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Caffeine and Theobromine Levels in Chocolate Couverture and Coating Products

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ABSTRACT

Thirty-two samples of chocolate products were analysed by HPLC for caffeine and theobromine contents. Defatted residues of samples were extracted with 80% aqueous acetone. After extraction into boiling water, the methylxanthines were identified and quantified with the use of µ-Bondapak column and mobile phase of methanol:water:acetic acid (20:79:1). Levels of caffein and theobromine in 32 samples of chocolate products averaged 0.62-1.14 mg/g and 0.026-0.153 mg/g respectively. Mean values for theobromine and caffeine content for chocolate coating were 0.82 and 0.07 mg/g respectively. The chocolate coating made from fat substitute had theobromine and caffeine levels ranging from 0.36-0.70 mg/g and 0.027-0.061 mg/g respectively, with mean values of 0.49 mg theobromine/g and 0.039 mg caffeine/g. In local chocolate, the mean theobromine and caffeine levels respectively were 0.72 mg/g and 0.04 mg/g in milk chocolate, and 0.85 mg/g and 0.06 mg/g in dark chocolate. Meanwhile, for imported chocolate, the mean theobromine and caffeine levels respectively were 1.05 mg/g and 0.12 mg/g in dark chocolate; 0.76 mg/g and 0.04 mg/g in milk chocolate; and 0.74 mg/g and 0.03 mg/g in white chocolate. Compared with the local chocolates, imported chocolates had higher levels of theobromine and caffeine at 1.141 mg/g and 0.153^3mg/g. The average theobromine and caffeine concentrations in local chocolate were 0.082mg/g and 0.066mg/g. Theobromine concentration in chocolate samples is within the range of 0.62mg/g-1.141mg/g and the range of caffeine concentration is 0.026mg/g-0.153mg/g respectively. Bittersweet chocolates were found to have higher theobromine and caffeine concentrations than normal sweet chocolates and milk chocolates.

INTRODUCTION

Many health professionals have recently expressed their concern on the effects of caffeine on health. This subject has been reviewed recently by a number of authors (Hadorn, 1980; Hurst, Kreiser & Martin, 1980; Kreiser & Martin, 1978). Theobromine (3,7 dehydro 3,7 dimethyl-IH-purine-2,6-dione) commonly called 3,7-dimethylxnthine and caffeine (3,7 dihydro-1,3,7 trimethyl-1H-purine-2,6-dione) also known as 1,3,7-trime-thylxanthine are major compounds in the methylxanthine group in the plant *Theobroma cacao.* Theobromine and caffeine are found in chocolate products and cocoa, and are responsible for the bitterness of these products. Increasing

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consumer awareness of the effects of theobromine and caffeine content in chocolate and cocoa-based food and beverages on their health has led to greater interest in these alkoloids.

Both theobromine and caffeine have pharmacological effects in that caffeine stimulates the nervous system white theobromine is a diuretic. Recent interest in these two alkoloids, however, is centered on their potential reproductive toxicities (Roberts & Barone, 1983; Eteng *et al.,* 1997). Theobromine and caffeine are now known to cross the placental and blood brain barrier thus having the potential to induce fetus malformation by affecting the expression of genes vital for development (Morris & Weinstein, 1981). The developing fetus may not have developed enzymes for detoxification of these methylxanthine alkaloids via demethylation. Excessive caffeine in take that exceeds 2 - 3g dose or when the blood caffeine concentration is 0.5 - 1.0mM can cause death (McGee, 1980). The United States Food and Drud Administration (FDA) has removed caffeine from the Generally Regarded as Safe (GRAS) drugs list in 1980 and issued a warning to expecting mothers to reduce intake of foods and drinks with caffeine. The average daily consumption of caffeine is estimated at 200 - 300 mg/day which is equivalent to 2-3 cups of brewed coffee per day (Narod, Desanjose & Victoria, 1991). Although cocoa may supply 230-280 mg of theobromine per cup, there is a lack of data on the average daily consumption levels or intake of theobromine in the diet. Caffeine content data on chocolate products are fairly limited but also show variable levels. Burg (1975) noted the differences in caffeine content of American cocoa and South American cocoa (6 vs 42 mg/5-oz cup, respectively). FDA (1980) cites a single value of 5mg/cup and Gilbert (1981) states that cocoa usually contains less than 40 mg/cup. Zoumas, Kreiser & Martin, (1980) also report average caffeine contents of 6 mg/1-oz serving for milk chocolate, 20 mg/1-oz serving for sweet chocolate (dark, bittersweet or semi-sweet chocolate).

