CAFFEINE CONTENT OF NATIONAL AND STORE

BRAND CARBONATED BEVERAGES

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CAFFEINE CONTENT OF NATIONAL AND STORE

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THESIS ABSTRACT

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Caffeine is a well-known stimulant which is added as an ingredient to various carbonated soft drinks. Caffeine has drawn more attention due to its physiological effects beyond that of its stimulatory effect. Consumers are interested in knowing the exact amounts of caffeine existing in beverages. However, limited data exist, especially for store brand beverages. Therefore, the caffeine content of 56 types of national and 75 types of store brand carbonated beverages were analyzed.

The caffeine determination was accomplished by utilizing high performance liquid chromatography (HPLC) equipped with a UV/Visible detector. The mobile phase consisted of 20%:80% (v/v) acetonitrile and deionized water. The chromatographic

separation occurred on two C-18 columns. Each beverage sample was diluted 3-fold with deionized water. Duplicate analyses of multiple lots were performed on all beverage samples.

Some of the more popular national brand carbonated beverages analyzed for caffeine in this study were Coca-Cola (33.9 mg/12 oz), Diet Coke (46.3 mg/12 oz), Pepsi (38.9 mg/12 oz), Diet Pepsi (36.7 mg/12 oz), Dr. Pepper (42.6 mg/12 oz), Diet Dr. Pepper (44.1 mg/12 oz), Mt. Dew (54.8 mg/12 oz), and Diet Mt. Dew (55.2 mg/12 oz). The caffeine content of Vault Zero (74.0 mg/12 oz) and Ritz Cola (10.3 mg/12 oz) were the highest and lowest values in the national brand carbonated beverages. On the other hand, the caffeine content of Big Fizz Diet Cola (61.9 mg/12 oz) and IGA Cola (4.9 mg/12 oz) were the highest and lowest values determined in the store brand carbonated beverages. Most store brand carbonated beverages were found to contain less caffeine than their national brand counterparts. The national brand carbonated beverages exhibited better quality control than store brand ones.

New flavors, formulas, and brands of carbonated beverages continue to be introduced into the market. The food labels on the beverages simply provide the existence of caffeine, but no information about the exact amount. The lacking caffeine information results in consumers not knowing their caffeine ingestion levels. Through the present study, food companies, research and educational institutions, dietitians, and consumers will have access to more comprehensive and updated caffeine data on national and store brand carbonated beverages.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 - INTRODUCTION

The methylxanthines caffeine (1,3,7-trimethyxanthine), theobromine (3,7-dimethylxanthine), and theophylline (1,3-dimethylxanthine) can be normally found in cola nuts, coffee beans, cocoa beans, tea leaves, mate leaves and other kinds of plants (Paradkar and Irudayaraj 2002). Over 60 plant species containing caffeine have been found by investigators. While coffee and tea beverages naturally contain caffeine and other methylxanthines, caffeine serves as an ingredient in many carbonated soft drinks in the United States, including colas, pepper-type beverages, and citrus beverages. Although soda manufacturers may explain that the purpose of adding caffeine to soft drinks is to improve the flavor, only 8% of adults in a study were able to differentiate between caffeinated and caffeine-free colas at the concentration of caffeine contained in most colas (Bernstein and others 2002). These products appeal to many consumers because they contain caffeine, which is a well-known stimulant. Caffeine has been a frequently researched subject due to its broad occurrence in nature, its long usage history, and its physiological effects.

Caffeine has drawn more attention in the past decades due to its physiological effects beyond that of its stimulatory effect. The Food and Drug Administration (FDA) defines caffeine as a generally recognized as safe (GRAS) substance. However, FDA specifies that the maximum amount in carbonated beverages is limited to 0.02% (FDA 2006). Therefore, the highest legal amount of caffeine allowed in a 355 mL (12 oz) can of soft drink is about 71 mg. Caffeine has attracted the interest of consumers and health professionals alike due to its wide consumption in the diet by a large percentage of the population and its pharmacological effects in humans (Mandel 2002). The human's saliva caffeine level, which demonstrates the extent of absorption, peaks around 40 mins after caffeine consumption (Liguori and others 1997). Its physiological effects on many body systems have been reported by researchers, including the central nervous, cardiovascular, gastrointestinal, respiratory, and renal systems (Nehlig and others 1992). The International Olympic Committee (IOC) defined caffeine as a drug and abuse is indicated when athletes have urine caffeine concentrations higher than 12 μg/mL (de Aragao and others 2005).

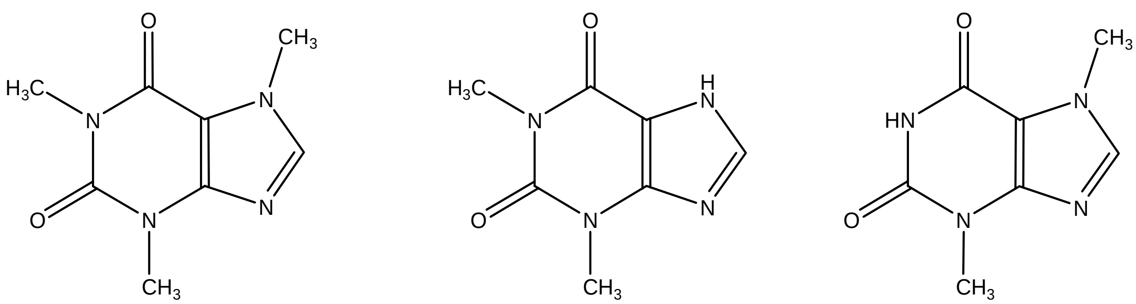
Caffeine contents in various foods and beverages have been analyzed, such as coffee, tea, carbonated beverages, caffeinated water, chocolate products, and chewing gum (Caudle and others 2001). The last large scale study involving the caffeine contents of carbonated beverages was conducted ten years ago where the caffeine contents of fountain and private-label store brand carbonated beverages were determined (Grand and Bell 1997). New flavors, formulas, and brands of carbonated beverages continue to be introduced into the market. Some manufacturers may gradually lower caffeine contents due to health concerns of the public while others may increase it to correspond to the demand for more caffeine by other consumers. Therefore, it is an important topic to investigate comprehensively the caffeine contents of national and private-label store brand carbonated beverages. The current data will be compared to previously published data in the literature and in databases. Caffeine data on new products will also be determined in the present study. Food companies, research and educational institutions, dietitians, and consumers will have access to the updated caffeine data.

Therefore, the specific objective of this research project is to measure the caffeine contents of national and private label carbonated beverages so that current data will be available to the scientific community and public.

CHAPTER 2 - LITERATURE REVIEW

Caffeine chemistry and general information

Caffeine (1,3,7-trimethyxanthine), theophylline (3,7- dimethylxanthine), and theobromine (1,3-dimethylxanthine) are in the family of alkaloid methylxanthines (Fig 2.1).



Caffeine | Theophylline | Theobromine

Figure 2.1 – Structure of methylxanthines

Caffeine is an odorless, white solid that has the form of needles or powder. Caffeine has a bitter taste. The molar mass of caffeine is 194.19 g/mol. Caffeine is slightly soluble in water due to its moderate polarity. Caffeine is a natural central nervous system stimulant, having the effects of reducing drowsiness and recovering alertness. Since it is widely consumed by humans, caffeine is considered the most frequently used psychoactive substance in the world (Lovett 2005).

Physiological effects of caffeine to human

Caffeine has numerous physiological effects on major organ systems, including the nervous system, cardiovascular system, digestive system, and respiratory system. Renal function and skeletal muscles are also affected by caffeine. Many of these physiological effects have been presented in detail in the literature (James 1991; Spiller 1998). Numerous studies have proven caffeine to be a stimulant to human's central nervous system (Rall 1985; Bruce and others 1986). Caffeine facilitates the conduction velocity in the heart and directly affects the contractility of the heart and blood vessels. Nevertheless, caffeine may significantly reduce cerebral blood flow by constricting of cerebral blood vessels. Caffeine provides a diuretic effect due to elevating the blood flow and glomerular filtration rate of the kidneys. Heartburn is an issue for some subjects' gastrointestinal system after consuming caffeine. The effects of caffeine to skeletal muscles are mainly the increasing occurrence of tremors (James 1991; Spiller 1998). Studies on caffeine's physiological effects continue, as demonstrated by the following summaries.

Liguori and others (1997) designed a study to verify the public's perception of coffee yielding greater pharmacological effects than colas. The time and amounts of peak caffeine levels and subjective effects between coffee and cola were compared in this study. After subjects ingested 400 mg caffeine contained in a 12 oz unsweetened coffee vehicle or 24 oz sugar-free cola vehicle, the subjects' saliva samples were collected at 30, 60, 90, 120, 180, and 240 min. The results demonstrated similar mean peak saliva caffeine levels between coffee (9.7 ± 1.2 µg/mL) and cola (9.8 ± 0.9 µg/mL). The time needed to reach the peak salivary caffeine level was similar for coffee (42 ± 5 min) and cola (39 ± 5 min). Therefore, peak caffeine absorption and time required to peak are not significantly affected by the beverage type (Liguori and others 1997).

Bernstein and others (2002) investigated caffeine dependence in teenagers due to the increasing consumption of caffeine in children and adolescents via soft drinks and other sources. Thirty-six subjects who consumed caffeine daily and had some features of caffeine dependence were selected by telephone screening and scheduled for further outpatient evaluation. The results indicated that 41.7% (n = 15) exhibited tolerance to caffeine, 77.8% (n = 28) demonstrated withdrawal symptoms, 38.9% (n = 14) failed to control use, and 16.7% (n = 6) continued to consume caffeine even though they perceived the side effects of caffeine (Bernstein and others 2002).

Watson and others (2000) examined the physiological and psychological effects of caffeine from soft beverages on healthy women subjects who were acutely withdrawn from caffeine. The researchers conducted the investigation after subjects' abstinence of caffeine and an overnight fast. After the subjects consumed caffeine through soft drinks, their physiological and psychological status were evaluated. The interesting findings were a 10% decrease in middle cerebral artery velocity and improvement in feelings of energy and hedonic mood ($p < 0.037$) in women consuming caffeine versus those not consuming caffeine. Tense mood remained unchanged after caffeine treatment. This study verified the hypothesis that women are affected physiologically and psychologically by caffeine provided by soft drinks after acute abstinence (Watson and others 2000).

Kaufman and Sachdeo (2003) observed the relationship between consuming caffeinated beverages and decreased seizure control via a case of a 49-year-old white male with a 36-year history of mixed seizure disorder. Adding caffeinated tea to this patient's diet caused an increase in seizure frequency. When the caffeinated tea was substituted by a decaffeinated tea beverage, the patient's seizure frequency retuned to baseline. After 4 years, the patient was re-challenged with caffeinated tea and experienced a similar reduction of seizure control. The authors suggested that the patient with epilepsy should be careful with his caffeine ingestion (Kaufman and Sachdeo 2003).

Shilo and others (2002) investigated whether caffeine may influence sleep quality and melatonin secretion. Caffeine serves as an inhibitor of the gland excreting melatonin which is believed to be highly related to sleep quality. The researchers conducted a double-blind study. The subjects randomly drank either regular or decaffeinated coffee on one day and consumed the alternate beverage 7 days later. Consumption of regular caffeinated coffee significantly decreased the total amount and quality of sleep as well as prolonged the time required for sleep induction. In addition, other sleep variables were worsened in the regular caffeinated trials. This study demonstrated individuals may improve their sleep quantity and quality by controlling caffeine ingestion (Shilo and others 2002).

Savoca and others (2005) assessed the association between caffeine consumption and ambulatory blood pressure patterns (BP) among adolescent subjects due to the increasing occurrence of essential hypertension in youth. Eighty-two healthy African-American and non-Hispanic white adolescents were recruited and given 4-day sodium-controlled diet containing a designated amount of caffeine. Ambulatory BP measurements were recorded every 20 min during daytime and every 30 min during nighttime. The results indicated caffeine was positively associated with daytime systolic and diastolic BP. More pronounced caffeine effects on the systolic BP of

African-American subjects were reported. There was no relationship between caffeine consumption and nighttime BP in both populations (Savoca and others 2005).

