Caffeine in Prepackaged Tea Leaves: A Food Labelling Concern

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Abstract

Being the second most consumed drink after water and more than coffee, chocolate, soft drinks, and alcohol all combined[1], tea is not only a popular drink but there are also many well-being claims[2] like aiding in digestion, controlling of weight, relieving stress, and even strengthening our minds and physical capacities. Caffeine in teas, when consumed at moderate levels, likewise, contributes to some favorable cognitive and mood effects in humans too[3,4,5,6], yet it has also been postulated to be associated with various negative reproductive issues, such as miscarriages, premature births, low birthweights, and extensive research, however inconclusive[7,8], is still being conducted between caffeine consumption and cardiovascular disease and osteoporosis[9]. This paper aims to determine the caffeine content of prepackaged brands of tea leaves available in the market via the HPLC-PDA method. Prepackaged tea leaves including white, black, oolong, green and pu-erh were examined. Using the Agilent TC-C_{18} column, caffeine contents in the tea leaves were studied via reversed-phase HPLC with a wavelength setting of 280 nm. Samples were isocratically eluted with a mobile phase consisting of 1% acetic acid and acetonitrile in the ratio of 8:2 with a flow rate of 1.0mL/min. Extraction of caffeine in boiling water was performed to mimic the real-life situation of tea drinking. The results provided innovative insights toward the future development of food composition and labeling regulation on prepackaged tea leaves, particularly in Asia where China, India, Russia and Japan alone have already accounted for some 50% of the world's tea consumption[10].

Key words: Prepackaged tea leaves, Caffeine, HPLC-PDA, Food composition and labeling

Introduction

Generally speaking, there are six types of teas, namely white, yellow, fully-fermented black, non-fermented green, semi-fermented oolong and the post-fermented pu-erh[11]. The tea is usually classified according to its manufacturing process. Green tea from China is sweet and fresh-tasting; India is well known for its flavorful and aromatic black tea; whereas Japan is famous for its astringent and fresh green tea[12]. In fact, all teas practically come from the same plant, *Camellia sinensis* and many close relatives, such as the Indian tea plant *Camellia assamica*, were later discovered[13,14]. Tea chemically composes of alkaloids such as theophylline and caffeine; minerals like selenium, zinc and chromium; chlorophyll; carbohydrates; amino acids as well as the phenolic acid, polyphenols, etc. The season, species and age can all affect its composition.

Caffeine is known to be present naturally in the tea as one of the main active ingredients. It is also found naturally in the coffee plant; cocoa plant and the kola nut yet it is also added into many different processed foods and beverages as an artificial ingredient[15]. Caffeine is known to have favorable cognitive and mood effects on humans[16] and is even known to exhibit some protective effects on the Parkinson's disease[17]. An inverse association has been found to exist between caffeine intake and the risk of Parkinson's disease[18] and the psychostimulant properties of caffeine can help reduce the cognitive decline in elderly women without dementia[19] while its withdrawal is proved to adversely affect the cognitive performance of those who are deprived of sleep[20]. Caffeine can also promote the secretion of cortisol in human, but the response of cortisol will decrease due to the tolerance effect through sustained daily caffeine consumption[21]. In fact, caffeine is also claimed to bring about increase in human blood pressure along with additional risks of cardiovascular dieseases[22,23,24] and this is why the tea becomes our prime target of study because the tea is most often, if not always, served hot when the solubility of caffeine is known to increase as temperature rises[25].

Materials and Methods

Sampling of Tea Leaf

Commercial brands of prepackaged tea leaves including, white, black, oolongs, green and pu-erh, available in market were used as the samples. Each tea leaf sample was performed in duplicate in the analysis in parallel with the spiked samples. While an ideal tea leaf sample should reflect, as far as possible, the properties of the bulk source of material, simple random sampling was deployed so as to minimize human bias. For reliable results, the samples were ensured to be of reasonable sizes and airtight containers were used to avoid sample

degradation due to moisture. Proper sample preservation procedures were carried out to prevent the changing of sample composition through biological, physical and chemical processes during storage[26].

Sample Preparation and Extraction

As previous research showed that boiling water is effective enough for extracting caffeine from tea leaves[27,28,29,30,31], traditional extraction methods, such as the Soxhlet extraction, microwave-assisted extraction, pressurized fluid extraction and ultrasonic extraction, etc., have not been used in this study. This is because by putting tea leaves in boiling water we could closely mimic the actual tea drinking habit of the people; and hence, creating a possibly better scenario of the real-life situation of caffeine intake.

