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Investigation of Caffeine Level in Homemade Coffee Liqueur

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Abstract: South Koreans are amongst the top worldwide consumers of coffee and the country is home to over 12,200 coffee shops in 2014. The food category providing highest caffeine (average 150.32 mg/day) to the South Korean consumer group was the coffee extracts such as the brewed coffee in coffeehouse. Coffee Liqueur is an alcoholic beverage that is bottled with added sugar and have added flavors that are derived from coffee nuts. There is currently no research report studying caffeine level of home-made coffee liqueur. In this study, we prepared the home-made coffee liqueur with a variety of liquors such as rum, Vodka, Korean traditional soju and fermented alcohol. Furthermore, different ratios of the roasted coffee bean and its powder with espresso shot were used to make the coffee liqueur. The caffeine level in the homemade coffee liqueurs are from 2.65-2.85 mg/mL. The caffeine extractability might be influenced by not the ratio of the powdered form of roasted coffee bean but the types of alcoholic liquor.

Key words: Coffee liqueur, caffeine, roasted coffee bean, HPLC, alcoholic, homemade

INTRODUCTION

This Caffeine is a very well-known chemical substance that alters perception, mood and consciousness by stimulating the brain function. The current definition of Caffeine in Food Additive Code in 2014, South Korea is the water or carbon dioxide extract (followed by separation and purification steps) of the seed of Rubiaceae Coffee (*Coffea arabica* LINNE) or the leaves of the *Camellia sinensis*, an evergreen shrub native to Asia. In 2015 the European Food Safety Authority (EFSA) published their scientific opinion on the safety of caffeine, advising that caffeine intakes from all sources up to 400 mg/day and single doses of 200 mg do not raise safety concerns for adults in the general population (Efsa, 2015). For children prior to adolescence an acceptable maximal daily intake of 2.5 mg/kg body weight was advised by Belgium's Superior Health Council. In Korea, Ministry of Food and Drug Safety (MFDS) assessed the caffeine exposure to Korean general population and consumers by oral intake of the general foods containing caffeine such as confectionaries (160 cases), cocoa products or chocolates (139 cases), beverage (38 cases), ice creams (50 cases) and other general food products (21 cases). MFDS used the caffeine monitoring data and Korean National Health and Nutrition Examination Survey (KNHNES) (2010-2012) to estimate the caffeine exposure to Korean population and consumers. Mean caffeine intake by general population, male and female were 67.8, 77.2 and 58.2 mg/day, respectively. Mean caffeine in take of 15-18 years consumer group in South Korea was 55.3 mg/day that is 0.9 mg/kg bw/day for the 15~18 years old population in Korea (the body weight was 60.7 kg). In adults mean caffeine intake of 30~49 years was 101.8 mg/day and the percent ratio of the Maximum Recommended Daily Intake (MRDI; 400 mg/day) was 25.5%. Mean caffeine intake by total consumer group, male and female were 102.6, 115.2 and 89.7 mg/day, respectively. And for the age group 30~49 of the consumer, the mean caffeine intake was the highest among all age groups as 129.01 mg/day that is 32.3% of the MRDI value. The food category providing highest caffeine (average 150.32 mg/day) to the consumer group was the coffee extracts such as the brewed coffee in coffeehouse followed by 102.85 mg/day of the instant coffee such as "Coffee mix" in which powder cream, sugar and instant coffee are mixed in a small sachet. Coffee products must be the major oral exposure sources providing caffeine to the Korean population. According to caffeine informer, the top coffee consuming country was Finland in 2013 data as 9.6 kg/capita whereas South Korea was nominated as 26th country to consume the most coffee in the world (2.6 kg/capita). According to the USDA National Nutrient Database, a 300 g cup of "coffee brewed from grounds" contains 120 mg caffeine whereas an Espresso (30 g) contains 64 mg (UDSA, 2013). Korean coffee imports data showed 27% increase in value of $527 million and 17% increase in volume of 134000 metric tons in 2014 compared to the year 2013. Korea's coffee industry has grown up 50% over the past three years, seeing an explosion in both import and consumption to its place as 11th largest coffee

market in the world. South Koreans are now amongst the top global consumers of coffee and the country is home to over 12200 coffee shops in 2014. Seoul itself boasts the highest concentration of coffee shops in any city in the entire world. Each of the various coffee formats has role to play for coffee lovers (USDA Foreign Agricultural Service, 2015).