Hartley and others (2004) compared the cardiovascular effects caused by caffeine in men and women. This study measured the blood pressure and hemodynamic responses to caffeine in a double-blind trial comparing age-matched, habitual users of caffeine. After giving caffeine or placebo treatments, the blood pressure, cardiac output, and vascular resistance were monitored at several situations including rest, during a stressful public-speaking simulation, reading aloud, and recovery. Caffeine exhibited the effects of elevating systolic and diastolic blood pressure in women (4.5 and 3.3 mm Hg, respectively) and men (4.1 and 3.8 mm Hg, respectively). The interesting finding was different hemodynamic mechanisms in men and women resulted in similar blood pressure responses to caffeine. According to the researchers' observation, the increase in cardiac output in women was responsible for their blood pressure responses, whereas increased vascular resistance caused the blood pressure responses in men (Hartley and others 2004).

Notarius and others (2006) designed a study to evaluate the effects of caffeine on post-exercise blood pressure in middle-aged subjects. In middle-aged men, persistently high systolic blood pressure after exercise is known to be associated with a potentially higher risk of subsequent myocardial infarction. Adenosine contributes to blood pressure lowering after exercise. The purpose of this study was to determine if caffeine blocked the effects of adenosine, causing an increase in blood pressure after exercise. After 72 h of caffeine abstinence, significant reductions in mean and diastolic blood pressures were observed in the post-exercise state (from 93 ± 2 to 85 ± 2 mm Hg and from 79 ± 2 to 73 ±

3 mm Hg, respectively). After caffeine infusion, the systolic and mean blood pressure were significant higher than those after the placebo treatment (by 9 ± 3 and 6 ± 2 mm Hg, respectively). These data indicated the possible relationship between caffeine and cardiovascular risk after exercise (Notarius and others 2006).

Despite the previously described physiological responses to caffeine, concrete data demonstrating risks associated with moderate consumption of caffeine are lacking. The American Dietetic Association states that consuming less than 300 mg caffeine/day does not cause physical problems (ADA 2004). Likewise, the International Food Information Council states that both the U.S. Food and Drug Administration and the American Medical Association consider consuming less than 300 mg caffeine/day to pose little risk (IFIC 1998). In Australia, the New South Wales Department of Health claims that less than 600 mg caffeine/day is not harmful (NSWHEALTH 2002). In order to limit consumption of caffeine to the recommended levels, data on caffeine contents in foods and beverages are needed.

Methods of analysis of caffeine in food

Many methods exist for determining the methylxanthine contents of food and beverages. Some of these methods include UV-Visible spectrophotometry, potentiometry, high performance liquid chromatography (HPLC), ion chromatography, high performance thin layer chromatography (HPTLC), capillary electrophoresis, micellar capillary electrophoresis, gas chromatography, and solid-phase microextraction gas chromatography (Armenta and others 2005). Of the above methods, HPLC has become one of the most commonly used analytical methods. The discussion below highlights some of the methodology studies that have involved the analysis of methylxanthines in food products.

One study demonstrated using an HPLC method with an octadecylsilyl (ODS) column and a water-acetonitrile-phosphoric acid mobile phase to analyze eight catechins and caffeine. Within 20 min, the catechins (epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, catechin, catechin gallate, gallocatechin and gallocatechin gallate) and caffeine were separated by an acetonitrile gradient. Two different types of Japanese green teas, Matcha and Sencha, both high and low grades for each tea, had their catechins and caffeine contents determined. The researchers found the caffeine contents were higher in Matcha tea than in Sencha tea (Goto and others 1996).

Wang and others (2000) applied an isocratic elution system to determine the contents of catechins, caffeine, and gallic acid in green and black tea. The separation system included a C18 reverse-phase column, a mobile phase of methanol/water/orthophosphoric acid (20/79.9/0.1), and an UV detector. The flow rate was set at 1.0 mL/min. The wavelength of detection was 210 nm. The validation of this method was confirmed by all analytes exhibiting good linearity within the range tested and correlation coefficients ranging from 0.988 to 1.000. The amounts of caffeine in Gunpower, roasted green tea (RGT), Sencha, Keemun, and Sri Lanka were found to be 23.9, 30.3, 28.9, 38.2, and 22.9 mg/100 mL, respectively (Wang and others 2000).

Mashkouri Najafi and others (2003) quantitated the caffeine existing in black tea leaves by Fourier transform infrared (FTIR) spectrometry. The caffeine of tea samples was extracted using $CHCl_3$ after wetting with an aqueous NH_3 solution. The spectrometric data were collected over the wave number range of 1800-1300 cm^{-1}. This method had a detection limit of 35 µg/mL, a sampling frequency of 6 h^{-1}, and a coefficient of variation of 0.8%. A black tea sample contained 3.68% w/w caffeine. The authors obtained similar results for the caffeine content from FTIR (3.68 ± 0.03% w/w) and a reference HPLC technique (3.60 ± 0.07% w/w). The advantages of the FTIR method for determining caffeine in tea leaves includes its quickness, precision, and accuracy, enabling it to be a possible alternative to the HPLC method (Mashkouri Najafi and others 2003). However, one potential shortcoming of this method is the fairly high detection limit.

Nishitani and Sagesaka (2004) developed an improved HPLC analytical method for simultaneously determining caffeine and the eight catechins as well as other phenolic compounds in tea. The proposed method provided additional ability to analyze phenolic compounds when compared with former HPLC methods. This procedure was based on an improved reverse-phase ODS column operated at 4°C, a binary gradient elution system of water-methanol-ethylacetate-phosphoric acid, and a photodiode array detector. The quantitative measurement of eight catechins and caffeine confirmed the validity of this proposed method. The detection limits of these analytes ranged from 1.4-3.5 ng per injection volume. The recovery rates of the analyses were in the range of 96-103%. The caffeine contents of Sencha, Matcha, Gunpowder, Tie Kuan yin, and Darjeeling determined in this study were 2.94±0.007, 3.62±0.005, 2.61±0.059, 2.51±0.019, and 3.24±0.016% (dry weight), respectively (Nishitani and Sagesaka 2004).

Caudle and others (2001) tried to improve the Association of Official Analytical Chemists (AOAC) official analytical method for analyzing methylxanthines in cocoa-based food products. Theobromine and caffeine contents could be obtained by reverse-phase HPLC. The AOAC method's degree of accuracy and precision was not reliable, especially for caffeine. In this study, the AOAC analytical method only showed recoveries of theobromine and caffeine to be 89.3 and 74.5%, respectively. The authors successfully changed from an organic extraction to an aqueous extraction and analyzed the samples via reverse-phase HPLC to improve the recoveries of theobromine and caffeine to 99.6 and 103.4%, respectively (Caudle and others 2001).

Zuo and others (2002) analyzed various substances in several green, Oolong, black and pu-erh teas by HPLC. They used a methanol-acetate-water buffer gradient elution system and a C-18 column; detection utilized a photodiode array detector. After multiple extractions with aqueous methanol and acidic methanol solutions, four major catechins, gallic acid and caffeine could be simultaneously determined within 20 min. This improved the previous studies' problem of catechins and caffeine remaining in tea residues after a single extraction. The results demonstrated that green teas contain higher amounts of catechins than Oolong, pu-erh, and black teas due to their fermentation processes reducing the levels of catechins significantly. An interesting finding was a lower caffeine content in Oolong teas, especially in Fujian Oolong tea (Zuo and others 2002).

Horie and others (1997) adapted capillary zone electrophoresis (CZE) in order to simultaneously determine the major compounds in green tea. Separation occurred in a fused-silica capillary column. The borax buffer was set at pH 8.0, and UV detection was at 200 nm. The major compounds in green tea were epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, catechin, caffeine, theanine, and ascorbic acid. The authors found the concentration of each compound was significantly different among each tea sample. One interesting finding was relatively lower caffeine contents in canned tea drinks. The authors concluded CZE is more appropriate for analyzing the properties and contents of green tea than HPLC due to its shorter analysis time and ability to separate more compounds (Horie and others 1997).

Schulz and others (1999) investigated the accuracy of rapidly predicting the amounts of polyphenol and alkaloid compounds in the leaves of green tea by a near-infrared reflectance spectroscopic (NIRS) method. The pretreatment of the NIR spectra with weighted multiple scatter correction effectively eliminated interferences of scatter and improved the final calibration results. The results were compared with those from analysis by HPLC. The potential of this NIRS method is demonstrated by the high correlation between its prediction and HPLC values for caffeine and major catechins. The authors claimed that the NIRS method may be an alternative technique to HPLC due to its high degree of accuracy for prediction and analysis time of less than 1 min per measurement (Schulz and others 1999).

Farah and others (2006) investigated the relationship between the Arabica coffee cup quality and the contents of sucrose, caffeine, trigonelline, and chlorogenic acids. The researchers applied reverse-phase HPLC analysis to determine each compound. Sucrose was analyzed by using 80% acetonitrile and 20% water as the mobile phase and a refractive index detector. For analyzing caffeine, the UV detector was set at 272 nm. The mobile phase was composed of 60% water and 40% methanol. The results demonstrated that the caffeine content was the highest in the highest quality sample and the lowest content was found in the poorest quality sample. However, trigonelline and

3,4-dicaffeoylquinic acid gave a better indication of high quality coffee (Farah and others 2006).

Huck and others (2005) compared the contents of caffeine, theobromine, and theophylline in 83 liquid coffee extracts determined by a NIRS method and HPLC coupled to mass spectrometry method. In the NIRS method, the spectra were recorded over a wave number range of 4008 to 9996 cm^{-1} with a resolution of 12 cm^{-1} in the reflectance mode. The authors obtained high robustness and reproducibility of the NIRS model for quantification of caffeine and theobromine. The lower limit of detection made it difficult for theophylline to fit the NIRS model and correctly be determined. Nevertheless, NIRS provides the coffee industry with an alternative method to quickly determine caffeine and theobromine (Huck and others 2005).

Chen and Wang (2001) analyzed the level of artificial sweeteners (sodium saccharin, aspartame, acesulfame-K), preservatives (benzoic acid, sorbic acid), caffeine, theobromine, and theophylline in carbonated cola drinks, fruit juice drink, fermented milk drink, preserved fruit, and one pharmaceutical preparation by an ion exchange chromatography method. Analytes were separated using an anion-exchange analytical column maintained at 40°C and detected by wavelength-switching ultraviolet absorption. The detection limits ranged from 4-30 ng/mL for all analytes. The average recoveries for samples ranged from 85 to 104%. In addition, the data obtained from this method were in good agreement with those determined by reference HPLC procedures. Two carbonated cola drinks were found to contain around 36 mg caffeine/12 oz (Chen and Wang 2001).

Chen and others (2006) investigated the feasibility of using near infrared (NIR) spectroscopy as a fast method which is non-destructive and less time consuming than other frequently used analytical methods for estimating the content of caffeine and total polyphenols in green tea. The calibration was performed by a partial least squares (PLS) algorithm. The result indicated that correlation coefficients of the prediction models were approximately 0.97 for the caffeine and 0.93 for total polyphenols. This method's potential to rapidly determine the caffeine and polyphenols of tea to control industrial processes has been proven by this study (Chen and others 2006).

Yao and others (2006) examined 20 leaf tea and 36 teabag samples obtained from Australian supermarkets. Each sample was prepared as a diluted tea solution, which was treated with lead acetate and hydrochloric acid solutions. After filtering and treating with a sulfuric acid solution, the measurement of caffeine was completed by using a UV/Visible spectrophotometer at 570 nm. The results showed that caffeine contents of black leaf tea and teabags were 3.89 and 3.87%, respectively. Similar results were found in the green leaf tea and teabags, 3.71 and 3.83%, respectively. These contents are generally higher than that claimed by the manufacturers (i.e., < 3%). This study revealed a need to establish quality control for both imported and Australian-made teas (Yao and others 2006).

Brunetto and others (2007) developed a reversed-phase HPLC method with an on-line sample cleanup to determine theobromine, theophylline, and caffeine in cocoa samples. The cocoa samples were prepared by an on-line solid-phase extraction of analytes and loaded into a home-made dry-packed precolumn with ODS-C18 in a column-switching system. The mobile phase consisted of 20% of methanol in water, under isocratic conditions, at a flow-rate of 1.4 mL/min. Chromatographic separation was performed on a NOVA-PAK C18 column (150 mm x 3.9 mm, 4 µm). The procedure demonstrated a recovery of over 95% with coefficients of variation less than 3.2%. The precolumn proved its long average life span by showing no signs of deterioration after approximately 1000 injections of sample cocoa extracts (Brunetto and others 2007).