High-pressure liquid chromatography has recently been utilised to identify and quantify simultaneously theobromine and caffeine levels in cocoa and chocolate products (Blauch & Tarka, 1983; Kim & Keeney, 1983; Kreiser & Martin, 1980). Much of the research carried out involved the analysis of commercial cocoas, chocolate liquors, different types of chocolate and cocoa beverages (Timbie *et al.,* 1978). Due to the wide range of reported values for methylxanthine content in cocoa products, especially in cocoa powder and cocoa liquor, this study was undertaken to determine theobromine and caffeine content in popular brands of local and imported chocolates.

MATERIALS AND METHODS

Preparation of samples

Popular brands of chocolate products were purchased from a local supermarket and retail food outlet in Kuala Lumpur. The criteria for a chocolate product is that its shelf life must be at least 6 months from the date of manufacture and must be stored in dry and cool conditions. The samples were divided into four groups. Group I comprised 8 samples of local commercial chocolates, Group 2 consisted of 8 samples of imported chocolate, Group 3 had 8 samples of chocolate courverture and Group 4 had 8 samples of chocolate substitutes. Theobromine and caffeine

contents were extracted from the samples and analysed by HPLC according to the methods developed by Kreiser & Martin (1978). All samples were defatted before analysis.

Apparatus and reagents

Analysis was carried out by liquid chromatography using a Model M6000A solvent delivery system (Water Associates, Inc.) and a Model 7120 sample injector system (Rheodyne Inc., CA) with a 20 µl sample loop. An µ-Bondapak 10µm column (30 cm x 4.0 mm) was used to separate the methylxanthines. The solvent flow rate was 1.0 ml/min. Waters spectrophotometer Model M440, with a wave length of 280 nm was used as the detector. The mobile phase used for analysis of the chocolate was methanol: acetic acid: water (20:1:79, v/v). Quantitation analysis was carried out by using the individual standard curves for each type of methylxanthines. Methylxanthine peak heights were obtained from the chart recorder after injection of each sample filtrate into the HPLC. Approximate retention times for theobromine was 8.95 min and for caffeine, it was 3.82 mm. Linear calibration curves were constructed by plotting the methylxanthine peak height to internal standard peak height ratio against methylxanthine concentration.

RESULTS AND DISCUSSION

Determination of theobromine and caffeine compounds

Figure 1 shows the chromatogram for theobromine and caffeine compounds that had been separated in the mobile phase. The results showed that both types of methylxanthine analysed gave good separation resolution. Chromatogram 1 is the result of chocolate sample extraction analysis. Co-elution peak 'a' with standard theobromine can be seen clearly in chromatogram II. Peak ‘b’ was identified as caffeine through the spiking test in chromatogram III.

Theobromine and caffeine levels in local and imported chocolate

Based on chromatogram results, theobromine and caffeine levels in local and imported chocolates were reported in mg/g. The results for theobromine levels in 8 of the local samples analysed are presented in Tables 1 and 2. Among the local (Group 1) and imported chocolates (Group 2), dark chocolate from the imported group had the highest level of theobromine and caffeine which were 0.83 mg/g and 0.058 mg/g respectively compared with local chocolate which were 0.75 and 0.041 mg/g respectively. This is due to dark chocolate containing a higher level of chocolate liquors than milk chocolate. Thus the theobromine content is higher than that in milk chocolate. However, it was found that theobromine and caffeine content in local milk and white chocolates for both sources ranges from 0.0728 — 0.028mg/g.