Coffee Liqueur is an alcoholic beverage that is bottled with added sugar and have added flavors that are derived from coffee nuts. There are a couple of well-known brands of coffee liqueur such as Allen's Coffee Brandy, Cafe Rica, Cafe del Fuego, Kahlua, Kamora, Tia Maria, Toussaint Coffee Liqueur, etc. (Coffee Liqueur, 1962). Among them, the most famous coffee Liqueur brand is Kahlua. Kahlua is the second largest single liqueur brand in the world. Kahlua has a thick creamed coffee taste due to the ingredients such as fine sugar cane, corn syrup, etc. Because of the thick taste, most of coffee liqueurs are consumed through a variety of cocktails such as white Russian, One of those things, white cuban, Mudslide, B52 Shot, etc. But, coffee Liqueur could be easily made in home due to the simple recipe with coffee, liquor, sugar and vanilla syrup. There is no fixed recipe of coffee liqueur. But different forms of coffee could be mixed with high alcohol liquors (usually over 40°). In this study, we prepared the home-made coffee liqueur with a variety of liquors such as vodka, Korean traditional soju, rum etc and the different ethanol percentages of the liquor were also compared. Furthermore, different ratios of the roasted coffee bean and its powder with espresso shot were used to make the coffee liqueur. There is currently no research report studying caffeine level of home-made coffee liqueur. The analyses were performed by simple pre-treatments such as filtration followed by High Performance Liquid Chromatography (HPLC). Caffeine exposure via home-made coffee liqueur was estimated and compared to the MRDI amount of caffeine in Korea. This result will help the coffee lovers to make a smart habit of consuming home-made coffee liqueur to minimize any health hazard due to caffeine.

MATERIALS AND METHODS

Experimental

Materials and sample preparation: Ethiopia Sidamo G2 coffee bean was used as a major ingredient to make the home-made coffee liqueur. Ethiopia Sidamo G2 is a typical Arabica coffee, Bourbon variety. The cultivation area for the coffee bean is located at an altitude of 1600~2000 m in Sidamo, Ethiopia. Within the region of Sidamo, the cultivation area lies on the valley town of Yigacheffe. The flavoring profile of Ethiopia Sidamo G2 is a full of rounded mouthfeel and lower-toned fruit notes such as peach, apricot, orange and raspberry.

Table 1: The recipes of the homemade coffee liqueur

Ingredients	Recipe A	Recipe B	Recipe C
Sugar	130 g	130 g	130 g
Roasted coffee bean	93 g	37 g	65 g
Roasted coffee bean powder	37 g	93 g	65 g
Espresso liquid	25 mL	25 mL	25 mL
Vanilla syrup	14 mL	14 mL	14 mL
Liquor (40% ethanol)	350 mL	350 mL	350 mL

Table 2: HPLC operating condition for the analysis of caffeine in coffee liqueur

Holding time (min)	Mobile Phase A	Mobile Phase B
Gradient mode condition		
10	75	25
15	25	75

Column: Agilent Eclipse plus C_{18} 5 μm (4.6×250 mm); mobile Phase A: 0.05 N phosphoric acid solution; Mobile phase B: 60% of Acetonitrile and 40% of water; Injector: Rheodyne Injector with 20 μL loop; Detector: PDA UV/VIS at 272 nm