Pura Naik (2001) modified a HPLC method for determining caffeine and theobromine contents in aqueous cocoa extracts. Instead of directly injecting the extracts on the column, the improved method can successfully remove the interfering cocoa pigments by passing them through a Sep-pak C18 cartridge which was also used to separate the theobromine and caffeine. This method enhanced the efficiency of the column and prolonged its life. After this treatment, the recoveries of caffeine and theobromine were 98.0-100.1 and 97.8-100%, respectively. The modified method displayed good resolution and sharp peaks on chromatograms that favored correct determination of theobromine and caffeine (Pura Naik 2001).

Thomas and others (2004) measured the contents of caffeine, theobromine, and theophylline in a food-matrix standard reference material (SRM) 2384, Baking Chocolate by a reverse-phase HPLC method. The stationary phase was composed of an inactive silica support to which C-18 was bonded. The mobile phase consisted of 10% acetronitrile/90% water (pH acidified to 2.5 with acetic acid). The flow rate was at 1.5 mL/min and UV detection was at 274 nm. The results of each sample could be obtained within 15 min. The results showed the reproducibility for caffeine, theobromine, and theophylline determinations was 5.1, 2.3, and 1.9%, respectively. This method had a limit of determination for all analytes at levels less than 100 ng/mL or 0.1 µg/mL. The measurements of caffeine, theobromine, and theophylline of SRM 2384 Baking

Chocolate were comparable with those from National Institute of Standard and Technology (Thomas and others 2004).

Abourashed and Mossa (2004) applied HPTLC densitometric analysis to determine the level of caffeine in several herbal products and energy drinks. The HPTLC plates were made of pre-coated silica gel. The solvent system contained 85% ethyl acetate and 15% methanol. The wavelength for detecting caffeine was set at 275 nm. The proposed method had a mean recovery of 98.9 ± 3.5% with a coefficient of variation less than 5%. The caffeine ranges of herbal products and energy drinks in this study were found at 4.76-13.29% (w/w) and 0.011-0.032% (w/w), respectively. The HPTLC method demonstrated effective determination of caffeine for stimulant herbal products and carbonated energy drinks (Abourashed and Mossa 2004).

Armenta and others (2005) applied a solid-phase Fourier transform-Raman (SP-FT-Raman) spectrometry-based method to determine caffeine contents in commercial energy drinks. The caffeine content of each sample was obtained from setting Raman intensity between 573 and 542 cm^{-1} with a two points corrected baseline between 580 and 540 cm^{-1}. The limit of detection of SP-FT-Raman method was 18 µg/mL. The combination of FT-Raman and solid-phase increased the sensitivity of detecting caffeine by a factor of 31 times when compared with using direct Raman measurement alone. The results of caffeine contents obtained from SP-FT-Raman method and liquid chromatography (LC) found no significant differences between the two methods. The SP-FT-Raman method displayed higher sampling frequency than the LC method. However, the LC method had a lower detection limit (0.05 µg/mL). The reduced reagent consumption and waste generation are also benefits of this method as compared to the LC method (Armenta and others 2005).

Lucena and others (2005) manipulated a continuous flow autoanalyzer for sequential determination of total sugars, class IV caramel and caffeine contents in 20 different soft drink samples. This apparatus consisted of on-line coupling of a continuous solid-phase extraction unit and two detectors which were UV-visible and evaporative light scattering (ELSD) detectors. The caffeine has the property of being retained on the sorbent column and other compounds can be preferentially determined due to their low affinity to the sorbent column. The caffeine can be detected later by the ELSD after it has been eluted with acetonitrile and the signal registered in the ELSD. In order to evaluate the performance of this analyzer, the authors carried out a recovery test. The results ranged from 90 to 102%. Unspecified colas were found to contain caffeine ranging from 14.9 mg/12 oz to 49.7 mg/12 oz (Lucena and others 2005).

Walker and others (1997) utilized capillary electrophoresis (CE) to simultaneously analyze the aspartame, benzoic acid, and caffeine contents of carbonated beverages in 2 min with 20 mM glycine buffer at pH 9.0 and detection at 215 nm. Good reproducibility for both peak area and migration times were observed (2.0-3.8% and 0.13-0.37%, respectively). The spiked recovery of the analytes ranged from 98 to 114%. The results of soft drinks samples in this study were comparable with those data evaluated by HPLC, but slightly higher in some cases using CE. The main advantages of CE over HPLC are relatively simpler operation, lower cost, no organic mobile solvents, and a shorter analysis time (Walker and others 1997).

Existing data on caffeine contents

Over the past thirty years, information about the caffeine content of carbonated beverages has been reported. Bunker and McWilliams (1979) and Strohl (1985) analyzed several beverages, whose data are listed in Table 2.1. Grand and Bell (1997) also determined the caffeine content of national brand carbonated beverages as well as store-brand beverages. For comparison, some of their national brand data also appear in Table 2.1. As seen in this table, caffeine levels appear to fluctuate in some beverages (e.g., Coca-Cola, Dr. Pepper). Table 2.2 reports some of the store-brand caffeine data from Grand and Bell (1997). Store-brand beverages generally contained less caffeine than their national brand counterparts, however very little data is available regarding these beverages in the literature.

The American Beverage Association (ABA) has reported caffeine contents provided to them by manufacturers on their website (ABA 2006). This data is tabulated in Table 2.3. However, store-brand data is not included in their information. A revised website was posted in the Autumn of 2006 in which this table no longer existed. Unfortunately, the ABA currently directs consumers to manufacturer's websites.

The USDA National Nutrient Database for Standard Reference, Release 19 lists 21 carbonated beverages, with 8 containing caffeine. This database groups similar products together with a single caffeine value (Table 2.4), but as seen in Tables 2.1-2.3, caffeine values range from 5.2 to 70 mg/12 oz. For example, the USDA database lists a sugar-sweetened carbonated cola as containing 29 mg caffeine/12 oz (USDA 2006). However, Coca-Cola, Pepsi Cola, and RC Cola are reported to have 34, 37, and 43 mg caffeine/12 oz, respectively (Table 2.3). Three beverage groups (cream soda, orange, and root beer) in the USDA database were reported as containing no caffeine, but some ingredient lists on product labels indicate the addition of caffeine to these beverages.

Several shortcomings exist with respect to the caffeine data that is currently available. As mentioned above, data on most private label store brand carbonated beverages have not been reported. New or reformulated beverages have either not had their caffeine contents evaluated or their values may have changed. Broad classifications of beverage types with corresponding caffeine values are misleading to consumers. The current study will provide more comprehensive and up-to-date information about the caffeine contents of carbonated beverages. In addition, the extent of quality control during manufacturing may be estimated through this project.

Table 2.1-Caffeine content (mg/12 oz) in national beverages as listed in the literature

Beverage	Bunker and McWilliams (1979)	Strohl (1985)	Grand and Bell (1997)
Coca-Cola	64.7	39.3	29.7
Diet Coke	-	47.7	40.8
Tab	49.4	53.5	49.1
Pepsi	43.1	38.2	32.1
Diet Pepsi	-	38.5	30.3
RC Cola	33.7	35.4	42.7
Diet RC Cola	33.0	-	48.7
Dr. Pepper	60.9	47.4	42.4
Diet Dr. Pepper	54.2	39.7	40.8
Mountain Dew	54.7	-	54.5
Mr. Pibb	-	-	42.9
Mello Yello	-	-	50.4
Shasta Cola	-	-	42.0
Shasta Diet Cola	-	-	37.3
Kick	-	-	55.8

Table 2.2-Caffeine content (mg/12 oz) in store-brand beverages reported by Grand and Bell (1997)

Beverage	mg/12 oz
Store brand cola products	
Winn-Dixie Chek Diet Cola	28.6±0.9
Kroger Big K Diet Cola	28.6±1.6
Winn-Dixie Chek Cola	27.0±1.0
Wal-mart Sam's Choice Diet Cola	12.8±0.2
Kmart American Fare Diet Cola	12.2±0.1
Kmart American Fare Cola	11.9±0.5
Wal-mart Sam's Choice Cola	11.8±0.5
Kroger Big K Cola	5.2±0.4
Store brand pepper products	
Wal-mart Sam's Choice Southern Lightening	29.2±1.3
Winn-Dixie Dr. Chek	17.9±1.2
Kroger Diet Dr. K	16.8±0.6
Kroger Dr. K	16.4±0.9
Store brand citrus products	
Winn-Dixie Chek Kountry Mist	52.3±1.5
Wal-mart Sam's Choice Green Lightning	48.6±0.9
Kroger Big K Diet Citrus Drop	26.6±0.6
Kroger Big K Citrus Drop	26.3±1.4

Table 2.3-Caffeine data for selected carbonated beverages from the American Beverage Association (2006)

Company	Products	mg/12 oz
The Coca-Cola Company	Barq's Root Beer	22
	Cherry Coca-Cola	34
	Diet Cherry Coca-Cola	34
	Coca-Cola classic	34
	Diet Coke	45
	Diet Coke with lemon	45
	Diet Coke with lime	45
	Diet Coke with Splenda	34
	Coca-Cola Zero	34
	Coca-Cola C2	34
	Mello Yello	51
	Pibb Zero	40
	Pibb Xtra	40
	TAB	47
	Vanilla Coke	34
	Diet Vanilla Coke	45
	Vault	70
Dr Pepper/7 Up	A&W Cream Soda	29
	Dr Pepper	41
	Diet Dr Pepper	41
	Sun Drop Regular	63
	Diet Sun Drop	69
	Sunkist Orange Soda	41
	Diet Sunkist Orange Soda	41
	Royal Crown Cola	43
Pepsi-Cola Company	Mountain Dew	55
	Diet Mountain Dew	55
	Code Red Mt. Dew	55
	Diet Code Red Mt. Dew	53
	Pepsi-Cola	37
	Diet Pepsi-Cola	36
	Pepsi One	55
	Wild Cherry Pepsi	38
	Diet Wild Cherry Pepsi	36

Table 2.4-Caffeine contents per beverage type as tabulated by USDA (2006)

Beverage	mg/12 oz
Carbonated beverage, cola, contains caffeine	29
Carbonated beverage, cola, with higher caffeine	99
Carbonated beverage, lemon-lime soda, contains caffeine	55
Carbonated beverage, low calorie, cola or pepper-type, with aspartame, contains caffeine	43
Carbonated beverage, low calorie, cola or pepper-type, with sodium saccharin, contains caffeine	39
Carbonated beverage, low calorie, other than cola or pepper, with aspartame, contains caffeine	53
Carbonated beverage, pepper-type, contains caffeine	37
Carbonate beverage, reduced sugar, cola, contains caffeine and sweeteners	9*
Carbonated beverage, root beer	0
Carbonated beverage, orange	0
Carbonated beverage, cream soda	0

* per 100 grams.

Objectives

The objective of this project is to collect data on the caffeine contents of over 120 types of national and store brand carbonated beverages. Values for the national brand products will be compared to manufacturer information. Store brand data are generally lacking and will be compared to national brand data and the USDA database.

CHAPTER 3 - MATERIAL AND METHODS

Chemicals and reagents

Anhydrous caffeine used for preparation of the standard solutions was purchased from Sigma (St. Louis, MO, USA). The acetonitrile for the mobile phase was HPLC grade (Fisher Scientific, Pittsburgh, PA, USA). Deionized water was obtained from a water purification system ($18M\Omega cm^{-1}$ quality). Sodium phosphate monobasic and HPLC-grade 85% phosphoric acid was obtained from Fisher Scientific (Pittsburgh, PA, USA).