Determining Caffeine in the Tea Leaf

High-performance liquid chromatography (HPLC) is an important separation method used for separating mixtures of similar analytes and both quantitative and qualitative information can be provided via the resulting chromatograms. With chromatograms, different analytes will show signals of different areas, heights and times of appearance. The time of first appearing of a signal represents the elution time, while the height and area of the signal will be proportional to the quantity of the analytes of interest. Identification and confirmation is achieved by comparing the retention time with the standards. Quantification is achieved by means of using calibration curves in which a range of concentration levels are used for the testing.

In addition to being simple, convenient, automatic and efficient, analyzing tea leaves using HPLC provides the advantage of producing a large peak for the caffeine signal since for the purpose of quantitative analysis, peak areas are usually measured instead of peak heights. Reversed phase columns, including octadecyl ODS (C_{18}) or octyl (C_8) of which the respective column diameters would help determine the HPLC flow rate[32], are also commonly used with low molecular weight compounds like caffeine (at 194.1906 g/mol).

Optimisation and Method Validation

In this study, optimization of the HPLC mobile phase solvent including the required flow rate (1.0mL/min); the ratio between the two solvents used (A and B, which is 8:2) and the percentage (1%) of acetic acid in 'Solvent A' of the mobile phase were carefully planned and determined. Other optimization procedures were performed including the volume of injection in HPLC; wavelength setting (280nm) in the detector and the time for complete extraction of caffeine from the tea leaves.

Method validation techniques were also used to monitor closely the experimental procedures throughout and various items common to method validation were checked to ensure accuracy. These included spike recovery, precision, limit of detection (LOD), limit of quantitation (LOQ), instrumental detection limit (IDL), method detection limit (MDL), linearity etc.[29].

Result

Summary for Optimization on Caffeine Analysis in Tea Leaf

A summary of the optimized instrumental setting for Agilent 1200 series HPLC system coupled with PDA detector on the caffeine analysis in tea leaves is tabulated in Table 1 below.

Table 1. Optimisation on Caffeine Analysis in Tea Leaf

Optimised instrumental setting of Agilent 1200 series HPLC system coupled with PDA detector on the caffeine analysis in tea leaves	
Optimisation of mobile phase solvent in Channel A and Channel B (Channel A : Acetic acid in deionized water) (Channel B: Acetonitrile)	
Percentage of acetic acid in 'Solvent A' of the mobile phase	1% acetic acid
Ratio of solvent A to solvent B	8:2 isocratic
Flow rate of the mobile phase	1.0 mL/min
Optimisation of volume of Injection in HPLC: 20μL	
Optimisation of wavelength setting in DAD detector: 280nm	
Optimisation of the time for complete extraction of caffeine from the tea samples: Extraction by boiling water for 60 min	
Column: Agilent TC-C18, 5μm, 4.6 x 150mm, USA (PN 518935-902, SN USFMA02121)	

Summary of the Method Validation

A summary of the method validation for Agilent 1200 series HPLC system coupled with PDA detector on the caffeine analysis in tea leaf is tabulated in Table 2 below.

Table 2. Summary of Method Validation

Method validation for Agilent 1200 series HPLC system coupled with PDA detector on the caffeine analysis in tea leaf	
Limit of Detection(LOD) / Method Detection Limit (MDL)	45.3 ppb
Limit of Quantitation (LOQ)	151 ppb
Linearity	R > 0.9999 Slope: 46.75 Y-intercept : 5.0902 (from 2.5 to 125 ppm, plus the blank)
Reporting limit of the test method	2.5 ppm
Instrument Detection Limit (IDL)	24.8 ppb
Accuracy (analytical recovery, obtained by analyzing the spiked sample)	95.6 %
Precision (RSD)	Low caffeine tea sample: 5.0%
	Medium caffeine tea sample: 3.21%
	High caffeine tea sample: 1.74%