The ingredients of coffee liqueur are the roasted coffee bean, roasted coffee bean powder, espresso shot (liquid), white sugar, vanilla syrup and the liquor (40% ethanol). The coffee bean was roasted by the automatic coffee roaster, Gene Cafe CBR-101 (Genesis, Ansan, Korea). The roasting was performed at 180°C for 5 min followed by heating at 250°C for 9.3 min. One batch amount of bean for roasting was 180 g. The color of the roasted bean was medium brown. The roasted bean was ground by the automatic coffee grinder, SP7426 (Wiswell, China). About 60 g of roasted coffee bean was ground for 20 sec for the espresso and 10 sec for the ingredient of coffee liqueur. The roasted coffee bean was used within 5 h. The espresso shot was prepared by an automatic espresso machine (Saeco Royal Type Sup 016, Bologna, Italy). The automatic brewing for one espresso coffee shot (25 mL) took 20 sec. One shot of espresso equal to 7 g of the roasted coffee bean. Coffee liqueurs were prepared by six different recipes (Table 1 and 2). The Recipe A-C were used for comparing the caffeine concentration according to the ratio of the roasted coffee bean and the ground roasted coffee bean. The recipe containing the highest amount ratio of the roasted coffee bean was recipe A (93 g) followed by recipe C (65 g) and Recipe B (37 g). And the recipe with the highest amount ratio of the ground roasted coffee bean (powder) was Recipe B (93 g) followed by Recipe C (65 g) and recipe A (37 g). Four different liquors were used for the preparation of the coffee liquors made by Recipe A, B and C. The liquors were rum (Barcardi superior, San Juan, Puerto Rico), vodka (Smirnoff No. 21, Leven, UK), Korean traditional soju (Hwayo 41°, Yeoju, Korea) and the fermented 95% ethanol (Korea Ethanol Supplies Company, Seoul, Korea). Ethanol percent of the fermented 95% ethanol was adjusted to 40% by diluting it with distilled water. However, the ethanol percentage 41 of the Korean traditional soju was not modified. Other liquors such as rum and vodka were 40% of ethanol. White sugar was from Cheiljedang (Seoul, Korea). The vanilla syrup

was the product of MONIN (Rawang, Malaysia). All ingredients were purchased in a local market in Seoul, Korea. The weight of each ingredient was measured by table top balance (FX-2000i, Cass, Yangju, Korea).

For the preparation of coffee liqueur, the roasted coffee, powder, espresso coffee shots, sugar, vanilla syrup are mixed in a 1 L size glass jar followed by adding of 40% alcoholic liquor (The amount of each ingredient is in Table 1). Each of rum, Vodka, Korean traditional soju and the fermented ethanol was used for the Recipe A, B and C therefore, total twelve coffee liqueurs were prepared. The prepared coffee liqueur ingredients mixture was stored in room temperature for 21 days followed by filtration of the infusion with a double layered coffee filter. The final coffee liqueur infusion was preserved in the 4°C until the instrumental analysis. The infusion was diluted 40 times by distilled water (and mixed with IS) and filtered via 0.45 μm Nylon filter (Sigma-Aldrich, St. Louis, USA) just before HPLC analysis. The analytical standards of caffeine and the Internal Standard (IS) acetaminophene (paracetamol) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Instrumental: HPLC analysis method using the most common reversed phase column was adapted (Belal *et al*., 2014). The analysis was performed by Prominence HPLC equipped with a photo-diode array UV/Vis detector (Shimadzu, Tokyo, Japan). The HPLC operating condition is presented in Table 2.

Method validation: The analysis method validation was performed for accuracy (recovery), precision, Limit of Quantitation (LOQ), Limit of Detection (LOD) and ruggedness by the method of drug equivalency testing method validation protocol, Korea. The concentrations of calibration standards in the ethanol matrix obtained for plotting the calibration curve were 25, 50 and 75 μg/mL with IS 50 μg/mL. The accuracy test was done by the three different solutions with caffeine concentrations 1000, 2000 and 5000 μg/mL in 40% ethanol solution followed by 40 times dilution with distilled water (the final concentrations of caffeine were 25, 50 and 75 μg/mL). All analysis in each concentration point was replicated 3 times. The precision test was performed at 5000 μg/mL with 6 times replication. The LOQ was calculated by the equation "LOQ = 10×σ/S" The symbol σ is the standard deviation mean of the intercept and S is mean of slope from the calibration curve acquired by the six concentrations of caffeine (15, 25, 50, 100, 150 and 250 μg/mL). The LOD was estimated by the equation "LOD = 3.3×σ/S". The ruggedness was estimated by the 4 times replicate analyses at an interval of 8 h with the caffeine concentration 5000 μg/mL in 40% ethanol solution.