Preparation of standard solution

Caffeine (around 25 mg) was weighed with an electric balance and transferred into a 250 mL volumetric flask. Deionized water was added to get a 250 mL bulk standard solution. Sonication was applied to completely dissolve the caffeine. One vial was filled and labeled with the bulk standard. The 2nd, 3rd, and 4th vials were obtained through consecutive 2-fold dilution with deionized water by pipetting (Precision Pipette, Atlanta, GA, USA). A second bulk solution was prepared using about 15 mg caffeine/250 mL water. The second bulk was diluted in the same manner as described above. The eight standard solutions were stored at 4°C in the refrigerator. These eight standard solutions were analyzed during each day's analysis to prepare the appropriate standard curve.

Preparation of mobile phase

Volumetric flasks were used to measure 250 mL of acetonitrile and 1000 mL of deionized water to achieve 20 % acetonitrile concentration (v/v). Sodium phosphate monobasic (1 g) was dissolved into the solution. The purpose of adding sodium phosphate monobasic was to increase the mobile phase's resistance to pH change. Phosphoric acid was added to acidify the solution to pH 3. The solution was vacuum filtered through a 0.45μm nylon filter. The solution was poured into a storage bottle and degassed by sonication.

Samples and sample preparation

The national-brand prepackaged (e.g., cans, bottles) carbonated beverages were collected across the southeastern United States (Table 3.1). The samples were stored at room temperature until analysis. The store-brand beverages were acquired from Bruno's, Food Lion, Dollar General, IGA, Winn-Dixie, Kroger, Ingle's, Piggy Wiggly, Publix, Save-a-lot, 7-Eleven, Rite-Aid, Walgreens, Supervalu, and Wal-Mart (Table 3.2). The cola, citrus, and pepper-type carbonated beverages as well as their diet varieties were analyzed in the present study. Average caffeine contents of each carbonated beverage were determined from a minimum of two different lots. The beverages analyzed in this study were purchased from June 2005 to July 2006.

Each beverage (50 mL) was poured into an Erlenmeyer flask and degassed in a sonicator. Each sample was diluted 3-fold with deionized water (1 mL sample + 2 mL water). Duplicate dilutions were performed on all samples. An aliquot of these diluted samples was injected into the HPLC system to quantitate the caffeine concentration.

Table 3.1-The list of national carbonated beverages

Manufacturers of national beverages	Products
Coca-Cola Company, Atlanta, GA.	Coca-Cola, Diet Coke, Cherry Coke, Diet Cherry Coke, Coke with Lime, Diet Coke with Lime, Vanilla Coke, Diet Vanilla Coke, Coca-Cola C2, Diet Coke with Splenda, Coke Zero, Coca-Cola Black Cherry Vanilla, Diet Coca-Cola Black Cherry Vanilla, Tab, Pibb Xtra, Pibb Zero, Vault Citrus, Vault Zero, Barq's Root Beer, and Mello Yello
Pepsico, Inc., Somers, NY.	Pepsi, Diet Pepsi, Cherry Pepsi, Diet Cherry Pepsi, Pepsi with Lime, Diet Pepsi with Lime, Vanilla Pepsi, Diet Vanilla Pepsi, Pepsi One, Mt. Dew, Diet Mt. Dew, Mt. Dew Code Red, Diet Mt. Dew Code Red
National Beverage Co, Ft. Lauderdale, FL.	Faygo Cola, Faygo Moon Mist, Ritz Cola, Shasta Cola
Carolina Beverage Corporation, Salisbury, NC.	Cheerwine, Diet Cheerwine
Dr Pepper/Seven Up, Inc., Plano, TX.	Dr. Pepper, Diet Dr. Pepper, Dr. Pepper Berries & Cream, Diet Dr. Pepper Berries & Cream, Cherry Vanilla Dr. Pepper, Diet Cherry Vanilla Dr. Pepper, RC Cola, Diet RC, SunDrop, Diet SunDrop, A & W Cream Soda, Sunkist, and Diet Sunkist
Buffalo Rock Company, Birmingham, AL.	Dr. Wham, Diet Dr. Wham
Big Red, Ltd. Waco, TX.	Big Red
Clayton Dist Co., Inc., Austell, GA.	Red Rock Cola

Table 3.2-The list of store-brand beverages

Manufacturers of store-brand beverages	Products
Kroger, Cincinnati, OH.	Big K Cola, Big K Diet Cola, Big K Cherry Cola, Big K Cherry Diet Cola, Dr. K, Diet Dr. K, Big K Citrus Drop, Big K Diet Citrus Drop, Big K Cola with Lime, Big K Diet Cola with Lime
Winn-Dixie Stores, Jacksonville, FL.	Chek Cola, Chek Diet Cola, Chek Cherry Cola, Chek Vanilla Cola, Chek Diet Vanilla Cola, Chek Diet Cola with Lime, Chek Mate Cola, Dr. Chek, Diet Dr. Chek, Chek Kountry Mist, Chek Diet Kountry Mist, Chek Red Alert
Wal-Mart Stores Inc, Bentonville, AK.	Sam's Cola, Sam's Diet Cola, Dr. Thunder, Diet Dr. Thunder, Sam's Mountain Lightning
Deep South Products, Inc., Fitzgerald, GA. (Bruno's)	Rally Cola, Rally Diet Cola, Ramp, Ramp Red, Dr. Bob, Diet Dr. Bob
Publix Super Markets, Inc., Lakeland, FL.	Publix Cola, Publix Diet Cola, Publix Cherry Cola, Dr. Publix, Publix Citrus Hit
DolgenCorp, Inc., Goodlettsville, TN. (Dollar General)	Clover Valley Cola, Clover Valley Diet Cola, Dr. Topper, Clover Valley Citrus Drop
Save-a-lot Food Stores, Inc., Earth City, MO.	Bubba Cola, Diet Bubba Cola, Mt. Holler, Dr. Pop, Diet Dr. Pop
Piggy Wiggly Corp, Memphis, TN.	Piggy Wiggly Cola, Piggy Wiggly, Piggy Wiggly Diet Cola, Mt. Yeller, Dr. Pig
7-Eleven Inc., Dalla, TX.	Big Gulp Cola, Big Gulp Diet Cola
Supervalu Inc., Eden Prairie, MN.	Superchill Cola, Superchill Diet Cola, Mt. Chill, Dr. Chill
Food Lion LLC. Salisbury, NC.	Food Lion Cola, Food Lion Diet Cola, Mt. Lion, Dr. Perky
Ingle’s Markets, Inc., Asheville, NC.	Laura Lynn Cola, Laura Lynn Diet Cola, Dr. Lynn, Diet Dr. Lynn, Laura Lynn Cherry Cola
IGA, Inc., Chicago, IL.	IGA Cola, IGA Diet Cola, IGA Spring Mist, Dr. IGA
Walgreens Co., Deerfield, IL.	Walgreens Cola, Walgreens Diet Cola
Rite-Aid Corp., Harrisburg, PA.	Big Fizz Cola, Big Fizz Diet Cola

Apparatus

The caffeine content was determined by isocratic reverse-phase high performance liquid chromatography (HPLC) equipped with a UV/Visible detector adapted from that used by Grand and Bell (1997). The injector with a 20 μL loop introduced a known sample volume into the system. The chromatographic separation occurred on a Prodigy 150-mm x 4.6-mm C-18 column (Phenomenex, Torrance, CA, USA) in series with a Novapak C-18 150-mm x 3.9-mm C-18 column (Water, Eatontown, NJ, USA). The mobile phase consisted of 20%:80% (v/v) acetonitrile and deionized water, acidified to pH 3 with phosphoric acid. The combination of these two analytical columns was designed to eliminate the interference of caffeine separation caused by other components in some samples, such as colors, artificial sweeteners, flavors, and preservatives. The wavelength of detection was set at 254 nm and flow rate was set at 1 mL/min. Caffeine eluted around 4.1 min. Data were recorded by a Hewlett Packard HP3395 integrator (Palo Alto, CA, USA). Sample chromatograms for Diet Coke and Dr. Pepper are shown in Figure 3.1 and 3.2, respectively.

Test for HPLC recovery and variability

Specific amounts (12.6 mg and 43.1 mg) of caffeine were measured and put into different 250 mL volumetric flasks. Degassed caffeine-free diet coke (250 mL) was added to each volumetric flask to obtain two spiked samples. A 1 mL aliquot of the first spiked sample was transferred to 5 vials and diluted 3-fold with deionized water. The same method was used to treat the second spiked sample to obtain another 5 diluted solutions. Samples were analyzed using the HPLC method described previously; using the standard

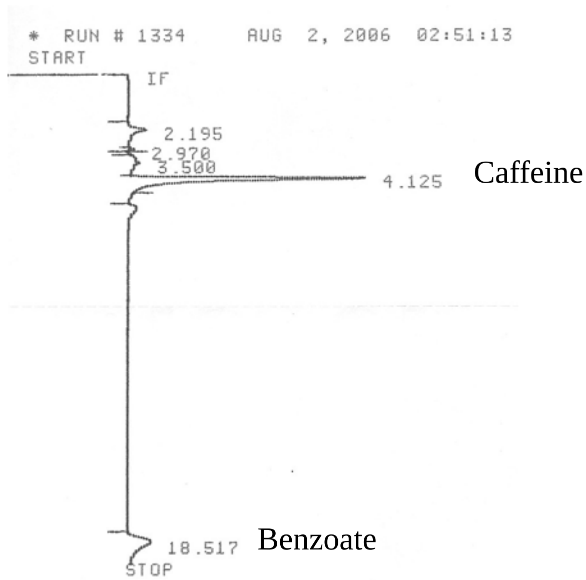


Figure 3.1- Chromatogram of Diet Coke HPLC analysis by two C-18 columns using 20%/80% (v/v) acetonitrile and deionized water as mobile phase.

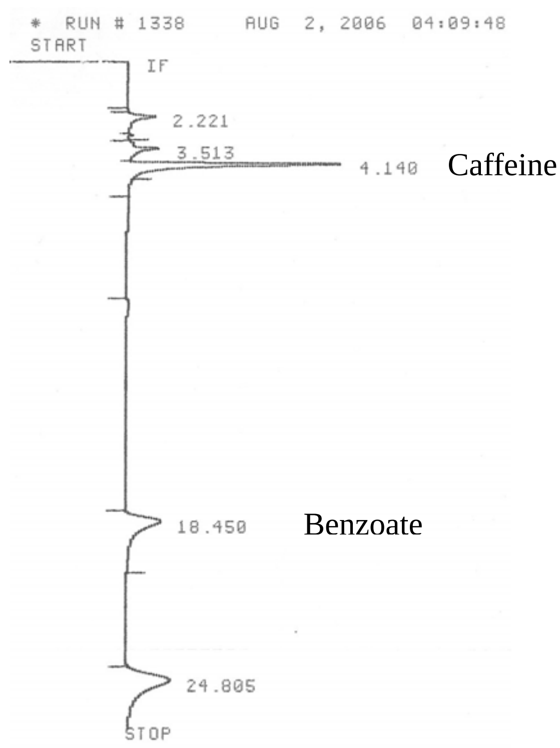


Figure 3.2- Chromatogram of Dr. Pepper HPLC analysis by two C-18 columns using 20%/80% (v/v) acetonitrile and deionized water as mobile phase.

calibration curve, the concentration of each sample was calculated. The coefficients of variation were determined from the standard deviation of the measurements divided by the sample's average. The percent recovery was calculated by the average of the measurements divided by the original concentration. The percent recovery and coefficients of variation were 96.7 to 100.8 % and 0.6%, respectively for this analytical method. These values are similar to that found by Grand and Bell (1997).

<u>Data analysis</u>

The caffeine contents of the samples were calculated using the peak areas reported by the integrator and the standard curve. An example of the caffeine standard curve is shown in Figure 3.3. The caffeine content per 12 oz can was calculated. Every type of beverage had duplicate measurements per lot, which were averaged to give the mean caffeine content for the lot. Data from these duplicate dilutions were typically found to vary by less than two percent. The tables in the Appendix show the individually analyzed samples. The caffeine contents for the various lots were then averaged to give the mean caffeine content for the beverages.