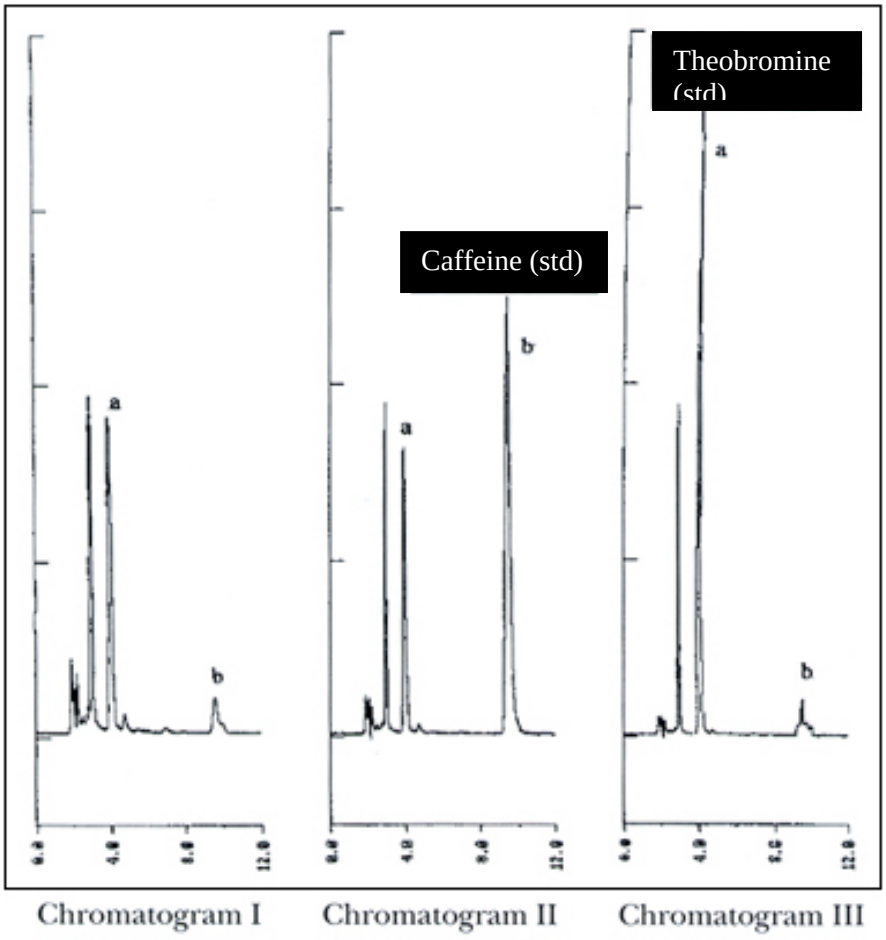

Figure 1. Chromatography of chocolate samples and standard on a µ-Bondapak 10µm column (30 cm x 4.0 mm) HPLC column. Mobile phase: methanol-acetic acid-water (20:1:79, v/v); flow rate = 1.0 ml/min; UV Detector, 280 nm. Standard containing of 20 µg of theobromine and caffeine. Chromatogram I-chocolate extracts, Chromatogram II - caffeine (standard) and chocolate samples. Chromatogram III - theobromine (standard) and chocolate samples

However, theobromine content in a local (Brand LC2) milk chocolate containing almonds was found to be less than that in plain milk chocolate (Brand LC1). Ingredients in milk chocolate such as almonds; peanuts and raisin decrease theobromine and caffeine compound levels (Kreiser & Martin, 1980).

Table 1. Theobromine and caffeine levels in Malaysian commercial chocolates[1]

Sample	Theobromine[2] (mg/g,)	Caffeine[2] (mg/g)
Brand LC1	0.88 ± 0.05	0.034 ± 0.01
Brand LC2	0.85 ± 0.03	0.058 ± 0.02
Brand LC3	0.74 ± 0.03	0.028 ± 0.02
Brand LC4	0.72 ± 0.05	0.034 ± 0.01
Brand LC5	0.71 ± 0.05	0.039 ± 0.01

Brand LC6	0.69 ± 0.06	0.029 ± 0.01
Brand LC7	0.69 ± 0.07	0.041 ± 0.01
Brand LC8	0.67 ± 0.06	0.066 ± 0.08
Mean	0.75 ± 0.05	0.041 ± 0.02

[1] Values are means ± SD; n=3
[2] All values within a column are not significantly different at $p \geq 0.05$

Table 2. Theobromine and caffeine levels in imported commercial chocolates[1]

Sample	Theobromine[2] (mg/g)	Caffeine[2] (mg/g)
Brand IC1	1.14 ± 0.01[b]	0.153 ± 0.02[b]
Brand IC2	0.96 ± 0.02[a]	0.082 ± 0.02[a]
Brand IC3	0.84 ± 0.02[a]	0.047 ± 0.02[a]
Brand IC4	0.81 ± 0.04[a]	0.039 ± 0.01[a]
Brand IC5	0.76 ± 0.04[a]	0.051 ± 0.01[a]
Brand IC6	0.74 ± 0.01[a]	0.028 ± 0.01[a]
Brand IC7	0.71 ± 0.02[a]	0.026 ± 0.01[a]
Brand IC8	0.69 ± 0.02[a]	0.031 ± 0.01[a]
Mean	0.83 ± 0.02	0.058 ± 0.01

[1] Values are means ± SD; n=3
[2] Within a column, values with the same superscripts are not significantly different ($p \geq 0.05$)

Furthermore, the higher level of theobromine in Brand LC1 could have been caused by the higher content of cocoa solid in the milk chocolate compared to Brand LC2. According to Beckett (1988), the average content of cocoa solid in milk chocolates is lower (15.7%) than the average content of cocoa solid in bittersweet chocolate and dark chocolate (61.7% and 39.6% respectively). Brand LC2 milk chocolates had the least theobromine content compared to other local chocolates and all milk chocolates. Kreiser & Martin (1980) reported that the difference in theobromine and caffeine levels in milk chocolate was influenced more by the type of cocoa beans than the cocoa content itself. Thus the lack of significant differences in theobromine levels in Brands LC3, LC4, LC5, LC6, LC7 and LC8 could be due to the local manufacturers using the same type of cocoa beans.