Statistical analysis: The mean, standard deviation and Relative Standard Deviation (RSD) % were determined. A two-way ANOVA was performed that examined the type of liquor and recipe on the caffeine level in the home-made coffee liqueurs. All statistical analyses were conducted by SPSS Version 21.0.

RESULTS AND DISCUSSION

Calibration curve, Limit of Quantitation (LOQ) and Limit of Detection (LOD): The correlation coefficient (R^2) of the caffeine calibration curve was 0.999 or better in the concentration range of 25~75 μg/mL by internal standard method. The Relative Standard Deviation (RSD) % of the triplicate analysis of each concentration point was from 0.021-0.036%. LOQ and LOD estimated by the standard curve in the range of 15~250 μg/mL was 2.42 and 0.80 μg/mL, respectively.

Selectivity, accuracy, precision and rugedness: Caffeine peak in HPLC chromatogram was eluted at 6.4 min that was well separated from the IS, acetaminophone eluted at 4.8 min (Fig. 1). The two peaks were well separated from the matrix derived peaks as shown in Fig. 2.

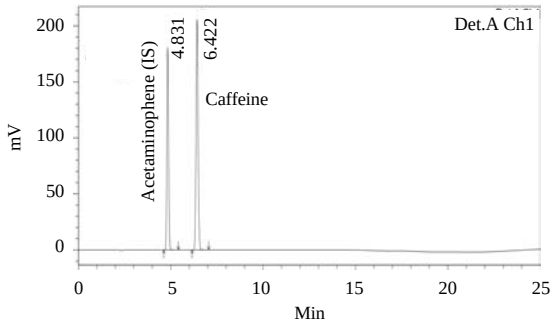

Fig. 1: HPLC-UV (272 nm) standard chromatogram of caffeine and acetaminophene (IS)

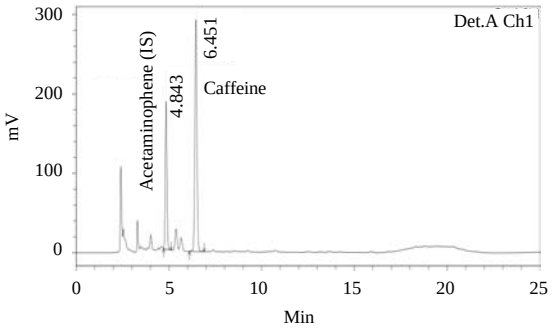

Fig. 2: HPLC-UV (272 nm) chromatogram of the detected caffeine in the coffee liqueur made with Korean traditional soju

Table 3: Caffeine concentrations in the home-made coffee liqueurs by three different recipes and four different liquors (μ/mL)

Variables	Recipe A	Recipe B	Recipe C	Average	RSD (%)
Rum	2683±16	2650±26	2652±53	2662	0.7
Vodka	2688±9	2737±5	2696±29	2707	1.0
Korean traditional soju	2828±14	2818±12	2794±48	2813	0.6
Fermented ethanol	2851±25	2816±11	2779±51	2815	1.3
Average	2763	2755	2730		
RSD (%)	3.2	2.9	2.5		

*Three replicated analyses were conducted

Table 4: Two-way Anova test result for the evaluation of the interaction between two independent variables (type of liquor and recipe) and their interaction (dependent variable: caffeine concentration)

Sources	Type 3 sum of squares	df	Mean square	F-values	Sig.
Corrected model	272300000[a]	12	22691274.800	25289.371	0.000
Type of liquor	160962.45	3	53654.150	59.797	0.000
Recipe	6947.669	2	3473.834	3.872	0.035
Type of liquor*recipe	8821.618	6	1470.270	1.639	0.180
Error	21534.367	24	897.265		
Total	272316832	36			