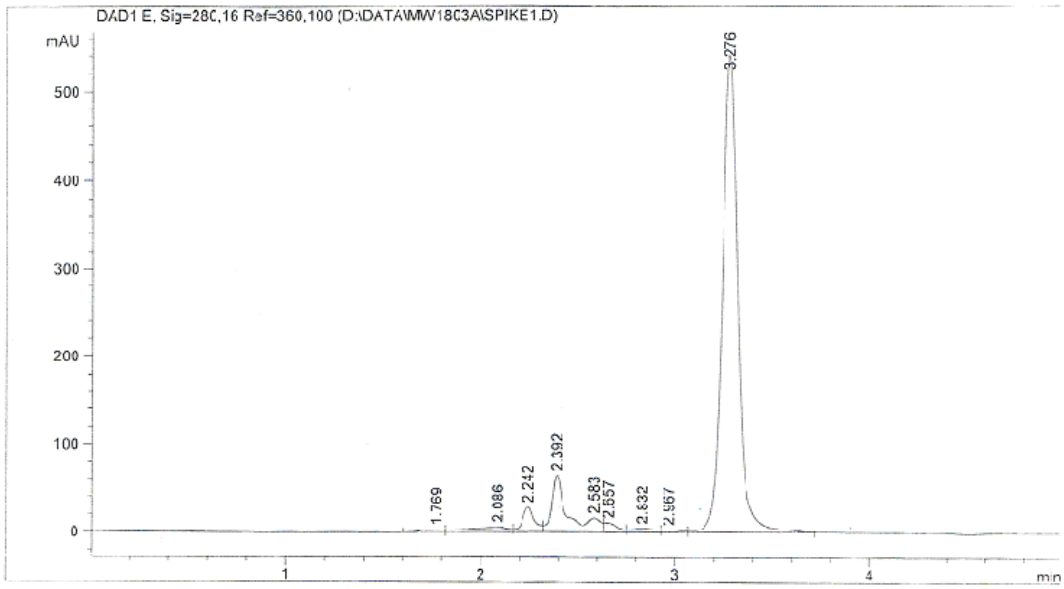
Chromatogram for a Spiked Tea Leaf Sample

The chromatogram for a spiked tea leaf sample is shown in Figure 1.

Results for Determination of Caffeine in Tea Leaf

A total of 34 tea leaf samples were collected in this study from the various tea brands found in the market from the different districts of Hong Kong. Calculations for caffeine content were performed for each of the 34 tea leaf samples followed by the average caffeine content for each type of tea. Results showed that different levels of caffeine were detected in around 91% of the total samples. The caffeine content ranged from around 13mg/g to 38mg/g and the

Figure 1. Chromatogram for a spiked tea leaf sample



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 E, Sig=280,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.769	BV	0.1053	20.22171	2.64132	0.5507
2	2.086	VV	0.1874	83.12969	5.65728	2.2640
3	2.242	VV	0.0641	119.75230	27.89422	3.2614
4	2.392	VV	0.0652	293.11206	64.29832	7.9826
5	2.583	VV	0.0737	78.02158	15.24981	2.1249
6	2.657	VV	0.0675	47.01910	10.26155	1.2805
7	2.832	VV	0.1271	36.16020	4.25801	0.9848
8	2.967	VV	0.0939	19.29693	2.96553	0.5255
9	3.276	VB	0.0823	2975.15112	540.12854	81.0256
Totals :				3671.86537	673.35459	

overall order of the average caffeine content was found to be in the following sequence: pu-erh > black tea > white tea > oolong > green tea. As for the nutrient label on the packages, it was found that food labels were only present in around 21% of the packages. Over 97% of the packages had no mentioning of its caffeine content even when nutrient labels were available for the consumer.

Discussion

Knowing the fact that caffeine in itself is controversial in the sense it could elevate human blood pressure and increase cardiovascular risks and that the level of caffeine in any specific type of tea leaf can be associated with the method of its processing, the origin of the plants and their growing conditions[33,34,35], it is high time that the government, be it Hong Kong, Chinese, Indian, Russian or Japanese, took a closer look at the possibility of introducing new legislations that require beverage companies to list caffeine contents in the ingredients list on their product labels. This is especially alarming when it is reported that China, India, Russia and Japan together have already consumed some 50% of the world's tea leaf supply, and that three of these countries, namely China, Russia and India (which amount up to 44% of the world's tea leaf consumption), are all listed as regions with either the relatively high or high regions of global cardiovascular diseases (CVD) mortality rates as reported by the World Health Organization[36] in 2011, as shown in Figure 2 and Figure 3 below.

