 ± 07	123.2 ± 0.6
50 : 50	(40 : 60)	79.1 ± 0.4	159.7 ± 0.6	124.3 ± 0.6
40 : 60		84.7 ± 0.7	174.7 ± 1.3	133.8 ± 1.1
30 : 70		84.0 ± 0.7	182.2 ± 1.3	134.5 ± 1.1
60 : 40	R : L	72.6 ± 0.8	144.8 ± 1.4	113.7 ± 1.2
50 : 50	(20 : 80)	78.1 ± 0.7	158.0 ± 1.2	122.8 ± 1.1
40 : 60		74.3 ± 0.5	156.6 ± 0.9	118.1 ± 0.7
30 : 70		73.7 ± 1.7	164.3 ± 3.0	119.0 ± 2.6
60 : 40	R : A	95.6 ± 0.3	185.3 ± 0.5	148.7 ± 0.4
50 : 50	(80 : 20)	93.0 ± 0.2	184.2 ± 0.4	145.4 ± 0.3
40 : 60		100.6 ± 0.2	202.8 ± 0.3	158.1 ± 0.3
30 : 70		94.2 ± 1.0	200.1 ± 0.2	150.0 ± 1.6
60 : 40	R : A	82.2 ± 5.4	161.8 ± 9.5	128.4 ± 8.2
50 : 50	(60 : 40)	86.7 ± 5.7	173.1 ± 10.0	135.8 ± 8.6
40 : 60		86.7 ± 0.6	178.3 ± 1.0	136.9 ± 0.8
30 : 70		86.2 ± 0.8	186.2 ± 1.5	138.0 ± 1.2
60 : 40	R : A	75.9 ± 3.4	150.7 ± 6.0	118.7 ± 5.2
50 : 50	(40 : 60)	78.7 ± 1.6	159.1 ± 2.8	123.7 ± 2.4
40 : 60		74.5 ± 1.9	156.9 ± 3.3	118.4 ± 2.8
30 : 70		76.1 ± 1.4	168.4 ± 2.5	122.6 ± 2.2
60 : 40	R : A	77.6 ± 0.2	153.6 ± 0.3	121.3 ± 0.3
50 : 50	(20 : 80)	74.3 ± 1.0	151.3 ± 1.8	117.0 ± 1.6
40 : 60		73.1 ± 2.2	154.4 ± 3.9	116.3 ± 3.4
30 : 70		77.0 ± 8.1	169.9 ± 14.2	123.9 ± 12.3
60 : 40	L : A	51.0 ± 0.4	107.0 ± 0.6	81.0 ± 0.5
50 : 50	(80 : 20)	51.6 ± 0.2	111.5 ± 0.4	82.6 ± 0.4
40 : 60		49.9 ± 0.9	113.8 ± 1.6	81.1 ± 1.4
30 : 70		45.1 ± 3.3	113.9 ± 5.9	75.5 ± 5.1
60 : 40	L : A	55.3 ± 1.3	114.5 ± 2.3	87.5 ± 2.0
50 : 50	(60 : 40)	53.2 ± 1.2	114.3 ± 2.1	85.0 ± 1.9
40 : 60		51.6 ± 1.4	116.8 ± 2.4	83.7 ± 2.1
30 : 70		47.6 ± 2.4	118.3 ± 4.1	79.3 ± 3.6
60 : 40	L : A	62.1 ± 5.4	126.5 ± 9.5	97.8 ± 9.2
50 : 50	(40 : 60)	67.9 ± 0.4	140.1 ± 0.7	107.3 ± 0.6
40 : 60		64.8 ± 0.8	139.9 ± 1.4	103.7 ± 1.2
30 : 70		69.3 ± 0.3	156.4 ± 0.5	112.2 ± 0.4
60 : 40	L : A	66.3 ± 0.2	133.8 ± 0.3	104.2 ± 0.3
50 : 50	(20 : 80)	71.4 ± 0.5	146.2 ± 1.0	112.6 ± 0.8
40 : 60		74.9 ± 0.3	157.5 ± 0.5	118.9 ± 0.5
30 : 70		76.1 ± 0.7	168.3 ± 1.2	122.5 ± 1.0

[1] R *Robusta* L *Liberica* A *Arabica*

[2] Coffee content determined by using the *robusta*, *liberica* and *arabica* calibration graph respectively

Standard MS 1360 [2] assumes all coffee species to contain 0.9% caffeine, but this value is closer to *liberica*'s caffeine content and much lower than *robusta*'s or *arabica*'s caffeine content. Using this value will overestimate the coffee content of products containing a high ratio of *robusta* or *arabica*. Coffee manufacturers using *robusta* beans are able to use less than 50% coffee and still meet the Regulation while manufacturers using *liberica* or *arabica* beans may need to use more than 50% coffee beans. More

extensive studies are required to determine the caffeine content of coffee beans used by local coffee mixture manufacturers. From an enforcement point of view, it may also be necessary to conduct invoice and purchasing data check in order to correlate the amount of beans bought and amount of coffee mixtures produced. However, it is also important to bear in mind that certain loopholes may exist if caffeine is used as the only indicator of coffee content. For example, it is possible for manufacturers to use less coffee beans and add caffeine separately to meet the requirement stated by the Food Regulations 1985 [1]. Organoleptic requirements can be met by adding coffee flavouring.

Conclusion

HPLC is a suitable method for caffeine analysis. HPLC without a defatting step had caffeine recovery of 98.6% and coefficient of variation of 1.4%. Caffeine recovery in a coffee extract system was in the range of 90.9 to 98.1% while its CVs were in the range of 4.8 to 6.6%. Coffee content of *robusta*, *liberica* and *arabica* showed linear relationships with caffeine concentrations. Accuracy of coffee content determination depends on the type and ratio of coffee species used in coffee mixtures as well as the calibration graph used. Determination of coffee content of coffee mixtures containing only one specie of coffee beans was accurate if the calibration graph of the same species was used.

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