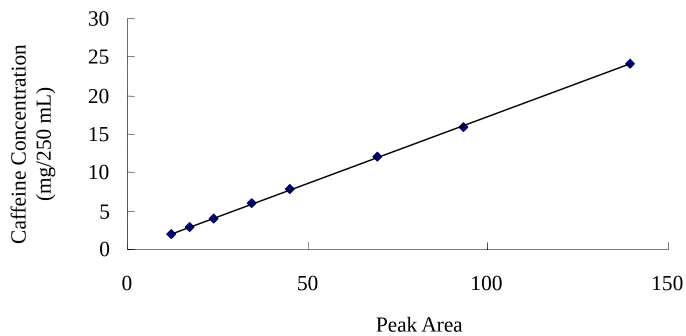


Figure 3.3-Standard curve for caffeine as analyzed by HPLC using two C-18 columns and 20%/80% (v/v) acetonitrile and deionized water as mobile phase detected at 254 nm.

CHAPTER 4 - RESULTS AND DISCUSSION

Recovery and variability studies

The five caffeine readings from adding 12.6 mg caffeine to 250 mL Caffeine-Free Diet Coke were 12.46, 12.61, 12.82, 13.03, and 12.56 mg. The average caffeine content was 12.70 mg, giving a recovery of 100.8%. The standard deviation was 0.23 mg; the coefficient of variation was therefore 1.8%. The other five caffeine readings from 43.1 mg caffeine added to 250 mL Caffeine-Free Diet Coke were 41.67, 41.41, 42.08, 41.67, and 41.52. The average caffeine content was 41.67 mg, and the standard deviation was 0.26 mg. The recovery was 96.7%, and the coefficient of variation was 0.6%.The recoveries and coefficients of variation for the analytical method were both acceptable.

National brand colas

The caffeine contents of 31 national brand colas are listed in Table 4.1. The caffeine contents of this group ranged from 10.3 to 57.1 mg per 12 oz. The highest value (57.1 mg/12 oz) was found in Pepsi One. Except for lower caffeine contents of Ritz Cola and Red Rock Cola and the higher caffeine content of Pepsi One, the rest of the samples contained between 33 and 48 mg caffeine/12 oz. The caffeine values of national brand colas samples determined in a previous study (Grand and Bell 1997) were 11-16% lower for Coke, Diet Coke, Pepsi, and Diet Pepsi than in the current study. Caffeine values for Tab, RC Cola, and Shasta Cola were similar to those reported previously

(Grand and Bell 1997). The U.S. Department of Agriculture (USDA) National Nutrient Database for standard Reference, Release 19 (2006) gave caffeine contents of

Table 4.1-Caffeine contents (mg/12 oz) of national brand colas (mean ± standard deviation)

Beverage	Lot values	Overall mean
Pepsi One (n=2)	54.7, 59.4	57.1±3.3
Diet Cheerwine (n=2)	47.3, 48.9	48.1±1.1
Tab (n=2)	46.7, 49.5	48.1±1.9
Cheerwine (n=2)	46.5, 48.6	47.5±1.4
Diet RC (n=2)	46.2, 48.5	47.3±1.6
Diet Coke (n=3)	44.4, 47.8, 46.8	46.3±1.7
Diet Coke with Lime (n=2)	44.4, 48.2	46.3±2.7
RC Cola (n=4)	42.3, 50.9, 42.0, 45.7	45.2±4.1
Diet Vanilla Coke (n=1)*	44.5	44.5
Shasta Cola (n=2)	41.3, 44.5	42.9±2.2
Faygo Cola (n=2)	39.6, 43.8	41.7±3.0
Diet Cherry Pepsi (n=2)	38.2, 42.8	40.5±2.7
Cherry Pepsi (n=2)	37.4, 41.9	39.7±3.2
Pepsi (n=3)	38.0, 39.9, 38.7	38.9±1.0
Pepsi with Lime (n=2)	36.7, 40.0	38.4±2.0
Diet Vanilla Pepsi (n=1)*	38.1	38.1
Vanilla Pepsi (n=1)*	37.4	37.4
Diet Coca-Cola Black Cherry Vanilla (n=2)	37.8, 35.8	36.8±1.4
Diet Pepsi (n=3)	36.1, 36.7, 37.4	36.7±0.6
Diet Pepsi with Lime (n=2)	36.0, 36.9	36.4±0.9
Coke Zero (n=2)	34.0, 37.6	35.8±2.6
Coca-Cola Black Cherry Vanilla (n=2)	35.8, 34.3	35.1±1.1
Diet Cherry Coke (n=2)	33.6, 36.3	35.0±2.0
Cherry Coke (n=2)	33.2, 35.7	34.4±1.8
Coca-Cola C2 (n=2)	33.4, 35.4	34.4±1.5
Diet Coke with Splenda (n=2)	33.5, 35.3	34.4±1.3
Coca-Cola (n=3)	33.1, 34.6, 34.1	33.9±0.9
Coke with Lime (n=2)	32.9, 34.4	33.6±1.1
Vanilla Coke (n=1) *	33.3	33.3
Red Rock Cola (n=2)	25.4, 26.8	26.1±1.0
Ritz Cola (n=2)	9.7, 10.9	10.3±0.9

*These products have been discontinued.

29 mg/12 oz beverage for regular cola products, which was lower than the most of the values determined in the present study. The measured caffeine contents of the cola beverages were similar to those reported in the database of the American Beverage Association (2006).

Only one lot of Vanilla Coke, Diet Vanilla Coke, Vanilla Pepsi, and Diet Vanilla Pepsi were obtained because additional lots were no longer available. Large variations were found between the first and second lots (42.3 and 50.9 mg/12 oz, respectively) of RC Cola. Therefore, third and fourth lots were analyzed to clarify this question. The caffeine content of the third lot (42.0 mg/12 oz) was found to be similar with the first lot. The caffeine content of the fourth lot (45.7 mg/12 oz) was also close to the first and third lots.

National brand pepper-type drinks

The caffeine contents of 10 national brand pepper-type drinks are reported in Table 4.2. The caffeine contents of this group ranged from 39.4 to 44.1 mg per 12 oz. The lowest and highest caffeine concentrations were found in Cherry Vanilla Dr. Pepper and Diet Dr. Pepper, respectively. All samples in this group contained similar caffeine contents and no quality control issues were found between the two lots. The caffeine values of national brand pepper-type drinks from a previous study (Grand and Bell 1997) were comparable to this current study. The USDA nutrient database gave caffeine contents of 43 mg/12 oz beverage for diet pepper-type drinks products. On the other hand, the USDA nutrient database gave caffeine contents of 37 mg/12 oz beverage for regular pepper products (USDA 2006). The caffeine values for diet pepper-type beverages were similar between the present study and the data from USDA, but the regular pepper-type value (37 mg/can) from USDA was lower than all values determined in the present study. Compared with the caffeine contents from the American Beverage Association (2006), the Dr. Pepper, Pibb Zero, and Pibb Xtra caffeine values were similar to those from the present study. The caffeine content of Diet Dr. Pepper was slightly higher in the present study.

Table 4.2 - Caffeine contents (mg/12 oz) of national brand pepper-type drinks (mean ± standard deviation)

Beverage	Lot values	Overall mean
Diet Dr. Pepper (n=2)	42.4, 45.7	44.1±2.3
Dr. Pepper (n=3)	40.4, 44.4, 43.2	42.6±2.0
Diet Dr. Pepper Berries & Cream (n=2)	42.8, 41.2	42.0±1.1
Diet Dr. Wham (n=2)	42.5, 41.4	41.9±0.8
Dr. Wham (n=2)	41.4, 41.7	41.6±0.3
Pibb Zero (n=2)	41.4, 41.1	41.2±0.2
Dr. Pepper Berries & Cream (n=2)	40.8, 41.4	41.1±0.5
Pibb Xtra (n=2)	38.6, 42.1	40.3±2.5
Diet Cherry Vanilla Dr. Pepper (n=2)	39.4, 40.8	40.1±1.0
Cherry Vanilla Dr. Pepper (n=2)	38.6, 40.3	39.4±1.3

National brand citrus products

The caffeine contents of 10 national brand citrus products are reported in Table 4.3. The caffeine contents of this group ranged from 19.7 to 74.0 mg caffeine per 12 oz. The greatest caffeine content (74.0 mg/12 oz) was found in Vault Zero. Except for the lowest caffeine content of Faygo Moon Mist (19.7 mg/12 oz), the rest of the samples contained more than 49 mg caffeine per 12 oz. Large variations were found between the first and second lots (17.6 and 23.1 mg/12 oz, respectively) of Faygo Moon Mist. Therefore, a

Table 4.3 - Caffeine contents (mg/12 oz) of national citrus products (mean ± standard deviation)

Beverage	Lot values	Overall mean
Vault Zero (n=2)	72.8, 75.2	74.0±1.7
Diet SunDrop (n=2)	70.2, 72.9	71.5±1.9
Vault Citrus (n=2)	70.2, 71.1	70.6±0.7
SunDrop (n=2)	63.3, 66.2	64.7±2.0
Diet Mt. Dew Code Red (n=2)	54.5, 56.3	55.4±1.3
Diet Mt. Dew (n=2)	55.0, 55.4	55.2±0.3
Mt. Dew (n=2)	53.0, 56.5	54.8±2.5
Mt. Dew Code Red (n=2)	54.1, 54.5	54.3±0.3
Mello Yello (n=2)	48.3, 50.8	49.5±1.8
Faygo Moon Mist (n=3)	17.6, 23.1, 18.3	19.7±3.0

third lot was analyzed for clarification. The caffeine content of the third lot (18.3 mg/can) was found to be similar to that of the first lot. There may be a quality control issue with the second lot. The USDA nutrient database gave caffeine contents of 55 mg/12 oz beverage for regular caffeinated lemon-lime beverages (USDA 2006). For the purpose of this study, it is assumed that the lemon-lime caffeinated beverage classification by USDA refers to citrus products because there is no other citrus beverage category. Five out of 10 national brand citrus products were found to be similar to the data from USDA. The other 5 citrus products were quite different from USDA database. The caffeine contents of regular/diet SunDrop and Vault were 17-34% greater than listed by USDA. There were little differences in data from the present study and that from Grand and Bell (1997) for the caffeine contents of regular/diet Mountain Dew and Mello Yello. The data collected in the current study is also similar to that reported by the American Beverage Association (2006). One thing should be noticed is the USDA classification of “Carbonated beverage, low calorie, other than cola or pepper, with aspartame, contains caffeine” could include diet citrus beverages. This beverage category had caffeine levels of 53 mg/12 oz (USDA 2006). Thus, clear descriptions of database categories are recommended.

Miscellaneous national brand beverages

The caffeine contents of 5 miscellaneous national brand beverages are reported in Table 4.4. The caffeine contents of this group ranged from 22.4 to 41.5 mg per 12 oz. The caffeine content of Big Red (34.0 mg/12 oz) was similar to the majority of national brand cola beverages. The caffeine values of Sunkist samples from the previous study (Grand and Bell 1997) were comparable to this current study. The data are also comparable to that provided by the American Beverage Association (2006). The USDA nutrient database gave no caffeine content for carbonated orange products, but the regular/diet Sunkist beverages were determined to contain 40.6 and 41.5 mg caffeine per 12 oz, respectively. In addition, the USDA nutrient database gave no caffeine content for root beer products, but the caffeine content of 22.4 mg per 12 oz was found in Barq’s Root Beer (USDA 2006). The USDA Nutrient Database gave no caffeine content for cream soda products, but the A & W Cream Soda was determined to contain 28.6 mg caffeine per 12 oz. The USDA may provide some inaccurate data in this group when compared with previous and present studies. Because these products may or may not contain caffeine, careful evaluation of the products’ ingredient list is advised.

Table 4.4 - Caffeine contents (mg/12 oz) of miscellaneous national brand beverages (mean ± standard deviation)

Beverage	Lot values	Overall mean
Diet Sunkist (n=2)	41.2, 41.7	41.5±0.3
Sunkist (n=2)	40.4, 40.8	40.6±0.2
Big Red (n=2)	33.7, 34.4	34.0±0.5
A & W Cream Soda (n=2)	27.6, 29.6	28.6±1.4
Barq's Root Beer (n=2)	21.4, 23.3	22.4±1.4

Private-label store brand colas

The caffeine contents of 41 private-label store brand regular and diet colas are reported in Tables 4.5 and 4.6, respectively. The caffeine contents of regular colas ranged from 4.9 mg (IGA Cola) to 46.4 mg (Big Fizz Cola) caffeine per 12 oz. The caffeine contents of diet colas ranged from 10.3 mg (IGA Diet Cola) to 61.9 mg (Big Fizz Diet Cola) caffeine per 12 oz. The range of caffeine contents of this group was unlike the spread of national brand colas, being much wider. The large caffeine content range of these products prevents consumers from having a general idea about how much caffeine exists in the private-label store brand colas.