Figure 2. Global Distribution of CVD Mortality Rates in Males per 100K population

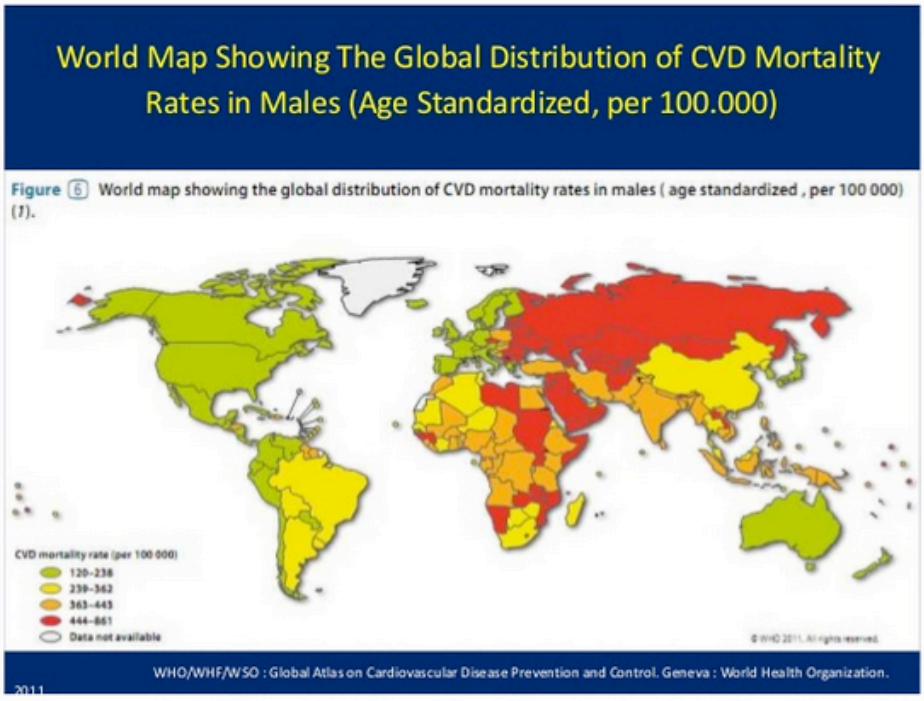
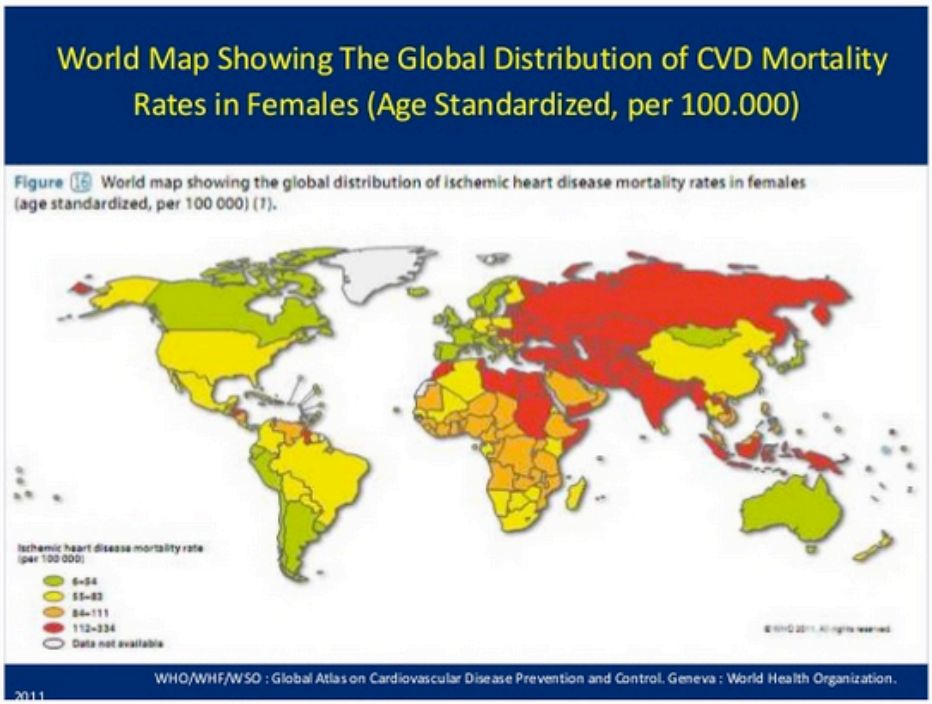
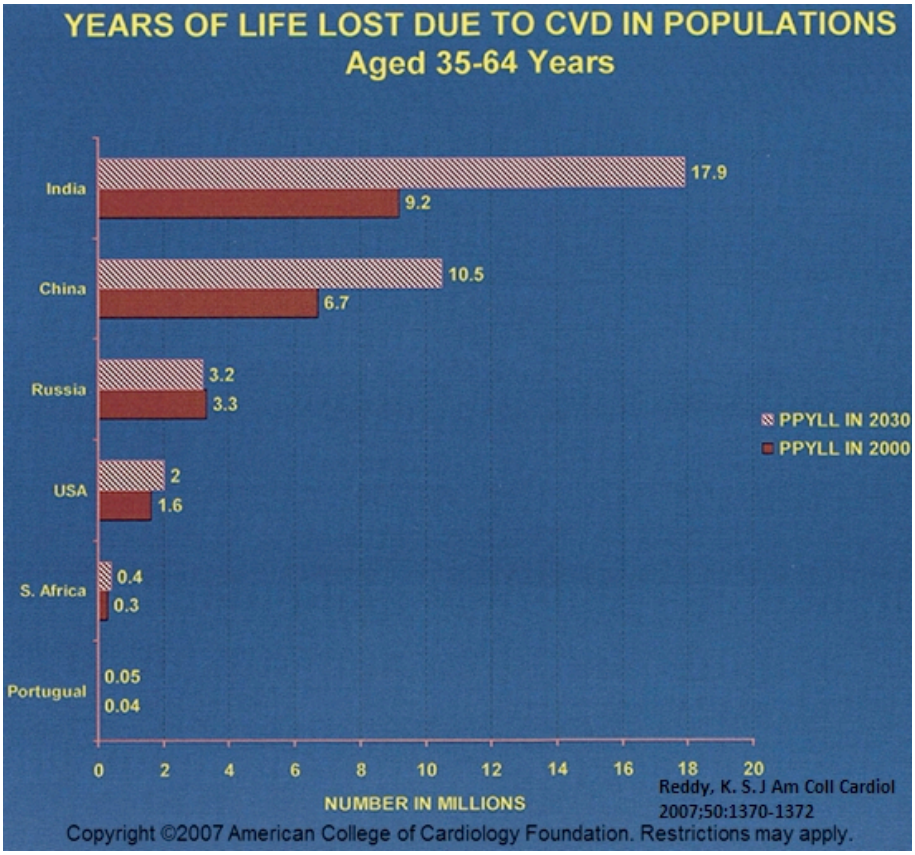