Caffeine content in imported chocolate is shown in Table 2. Bittersweet chocolate Brand IC1 had the highest caffeine level followed by Brand IC2 dark chocolate at 0.153 mg/g and 0.082 mg/g respectively. It is likely that different quantities of cocoa solid were used in these chocolates. Brand IC7 milk chocolate had the lowest caffeine level, even lower than that of white chocolate. Brand 1C8 milk chocolate was found to contain at least 25% of white chocolate mixture. White chocolate does not contain any cocoa solid.

Overall, the mean for theobromine content in local and imported chocolates are 0.75 mg/g and 0.83 mg/g respectively. The mean caffeine levels in local and imported chocolate are 0.041 mg/g and 0.057mg/g respectively. These results show that imported chocolates contain higher theobromine and caffeine levels compared to local chocolates. This could be due to the use of higher amounts of cocoa paste in imported chocolates than in local chocolates. Moreover, the

species of cocoa beans used by overseas manufacturers of imported products could be different from those used by local manufacturers. Kreiser & Martin (1978) have reported 1.1% theobromine content in cocoa paste in Malaysian cocoa beans whereas Trinidad cocoa beans contained 1.2% theobromine. Different species of cocoa beans contribute to different levels of theobromine and caffeine. This study found that dark chocolates contain a higher theobromine and caffeine content compared to milk chocolates. Kreiser & Martin (1980) reported similar findings.

Comparison among types of chocolate

Theobromine and caffeine contents of chocolate coating using chocolate couverture and substitute are summarized in Tables 3 and 4. Chocolate couverture is produced from cocoa liquor to which sugar and cocoa butter have been added. However, vegetable fats have been added to produce chocolate substitutes as a fat replacer of cocoa butter. Chocolate substitutes contain cocoa powder, natural fats substitute, sugar, milk and emulsifier. Mean values for theobromine and caffeine content for all chocolate coating were 0.82 and 0.07 mg/g respectively. The chocolate coating made from fat substitute had theobromine and caffeine levels that ranged from 0.36-0.70 mg/g and 0.027-0.061 mg/g respectively, with mean values of 0.49 mg theobromine/g and 0.039 mg caffeine/g. It is clear that adding increasing amounts of cocoa powder to the chocolate coating leads to increasing levels of theobromine and caffeine in the final chocolate product. Tables 5 & 6 show a summary of the mean values of theobromine and caffeine contents in different types of chocolates namely, dark chocolate (including bittersweet chocolate), milk chocolate (including milk chocolate mixed with other ingredients) and white chocolate. The average theobromine and caffeine contents in local dark chocolate were found to be 0.85 mg/g and 0.06 mg/g respectively compared to 1.05 mg/g and 0.12 mg/g in imported dark chocolate. Theobromine and caffeine content in local milk chocolates were 0.73 and 0.04 mg/g compared to 0.76 and 0.04 mg/g in imported milk chocolates. As there are no locally produced white chocolates, theobromine and caffeine contents in the imported white chocolate were found to be 0.737mg/g and 0.028mg/g respectively. The study found theobromine and caffeine levels in local chocolates to be lower than those in the imported ones.

Table 3. Theobromine and caffeine levels in chocolates coating (couverture)[1]

Sample	Theobromine[2] (mg/g)	Caffeine[2] (mg/g)
Brand CC1	0.82 ± 0.86[b]	0.082 ± 0.09[a]
Brand CC2	0.87 ± 0.74[b]	0.077 ± 0.10[a]
Brand CC3	0.95 ± 0.80[a]	0.089 ± 0.09[a]
Brand CC4	1.03 ± 0.95[a]	0.085 ± 0.08[a]
Brand CC5	1.04 ± 1.01[a]	0.093 ± 0.11[a]
Brand CC6	0.84 ± 0.65[b]	0.064 ± 0.07[a]
Brand CC7	0.88 ± 0.94[b]	0.062 ± 0.09[a]
Brand CC8	0.97 ± 0.94[a]	0.074 ± 0.12[a]
Means	0.93 ± 0.86	0.079 ± 0.09