The caffeine values of private-label store brand colas including Chek Diet Cola, Big K Diet Cola, and Sam's regular/diet Cola reported previously (Grand and Bell 1997) were comparable to those from the current study. The Big K Cola and Chek Cola were found to contain 6.5 times and 29% more caffeine contents than the data reported by Grand and Bell (1997). The USDA nutrient database gave caffeine contents of 29 mg/12 oz beverage for regular cola products (USDA 2006). The wide caffeine content range of private-label store brand regular/diet colas (from 4.9 to 61.9 mg per 12 oz) may not be appropriately compared with the USDA database. The lack of data regarding private-label store brand regular/diet colas in the American Beverage Association database prevents the public from having information about these products.

Table 4.5-Caffeine contents (mg/12 oz) of private-label store brand regular colas

Beverage	Lot values	Overall mean
Big Fizz Cola[l] (n=3)	54.3, 56.7, 28.2	46.4±15.8
Big K Cherry Cola[a] (n=2)	40.9, 45.0	43.0±2.9
Walgreen Cola[o] (n=3)	39.1, 42.9, 35.5	39.2±8.1
Big K Cola[a] (n=3)	36.5, 40.8, 39.1	38.8±2.2
Big Gulp Cola[i] (n=3)	39.3, 38.5, 38.1	38.6±0.6
Chek Vanilla Cola[b] (n=2)	34.6, 37.9	36.3±2.3
Bubba Cola[g] (n=3)	36.5, 33.6, 33.6	35.4±1.6
Chek Cola[b] (n=3)	36.4, 35.0, 32.8	34.7±1.8
Big K Cola with Lime[a] (n=2)	30.0, 30.6	30.3±0.5
Clover Valley Cola[f] (n=6)	21.3, 25.5, 33.8, 33.4, 34.5, 24.5	28.8±5.7
Chek Cherry Cola[b] (n=2)	25.4, 27.2	26.3±1.2
Chek Mate Cola[b] (n=2)	25.3, 27.2	26.2±1.4
Food Lion Cola[k] (n=3)	24.5, 26.0, 25.3	25.3±0.8
Laura Lynn Cola[m] (n=4)	22.2, 26.4, 24.8, 24.2	24.4±1.8
Superchill Cola[j] (n=3)	23.2, 24.3, 25.1	24.2±0.9
Publix Cola[e] (n=3)	21.9, 25.6, 21.7	23.1±2.2
Rally Cola[d] (n=3)	11.7, 14.6, 13.6	13.3±1.5
Piggy Wiggly Cola[h] (n=3)	11.4, 14.3, 12.3	12.7±1.5
Sam's Cola[c] (n=3)	11.7, 12.9, 13.6	12.7±1.0
Publix Cherry Cola[e] (n=2)	10.9, 13.9	12.4±2.1
Laura Lynn Cherry Cola[m] (n=2)	7.1, 9.6	8.4±1.8
IGA Cola[n] (n=3)	5.7, 3.7, 5.4	4.9±1.1

[a]Kroger, Cincinnati, OH; [b]Winn-Dixie Stores, Jacksonville, FL; [c]Wal-Mart Stores Inc, Bentonville, AK; [d]Deep South Products, Inc., Fitzgerald, GA; [e]Publix Super Markets, Inc., Lakeland, FL; [f]DolgenCorp, Inc., Goodlettsville, TN; [g]Save-a-lot Food Stores, Inc., Earth City, MO; [h]Piggy Wiggly Corp, Memphis, TN; [i]7-Eleven Inc., Dalla, TX; [j]Supervalu Inc., Eden Prairie, MN; [k]Food Lion LLC. Salisbury, NC; [l]Rite-Aid Corp., Harrisburg, PA; [m]Ingle’s Markets, Inc., Asheville, NC; [n]IGA, Inc., Chicago, IL; [o]Walgreens Co., Deerfield, IL.

Table 4.6-Caffeine contents (mg/12 oz) of private-label store brand diet colas

Beverage	Lot values	Overall mean
Big Fizz Diet Cola[l] (n=3)	60.5, 64.7, 60.5	61.9±2.4
Chek Diet Cola with Lime[b] (n=2)	42.8, 48.8	45.8±4.2
Walgreen Diet Cola[o](n=3)	54.2, 42.1, 38.6	45.0±6.74
Diet Bubba Cola[g] (n=4)	40.7, 45.3, 40.8, 41.3	42.0±2.2
Big K Cherry Diet Cola[a] (n=2)	38.6, 41.2	39.9±1.8
Publix Diet Cola[e] (n=3)	34.3, 38.5, 32.9	35.2±2.9
Superchill Diet Cola[j] (n=3)	33.8, 34.8, 35.0	34.5±0.6
Big Gulp Diet Cola[i] (n=2)	30.5, 33.1	31.8±1.9
Big K Diet Cola[a] (n=3)	28.5, 31.6, 30.0	30.0±1.6
Chek Diet Vanilla Cola[b] (n=2)	27.5, 30.3	28.9±2.0
Chek Diet Cola[b] (n=3)	26.3, 29.5, 26.7	27.5±1.7
Clover Valley Diet Cola[f] (n=7)	25.5, 13.2, 27.9, 28.0, 26.5, 25.0 14.0	22.9±6.4
Big K Diet Cola with Lime[a] (n=2)	18.8, 18.4	18.6±0.3
Sam's Diet Cola[c] (n=3)	12.4, 12.3, 14.5	13.1±1.3
Rally Diet Cola[d] (n=3)	10.7, 13.8, 14.6	13.0±2.1
Piggy Wiggly Diet Cola[h] (n=3)	10.2, 14.1, 11.5	11.9±2.0
Food Lion Diet Cola[k] (n=3)	11.6, 11.3, 12.8	11.9±0.8
Laura Lynn Diet Cola[m] (n=3)	11.6, 10.6, 11.7	11.3±0.6
IGA Diet Cola[n] (n=3)	10.9, 9.0, 11.1	10.3±1.2

[a]Kroger, Cincinnati, OH; [b]Winn-Dixie Stores, Jacksonville, FL; [c]Wal-Mart Stores Inc, Bentonville, AK; [d]Deep South Products, Inc., Fitzgerald, GA; [e]Publix Super Markets, Inc., Lakeland, FL; [f]DolgenCorp, Inc., Goodlettsville, TN; [g]Save-a-lot Food Stores, Inc., Earth City, MO; [h]Piggy Wiggly Corp, Memphis, TN; [i]7-Eleven Inc., Dalla, TX; [j]Supervalu Inc., Eden Prairie, MN; [k]Food Lion LLC. Salisbury, NC; [l]Rite-Aid Corp., Harrisburg, PA; [m]Ingle’s Markets, Inc., Asheville, NC; [n]IGA, Inc., Chicago, IL; [o]Walgreens Co., Deerfield, IL.

Private brand pepper products

The caffeine contents of 18 private label store brand pepper products are reported in Table 4.7. The caffeine contents of this group ranged from 18.2 to 59.8 mg caffeine per 12 oz. The lowest and highest caffeine concentration was found in Diet Dr. Lynn and Dr. IGA, respectively. The caffeine contents of the samples were distributed evenly within this range. The distribution of this group was different from national ones, which contained around 40 mg caffeine per 12 oz. The caffeine content of regular/diet Dr. K analyzed by Grand and Bell (1997) were quite lower than the present study. The caffeine content of Dr. Chek analyzed in the present study was slightly higher than the previous study. The USDA nutrient database gave caffeine contents of 43 mg/12 oz beverage for diet pepper-type drinks and 37 mg/12 oz beverage for regular pepper drinks. Obviously, the USDA database fails to fit the results of private label store brand pepper products. Neither does the database from American Beverage Association, where no values are reported. Thus, more comprehensive data on private label store brand pepper products is recommended for these public databases.

Table 4.7-Caffeine contents (mg per 12 oz) of private brand pepper products

Beverage	Lot values	Overall mean
Dr. IGA[l] (n=2)	62.4, 57.1	59.8±3.7
Diet Dr. Pop[g] (n=2)	55.3, 58.2	56.8±2.0
Dr. Pop[g] (n=5)	29.1, 46.2, 55.9, 53.7, 52.8	47.5±11.0
Dr. K[a] (n=2)	39.2, 43.2	41.2±2.8
Diet Dr. K[a] (n=2)	39.0, 42.5	40.7±2.5
Dr. Topper[f] (n=2)	35.8, 32.1	34.0±2.7
Dr. Publix[e] (n=2)	30.2, 33.0	31.6±2.0
Dr. Bob[d] (n=2)	30.2, 32.5	31.3±1.6
Dr. Pig[h] (n=2)	29.5, 32.8	31.2±2.3
Diet Dr. Bob[d] (n=2)	30.5, 31.3	30.9±0.6
Dr. Thunder[c] (n=2)	29.7, 31.5	30.6±1.3
Dr. Chill[i] (n=2)	28.6, 31.2	29.9±1.8
Diet Dr. Thunder[c] (n=2)	29.3, 30.5	29.9±0.8
Dr. Chek[b] (n=2)	23.5, 25.3	24.4±1.3
Diet Dr. Chek[b] (n=2)	21.4, 23.2	22.3±1.3
Dr. Lynn[k] (n=2)	18.7, 19.9	19.3±0.9
Dr. Perky[j] (n=2)	17.8, 19.9	18.8±1.5
Diet Dr. Lynn[k] (n=2)	17.5, 18.9	18.2±1.0

[a]Kroger, Cincinnati, OH; [b]Winn-Dixie Stores, Jacksonville, FL; [c]Wal-Mart Stores Inc, Bentonville, AK; [d]Deep South Products, Inc., Fitzgerald, GA; [e]Publix Super Markets, Inc., Lakeland, FL; [f]DolgenCorp, Inc., Goodlettsville, TN; [g]Save-a-lot Food Stores, Inc., Earth City, MO; [h]Piggy Wiggly Corp, Memphis, TN; [i]Supervalu Inc., Eden Prairie, MN; [j]Food Lion LLC. Salisbury, NC; [k]Ingle's Markets, Inc., Asheville, NC; [l]IGA, Inc., Chicago, IL.

Private-label store brand citrus products

The caffeine contents of 16 private-label store brand citrus products are reported in Table 4.8. The caffeine contents of this group ranged from 25.1 to 55.1 mg caffeine per 12 oz. The lowest and highest caffeine concentrations were found in Big K Diet Citrus Drop and Chek Kountry Mist, respectively. Ten beverages within this group contained over 50 mg caffeine per 12 oz. The USDA nutrient database gave caffeine contents of 55 mg/12 oz beverage for lemon-lime (i.e., citrus) products (USDA 2006). Most of this group's results are similar to the value from USDA. Kroger's Big K products contained approximately half the caffeine of the value listed by USDA. There is no data on the caffeine content of private-label store brand citrus products in the American Beverage

Table 4.8-Caffeine contents (mg per 12 oz) of private-label store brand citrus products

Beverage	Lot values	Overall mean
Chek Kountry Mist[b] (n=2)	51.7, 58.6	55.1±4.9
Ramp Red[d] (n=2)	53.9, 55.3	54.6±1.0
IGA Spring Mist[l] (n=2)	51.0, 57.3	54.2±4.4
Publix Citrus Hit[e] (n=2)	53.5, 54.8	54.1±1.0
Ramp[d] (n=2)	53.1, 54.5	53.8±1.0
Superchill Mt. Chill[i] (n=2)	54.0, 52.9	53.5±0.8
Chek Red Alert[b] (n=2)	54.5, 51.9	53.2±1.8
Save-a-lot Mt. Holler[g] (n=2)	52.8, 53.4	53.1±0.4
Piggy Wiggly Mt. Yeller[h] (n=2)	53.2, 53.0	53.1±0.1
CloverValley Citrus Drop[f] (n=2)	51.8, 52.1	52.0±0.3
Sam's Mountain Lightening[c] (n=2)	47.3, 45.8	46.5±1.0
Chek Diet Kountry Mist[b] (n=4)	49.4, 34.8, 50.6, 50.3	46.3±7.7
Food Lion Mt. Lion[j] (n=2)	30.9, 30.9	30.9±0
Laura Lynn Mt. Moon Drop[k] (n=4)	30.6, 16.8, 32.7, 30.0	27.5±7.2
Big K Citrus Drop[a] (n=2)	25.8, 26.6	26.2±0.5
Big K Diet Citrus Drop[a] (n=2)	24.8, 25.5	25.1±0.5

[a]Kroger, Cincinnati, OH; [b]Winn-Dixie Stores, Jacksonville, FL; [c]Wal-Mart Stores Inc, Bentonville, AK; [d]Deep South Products, Inc., Fitzgerald, GA; [e]Publix Super Markets, Inc., Lakeland, FL; [f]DolgenCorp, Inc., Goodlettsville, TN; [g]Save-a-lot Food Stores, Inc., Earth City, MO; [h]Piggy Wiggly Corp, Memphis, TN; [i]Supervalu Inc., Eden Prairie, MN; [j]Food Lion LLC. Salisbury, NC; [k]Ingle's Markets, Inc., Asheville, NC; [l]IGA, Inc., Chicago, IL.