Figure 3. Global Distribution of CVD Mortality Rates in Females per 100K population



In fact, similar legislations in the United States have already been enacted over forty years ago and the U.S. Food and Drug Administration (FDA) still requires that caffeine added to food be subjected to the Federal Food, Drug, and Cosmetic Act (FD&C Act). As a matter of fact, according to U.S. Food and Drug Regulations 21 CFR 182.1180, only caffeine levels up to 200 ppm is 'generally recognized as safe' (GRAS) for use in cola type beverages (which is consistent with the Current Good Manufacturing Practices or 'cGMPs'). Yet if we check the caffeine content we found from our samples, the figures ranged from around 13mg/g (or 13,000ppm) to 38mg/g (or 38,000ppm) which is way beyond the 200ppm benchmark of the U.S. – even though this is a substance that is naturally occurring in the tea leaves. If 200ppm of added caffeine is enough to trigger legislation in the U.S., why would such stunning high levels of caffeine in the range of 13,000-38,000ppm found among the 91% of the samples be not subject to any scrutiny? The topic of caffeine labelling, hence, should be subject of legislation as it is obviously related to public concern as well as a matter of the ever increasing public health expenditure across the world, particularly when the inconclusive occurrences of CVD related issues could still be taking away the highest numbers of years of lives in China, India, and Russia, places where tea is consumed most, see Figure 4.

Figure 4. Years of life lost due to CVD in populations



We therefore suggest that prepackaged tea leaves should never be exempted from any food labelling requirements, especially in countries where tea consumption is this high for both cultural and historical reasons. New legislations should be formulated and regulations should be in place to include caffeine content in the nutrient labels of all prepackaged tea leaves. By including caffeine figures it would not only act as an important source of nutrient information and reference for the citizens but also as a new line of defense for the health conscious public. When sodium, sugar, saturated fat and trans-fat that are all known to be associated with various diseases like diabetes, clogging of arteries, cardiovascular diseases and strokes, etc. are all required to be listed on the food labels, why on earth is caffeine not there?

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