[1] Values are means ± SD; n=3
[2] Within a column, values with the same superscripts are not significantly different ($p \geq 0.05$)

Table 4. Theobromine and caffeine levels in chocolates coating (substitute)[1]

Sample	Theobromine[2] (mg/g)	Caffeine[2] (mg/g)
Brand SC1	0.60 ± 0.43[b]	0.050 ± 0.06[a]
Brand SC2	0.40 ± 0.39[b]	0.029 ± 0.05[a]
Brand SC3	0.55 ± 0.75[b]	0.043 ± 0.08[a]
Brand SC4	0.69 ± 0.38[a]	0.066 ± 0.08[a]
Brand SC5	0.36 ± 0.35[b]	0.027 ± 0.03[a]
Brand SC6	0.70 ± 0.38[a]	0.061 ± 0.06[a]
Brand SC7	0.54 ± 0.32[b]	0.037 ± 0.06[a]
Brand SC8	0.65 ± 0.72[ab]	0.039 ± 0.06[a]
Means	0.56 ± 0.47	0.044 ± 0.06

[1] Values are means ± SD; n=3
[2] Within a column, values with the same superscripts are not significantly different ($p \geq 0.05$)

Table 5. Mean theobromine and caffeine levels in commercial chocolates[1]

Sample	Theobromine[2] (mg/g)	Caffeine[2] (mg/g)
	Local chocolate	
Dark Chocolate	0.85	0.06
Milk Chocolate	0.73	0.04
White Chocolate	ND	ND
	Imported chocolate	
Dark Chocolate	1.05	0.12
Milk Chocolate	0.76	0.04
White Chocolate	0.74	0.03

[1] Values are means ± SD; n=3

Table 6. HPLC analysis of theobromine and caffeine levels in chocolate1

Sample	Chocolates[1]		Chocolate using CB* [1]		Chocolate using CBS* 1	
	Theo-bromine	Caffeine	Theo-bromine	Caffeine	Theo-bromine	Caffeine
Average (mg/g)	0.74	0.06	0.93	0.08	0.56	0.04
Range (mg/g)	0.36-1.04	0.03-0.09	0.82-1.04	0.06-0.09	0.36-0.70	0.03-0.07
CV (%)	3.64	5.49	3.73	4.84	3.56	5.52

[1] Values are average ± SD; n=24
* CB = cocoa butter; CBS = cocoa butter substitute

Very high levels of theobromine and caffeine in chocolate can have adverse effects on consumers. A theobromine level of 50 oz. (1417.48 g) in chocolate milk and 15 oz in semi-sweet chocolate can cause toxicity to dogs that weigh 50 lb (Matissek 1997; Nightingle & Flamm, 1983). While there are several reports on the toxic effects of these methylxanthines on animals, they have not been extensively documented in humans. A recent study using human samples demonstrated that exposure to caffeine was related to poorer neuromuscular development and significant increases in breech presentation of fetuses (Legator & Zimmeving, 1979).

CONCLUSION

Cocoa based foods are consumed world wide and have been shown to be very nutritious, containing substantial amounts of amino acids, except for methionine and arginine. Vitamins, minerals and fat are also present an a high proportion. The high level of fat contributes to the high gross energy content of the cocoa bean. Despite its high nutritional value, the presence of caffeine and thebromine alkoloids may limit its potential as a nourishing food. This study found the highest levels of theobromine and caffeine to be in imported dark chocolate. Nevertheless, no significant difference was found between imported and local milk chocolates. Overall, local chocolates have lower amounts of theobromine and caffeine than imported chocolates. This indicates that imported chocolates contain more cocoa solid. Perhaps the higher cocoa content, as well as import duties result in imported chocolates being more expensive than local chocolates. Another factor that makes imported chocolate expensive is that overseas manufacturers do not use cocoa butter substitutes or vegetable oils such as palm oil in the making of pure chocolates. Many local chocolate manufacturers are known to use less than 5% cocoa butter substitute in their products.

Imported chocolates have a higher level of caffeine compared to local chocolates. It has been reported that 170 bars of imported dark chocolate (100g) can cause toxicity compared to 345 bars of local dark chocolate (Kreiser & Martin, 1978). They also reported that 517 bars of local milk chocolate can cause toxicity compared to 513 of imported milk chocolate. The toxicity level of white chocolate is relatively lower as it has lesser amounts of caffeine.

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