Association's database. The amounts of caffeine existing in samples (Chek Kountry Mist, Big K regular/diet Citrus Drop) were similar to the values of Grand and Bell (1997).

Quality control of store brand beverages

The quality control of national brand colas appeared much better than the store brand colas, which is why some samples in this group required more than two lots to clarify some questionable data. The colas under the Clover Valley label were the most variable.

Within the store brand pepper products, because big variations of caffeine values were observed between the first and second lots of Dr. Pop, the third, fourth, and fifth lots were obtained and analyzed.

Both Chek Diet Kountry Mist and Laura Lynn Mt. Moon Drop were found to have lower caffeine contents in the second lot. Therefore, third and fourth lots were analyzed to clarify this problem. The results of the third and fourth lot are similar to the first lot. In addition, one lot of Mt. Lion was found to contain no caffeine in the present study. Thus, there appears to be some quality control issues with selected store brand products.

The mean caffeine contents in different types of beverages

The average amounts of caffeine existing in each beverage classification are tabulated in Table 4.9. The amounts of caffeine in national brand cola and pepper groups were similar. The regular/diet citrus groups contained more caffeine than cola and pepper beverages in national-brand category. The caffeine contents of the store-brand beverages were on average lower than national-brand counterparts. In addition, the variation between store brands appears greater than between national brands.

Table 4.9-The caffeine contents (mg per 12 oz) in each classification (mean ± standard deviation)

Beverage Type	National-brand	Store-brand
Cola with sugar	35.9±8.9 (n=15)	26.6±11.9 (n=22)
Diet Cola	42.0±6.8 (n=15)	28.2±14.7 (n=19)
Pepper	41.0±1.2 (n=5)	33.3±11.6 (n=12)
Diet Pepper	41.9±1.5 (n=5)	33.1±14.0 (n=6)
Citrus	52.3±17.8 (n=6)	47.7±10.8 (n=12)
Diet Citrus	64.0±10.1 (n=4)	35.7±15.0 (n=2)

CHAPTER 5: SUMMARY AND CONCLUSIONS

Within the national brand beverage category, the lowest caffeine content was determined to be in Ritz Cola, which contained 10 mg caffeine per 12 oz. The highest caffeine values of the national brand beverages were observed among Vault Citrus, Vault Zero, and Diet SunDrop at 70-74 mg caffeine per 12 oz. There is approximately a 6-fold difference between the highest and lowest caffeine values within national brand beverages. Most colas and pepper-type products contained around 40 mg caffeine per 12 oz. A more robust extent of quality control appeared to exist in the national brand carbonated beverages as demonstrated by less lot to lot variability.

Within the store brand beverage category, the IGA Cola contained the lowest caffeine value (5 mg per 12 oz). The highest caffeine contents (around 60 mg per 12 oz) were found in Big Fizz Cola and Dr. IGA. The caffeine contents within beverage types were quite disperse for the store brand beverages. In addition, variability between lots was greater than for the national brand beverages.

The caffeine data of the current study may be used as a more extensive database to replace that removed from the American Beverage Association website and improve the vague classification of beverages by the USDA. The caffeine data determined in the present study suggests that consumers concerned about limiting daily caffeine ingestion from carbonated beverages may select the lower caffeine-containing store brand beverages. Consumers desiring caffeine may likewise select from the higher caffeine products. However, broad generalizations about the caffeine contents of carbonated beverages are difficult to make. The varied contents should be either accounted for in databases or caffeine values placed on food labels so consumers can be better informed.

REFERENCES

Abourashed EA, Mossa JS. 2004. HPTLC determination of caffeine in stimulant herbal products and power drinks. Journal of Pharmaceutical and Biomedical Analysis 36:617-20.

ADA. 2004. Caffeine and Coffee: are there health risks? American Diet Association. http://eatright.org/cps/rde/xchg/ada/hs.xsl/home_4293_ENU_HTML.htm. Accessed Oct 2, 2006.

Armenta S, Garrigues S, de la Guardia M. 2005. Solid-phase FT-Raman determination of caffeine in energy drinks. Analytica Chimica Acta 547:197-203.

Bernstein GA, Carroll ME, Thuras PD, Cosgrove KP, Roth ME. 2002. Caffeine dependence in teenagers. Drug and Alcohol Dependence 66:1-6.

Brunetto MdR, Gutierrez L, Delgado Y, Gallignani M, Zambrano A, Gomez A, Ramos G, Romero C. 2007. Determination of theobromine, theophylline and caffeine in cocoa samples by a high-performance liquid chromatographic method with on-line sample cleanup in a switching-column system. Food Chemistry 100:459-67.

Caudle AG, Gu Y, Bell LN. 2001. Improved analysis of theobromine and caffeine in chocolate food products formulated with cocoa powder. Food Research International 34:599-603.

Chen Q-C, Wang J. 2001. Simultaneous determination of artificial sweeteners, preservatives, caffeine, theobromine and theophylline in food and pharmaceutical preparations by ion chromatography. Journal of Chromatography A 937:57-64.

Chen Q, Zhao J, Huang X, Zhang H, Liu M. 2006. Simultaneous determination of total polyphenols and caffeine contents of green tea by near-infrared reflectance spectroscopy. Microchemical Journal 83:42-7.

de Aragao NM, Veloso MCC, Bispo MS, Ferreira SLC, de Andrade JB. 2005. Multivariate optimisation of the experimental conditions for determination of three methylxanthines by reversed-phase high-performance liquid chromatography. Talanta 67:1007-13.

Farah A, Monteiro MC, Calado V, Franca AS, Trugo LC. 2006. Correlation between cup quality and chemical attributes of Brazilian coffee. Food Chemistry 98:373-80.

FDA. 2006. Food additives status list. Food and Drug Adminitration. http://www.cfsan.fda.gov/~dms/opa-appa.html. Accessed July 6, 2006.

Goto T, Yoshida Y, Kiso M, Nagashima H. 1996. Simultaneous analysis of individual catechins and caffeine in green tea. Journal of Chromatography A 749:295-9.

Grand AN, Bell LN. 1997. Caffeine Content of Fountain and Private-Label Store Brand Carbonated Beverages. Journal of the American Dietetic Association 97:179-82.

Hartley TR, Lovallo WR, Whitsett TL. 2004. Cardiovascular effects of caffeine in men and women. The American Journal of Cardiology 93:1022-6.
Horie H, Mukai T, Kohata K. 1997. Simultaneous determination of qualitatively important components in green tea infusions using capillary electrophoresis. Journal of Chromatography A 758:332-5.
Huck CW, Guggenbichler W, Bonn GK. 2005. Analysis of caffeine, theobromine and theophylline in coffee by near infrared spectroscopy (NIRS) compared to high-performance liquid chromatography (HPLC) coupled to mass spectrometry. Analytica Chimica Acta 538:195-203.
James J. 1991. Caffeine and health. San Diego: Academic Press Inc. 26-32 p.
Kaufman KR, Sachdeo RC. 2003. Caffeinated beverages and decreased seizure control. Seizure 12:519-21.
Liguori A, Hughes JR, Grass JA. 1997. Absorption and Subjective Effects of Caffeine from Coffee, Cola and Capsules. Pharmacology Biochemistry and Behavior 58:721-6.
Lucena R, Cardenas S, Gallego M, Valcarcel M. 2005. Continuous flow autoanalyzer for the sequential determination of total sugars, colorant and caffeine contents in soft drinks. Analytica Chimica Acta 530:283-9.
Mandel HG. 2002. Update on caffeine consumption, disposition and action. Food and Chemical Toxicology 40:1231-4.
Mashkouri Najafi N, Hamid AS, Afshin RK. 2003. Determination of caffeine in black tea leaves by Fourier transform infrared spectrometry using multiple linear regression. Microchemical Journal 75:151-8.
Nehlig A, Daval J-L, Debry G. 1992. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Research Reviews 17:139-70.
Nishitani E, Sagesaka YM. 2004. Simultaneous determination of catechins, caffeine and other phenolic compounds in tea using new HPLC method. Journal of Food Composition and Analysis 17:675-85.
Notarius CF, Morris BL, Floras JS. 2006. Caffeine Attenuates Early Post-Exercise Hypotension in Middle-Aged Subjects. American Journal of Hypertension 19:184-8.
NSWHEALTH. 2002. Caffeine factsheet. Centre for Drug and Alcohol, New South Wales Department of Health. http://www.health.nsw.gov.au/public-health/dpb/publications/caffeine.html. Accessed Oct 4, 2006.
Paradkar MM, Irudayaraj J. 2002. Rapid determination of caffeine content in soft drinks using FTIR-ATR spectroscopy. Food Chemistry 78:261-6.
Pura Naik J. 2001. Improved high-performance liquid chromatography method to determine theobromine and caffeine in cocoa and cocoa products. J Agric Food Chem 49:3579-83.
Savoca MR, MacKey ML, Evans CD, Wilson M, Ludwig DA, Harshfield GA. 2005. Association of ambulatory blood pressure and dietary caffeine in adolescents. American Journal of Hypertension 18:116-20.

Schulz H, Engelhardt UH, Wegent A, Drews H, Lapczynski S. 1999. Application of near-infrared reflectance spectroscopy to the simultaneous prediction of alkaloids and phenolic substances in green tea leaves. J Agric Food Chem 47:5064-7.

Shilo L, Sabbah H, Hadari R, Kovatz S, Weinberg U, Dolev S, Dagan Y, Shenkman L. 2002. The effects of coffee consumption on sleep and melatonin secretion. Sleep Medicine 3:271-3.

Spiller G. 1998. Caffeine. New York: CRC Press. 225-30 p.

Thomas JB, Yen JH, Schantz MM, Porter BJ, Sharpless KE. 2004. Determination of caffeine, theobromine, and theophylline in standard reference material 2384, baking chocolate, using reversed-phase liquid chromatography. J Agric Food Chem 52:3259-63.

Walker JC, Zaugg SE, Walker EB. 1997. Analysis of beverages by capillary electrophoresis. Journal of Chromatography A 781:481-5.

Wang H, Helliwell K, You X. 2000. Isocratic elution system for the determination of catechins, caffeine and gallic acid in green tea using HPLC. Food Chemistry 68:115-21.

Watson JM, Lunt MJ, Morris S, Weiss MJ, Hussey D, Kerr D. 2000. Reversal of Caffeine Withdrawal by Ingestion of a Soft Beverage. Pharmacology Biochemistry and Behavior 66:15-8.

Yao L, Liu X, Jiang Y, Caffin N, D'Arcy B, Singanusong R, Datta N, Xu Y. 2006. Compositional analysis of teas from Australian supermarkets. Food Chemistry 94:115-22.

Zuo Y, Chen H, Deng Y. 2002. Simultaneous determination of catechins, caffeine and gallic acids in green, Oolong, black and pu-erh teas using HPLC with a photodiode array detector. Talanta 57:307-16.

APPENDICES

Table A1 – Caffeine content of national brand colas

Beverage type	Caffeine in 1st Lot		Caffeine in 2nd Lot		Caffeine in 3rd Lot	
Coca-Cola	32.57	33.58	34.26	34.96	33.65	34.6
Diet Coke	45.09	43.74	47.69	48.00	46.85	46.85
Cherry Coke	33.33	32.98	35.73	35.57		
Diet Cherry Coke	33.79	33.36	36.04	36.65		
Coke with Lime	32.92	32.84	33.99	34.77		
Diet Coke with Lime	44.17	44.52	47.71	48.72		
Vanilla Coke	33.45	33.17				
Diet Vanilla Coke	43.94	45				
Coca-Cola C2	32.6	34.1	35.04	35.82		
Diet Coke with Splenda	33.58	33.36	35.42	35.11		
Coke Zero	33.56	34.41	36.82	38.45		
Tab	46.19	47.24	48.71	50.24		
Pepsi	37.98	37.98	40.07	39.69	38.61	38.83
Diet Pepsi	35.59	36.67	36.4	36.95	36.79	38.03
Cherry Pepsi	37.25	37.62	42.2	41.58		
Diet Cherry Pepsi	38.6	37.69	42.65	42.89		
Pepsi with Lime	36.01	37.39	39.76	40.27		
Diet Pepsi with Lime	35.37	36.57	36.19	37.57		
Vanilla Pepsi	38.24	36.49				
Diet Vanilla Pepsi	37.47	38.63				
Pepsi One	54.08	55.39	59.4	59.48		
RC Cola	42.01	42.58	51.01	50.78	41.75	42.19
					45.75[a]	45.54[a]
Diet RC	46.06	46.34	47.98	48.99		
Faygo Cola	39.31	39.81	43.66	43.88		
Cheerwine	46.02	47.01	48.47	48.62		
Diet Cheerwine	47.25	47.39	48.4	49.41		
Ritz Cola	9.6	9.74	11.23	10.54		
Shasta	41.41	41.26	44.84	44.06		
Coca-Cola Black Cherry Vanilla	35.62	36.02	33.96	34.64		
Diet Coca-Cola Black Cherry Vanilla	37.91	37.60	35.37	36.29		
Red Rock Cola	25.38	25.38	26.67	26.91		

45.75[a], 45.54[a] are the 4[th] lot values of RC Cola.

Table A2 – Caffeine content of national brand pepper-type products

Beverage type	Caffeine in 1st Lot		Caffeine in 2nd Lot		Caffeine in 3rd Lot	
Dr. Pepper	40.59	40.16	44.16	44.55	43.28	43.03
Diet Dr. Pepper	42.75	42.04	45.66	45.74		
Dr. Pepper Berries & Cream	40.63	40.93	41.24	41.63		
Diet Dr. Pepper Berries & Cream	42.70	42.86	41.51	40.93		
Cherry Vanilla Dr. Pepper	38.43	38.67	39.91	40.75		
Diet Cherry Vanilla Dr. Pepper	39.31	39.38	40.36	41.20		
Dr. Wham	40.46	42.28	41.78	41.70		
Diet Dr. Wham	42.43	42.51	41.32	41.39		
Pibb Xtra	38.64	38.57	42.20	41.97		
Pibb Zero	41.43	41.28	41.78	40.44		

Table A3 – Caffeine content of national brand citrus products

Beverage type	Caffeine in 1st Lot		Caffeine in 2nd Lot		Caffeine in 3rd Lot	
Mt. Dew	53.00	53.00	56.16	56.85		
Diet Mt. Dew	54.46	55.54	55.31	55.55		
Mt. Dew Code Red	54.32	53.89	54.67	54.37		
Diet Mt. Dew Code Red	55.07	53.84	56.08	56.54		
Mello Yello	48.16	48.37	50.84	50.76		
SunDrop	63.13	63.41	65.89	66.43		
Diet SunDrop	69.63	70.71	72.65	73.11		
Vault Citrus	70.45	69.88	70.94	71.25		
Vault Zero	71.95	73.70	75.02	75.33		
Faygo Moon Mist	17.53	17.74	23.40	22.78	18.15	18.44

Table A4 – Caffeine content of national brand miscellaneous products

Beverage type	Caffeine in 1st Lot		Caffeine in 2nd Lot	
Barq’s Root Beer	21.56	21.27	23.57	23.10
A & W Cream Soda	27.26	27.96	29.23	29.92
Sunkist	40.34	40.48	40.99	40.52
Diet Sunkist	41.27	41.20	41.52	41.83
Big Red	33.24	34.09	34.66	34.11

Table A5 – Caffeine content of store brand regular colas

Beverage type	Caffeine in 1st Lot		Caffeine in 2nd Lot		Caffeine in 3rd Lot	
Big Fizz Cola	54.24	54.31	54.72	58.69	27.73	28.67
Big Gulp Cola	39.11	39.46	38	38.95	37.66	38.61
Big K Cherry Cola	40.82	41.03	44.95	45.10		
Big K Cola with Lime	29.80	30.11	30.23	30.95		
Big K Cola	36.35	36.7	40.57	41.03	38.69	39.41
Bubba Cola	36.73	36.23	33.48	33.64	35.69	36.84
Chek Cherry Cola	25.12	25.75	27.2	27.17		
Chek Cola	36.19	36.61	34.94	35.10	32.66	32.87
Chek Mate Cola	25.18	25.32	26.89	27.43		
Chek Vanilla Cola	34.35	34.85	37.55	38.23		
Clover Valley Cola	20.88	21.8	25.2	25.81	33.81	33.73
	33.23[a]	33.53[a]	34.1[b]	34.82[b]	25.26[c]	23.74[c]
IGA Cola	5.76	5.54	3.82	3.65	5.46	5.38
Laura Lynn Cherry Cola	6.54	7.61	10.02	9.24		
Laura Lynn Cola	21.67	22.68	26.12	26.66	24.23	25.41
					23.37[d]	24.97[d]
Piggly Wiggly Cola	11.35	11.35	13.96	14.66	12.52	12.16
Publix Cherry Cola	10.69	11.19	13.91	13.84		
Publix Cola	21.84	21.91	25.79	25.48	21.69	21.69
Rally Cola	11.34	11.99	14.45	14.75	14.69	12.45
Sam's Cola	11.69	11.69	12.98	12.75	13.46	13.75
Superchill Cola	23.16	23.3	24.3	24.31	25.45	24.64
Walgreen Cola	38.49	39.72	42.79	43.02	35.25	35.69
Food Lion Cola	24.34	24.55	26.24	25.77	25.28	25.35

33.23[a], 33.53[a] are the 4th lot values of Clover Valley Cola.
34.1[b], 34.82[b] are the 5th lot values of Clover Valley Cola.
25.26[c], 23.74[c] are the 6th lot values of Clover Valley Cola.
23.37[d], 24.97[d] are the 4th lot values of Laura Lynn Cola.

Table A6 – Caffeine content of store brand diet colas

Beverage type	Caffeine in 1st Lot		Caffeine in 2nd Lot		Caffeine in 3rd Lot	
Big Fizz Diet Cola	60.12	60.86	64.39	65.02	60.67	60.38
Big Gulp Diet Cola	30.04	30.89	32.54	33.64		
Big K Cherry Diet Cola	38.1	39.15	40.8	41.57		
Big K Diet Cola with Lime	19.11	18.57	18.29	18.53		
Big K Diet Cola	28.12	28.81	31.79	31.34	29.89	30.04
Chek Diet Cola with Lime	41.34	44.25	48.5	49.04		
Chek Diet Cola	25.96	26.67	28.96	30.04	26.67	26.74
Chek Diet Vanilla Cola	27.23	27.72	29.65	30.87		
Clover Valley Diet Cola	25.18	25.75	12.65	13.71	28.82	26.9
	27.13[a]	28.90[a]	26.26[b]	26.77[b]	25.01[c]	25.01[c]
	14.05[d]	13.9[d]				
Diet Bubba Cola	40.39	41.09	44.84	45.78	40.58	40.95
	40.81[e]	41.68[e]				
Food Lion Diet Cola	11.85	11.38	11.48	11.01	12.81	12.81
IGA Diet Cola	10.95	10.79	9.17	8.78	11.01	11.15
Laura Lynn Diet Cola	12.02	11.1	10.56	10.71	11.5	11.79
Piggy Wiggly Diet Cola	10.08	10.29	14.03	14.19	11.86	11.2
Publix Diet Cola	34.22	34.36	38.05	38.98	33.02	32.8
Rally Diet Cola	10.41	10.91	14.07	13.53	14.40	14.83
Sam's Diet Cola	12.18	12.53	12.45	12.14	14.76	14.32
Superchill Diet Cola	33.56	34.01	34.57	35.03	34.7	35.22
Walgreen Diet Cola	54.62	53.70	42.4	41.87	38.52	38.74

27.13[a], 28.90[a] are the 4th lot values of Clover Valley Diet Cola.
26.26[b], 26.77[b] are the 5th lot values of Clover Valley Diet Cola.
25.01[c], 25.01[c] are the 6th lot values of Clover Valley Diet Cola.
14.05[d], 13.9[d] are the 7th lot values of Clover Valley Diet Cola.
40.81[e], 41.68[e] are the 4th lot values of Diet Bubba Cola.

Table A7 – Caffeine content of store brand pepper-type products

Beverage type	Caffeine in 1st Lot		Caffeine in 2nd Lot		Caffeine in 3rd Lot	
Dr. IGA	62.21	62.59	56.41	57.82		
Diet Dr. Pop	54.83	55.82	57.99	58.38		
Dr. Pop	28.98	29.26	46.17	46.32	55.32	56.54
	53.14	54.17	52.21	53.3		
Dr. K	39.13	39.2	43.11	43.26		
Diet Dr. K	38.21	39.7	41.72	43.26		
Dr. Topper	35.12	36.53	32.32	31.78		
Dr. Publix	30.07	30.28	33.04	32.88		
Dr. Bob	29.87	30.58	32.84	32.07		
Dr. Pig	29.47	29.61	32.55	33.02		
Diet Dr. Bob	29.62	31.31	31.31	31.31		
Dr. Thunder	29.41	29.98	31.61	31.38		
Dr. Chill	28.46	28.69	31.3	30.99		
Diet Dr. Thunder	28.84	29.84	30.24	30.70		
Dr. Chek	23.42	23.49	25.28	25.28		
Diet Dr. Chek	21.07	21.78	23.14	23.29		
Dr. Lynn	18.56	18.78	19.77	20.08		
Diet Dr. Lynn	17.34	17.72	18.54	19.23		
Dr. Perky	17.71	17.85	19.86	19.86		

Table A8 – Caffeine content of store brand citrus products

Beverage type	Caffeine in 1st Lot		Caffeine in 2nd Lot		Caffeine in 3rd Lot	
Chek Kountry Mist	50.92	52.41	58.74	58.50		
Ramp Red	53.83	54.04	54.92	55.75		
IGA Spring Mist	49.98	52.08	57.04	57.50		
Publix Citrus Hit	53.39	53.53	54.16	55.48		
Ramp	53.04	53.11	54.38	54.68		
Superchill Mt. Chill	54.75	53.29	52.60	53.28		
Chek Red Alert	54.37	54.68	51.69	52.14		
Save-a-lot Mt. Holler	52.37	53.21	53.14	53.68		
Piggy Wiggly Mt. Yeller	52.90	53.45	52.45	53.54		
CloverValley Citrus Drop	51.48	52.11	52.14	52.14		
Sam's Mountain Lightening	47.21	47.35	45.77	45.84		
Chek Diet Kountry Mist	48.86	49.99	34.24	35.30	50.85	50.31
					50.49[a]	50.13[a]
Food Lion Mt. Lion	30.98	30.75	30.82	30.98		
Laura Lynn Mt. Moon Drop	30.26	30.95	16.67	16.98	32.68	32.61
					29.64[b]	30.22[b]
Big K Citrus Drop	25.73	25.95	26.49	26.71		
Big K Diet Citrus Drop	26.23	23.30	25.41	25.56		

50.49[a], 50.13[a] are the 4[th] lot values of Chek Diet Kountry Mist.
29.64[b], 30.22[b] are the 4[th] lot values of Laura Lynn Mt. Moon Drop.