[a]R^2 = 1.000 (Adjusted R^2 = 1.000)

Therefore, the selectivity of the targets peaks was satisfactory. The accuracy was estimated from the recovered caffeine amount after sample preparation and instrumental analysis. The average recovery was higher than 98%, calculated from the result of the quantitated caffeine comparing the added amount of caffeine, 1000, 2000 and 5000 μg/mL in the 40% ethanol solution. The precision was represented by RSD% as 1.2% that was satisfactory. The RSD% of the quantitated caffeine in the caffeine fortified 40% ethanol solution analyzed at 0, 8, 16 and 24 h was 1.1% that represented the satisfactory ruggedness of the analysis method.

Caffeine concentration in home-made coffee liqueurs: The caffeine concentration results of the twelve coffee liqueur samples are listed in Table 3. The highest average caffeine concentration (2851 μg/mL) was detected in the coffee liqueur made by Recipe A (consisted with the fermented liquor and lowest portion of the roasted coffee bean powder). The lowest average caffeine concentration (2650 μg/mL) was detected in the coffee liqueur made by Recipe B (consisted with the rum and the highest roasted coffee bean powder). This result is opposite to our initial assumption of the roasted coffee bean powder that it may increase the caffeine concertaion in the coffee liqueur due to its large surface for the possibility of alcoholic infusion. The range of RSD% of the average caffeine concentraions (acquired by the same liquor and three different recipes) are from 0.7-1.3% which suggest that there is not much difference between recipes comprised of different level of bean powder. But the RSD% of the average caffeine concentrations of the coffee liqueurs made with different liquors is high as 3.2% for Recipe A followed by 2.9% (for Recipe B) and 2.5% (for Recipe C). Therefore, the results suggest that the caffeine extractability might be influenced by the types of alcoholic liquor instead of the ratio of the powdered form of roasted coffee bean. No matter which recipe was applied, the caffeine concentrations extracted by the Korean traditional soju and the fermented ethanol infusion were higher than others. The lowest caffeine concentration was always observed in the coffee liqueur made with rum. If we consider the relatively low sugar content in the rum used in this research (3 g/L), the lowest caffeine extractability might be influenced by other characters of rum.

Statistical difference of home-made coffee liqueurs: As a result of the two-way ANOVA, a significant difference between the types of liquor and between the types of recipe were observed ($p<0.005$). But there was no statistically significant interaction between the effects of type of liquor and recipe on the caffeine level at the $p = 0.180$ (Table 4). The post hoc test, "Tukey's Honestly Significant Difference (HSD) "was conducted for the detail estimation of the difference between each group (type of liquor and recipe). As shown in Table 5, Korean traditional soju and the fermented ethanol positioned in the same subset. That means the caffeine level extracted by Korean traditional soju and the fermented ethanol showed similarity. But other liquors such as rum and vodka positioned in the different subsets those means the different caffeine level patterns were observed in rum and vodka, respectively. For the matter of the differences between Recipe A-C, Recipe B and C are positioned in same subset as recipe B and A positioned in the same subset too (Table 6). That means only Recipe A and C showed the different patterns of the caffeine level derived by the composition ratio of the powder form of roasted coffee bean (28 and 50% for the Recipe A and C, respectively). However, the Recipe B with the highest composition ratio of the roasted coffee bean powder (72%) was positioned in the same subset

Table 5: The homogeneous subset of post hoc test, Tukey HSD result for the type of liquor used for the preparation of coffee liqueur

		Subset for $\alpha = 0.05$		
Treatment group	N	1	2	3
Tukey HSD[a]				
Rum	9	2661.7778		
Vodka	9		2707.0556	
Korean traditional soju	9			2813.3778
Fermented ethanol	9			2815.1333
Sig.		1	1.000	0.999

[a]Uses harmonic mean sample size = 9

Table 6: The homogeneous subset of post hoc test, Tukey HSD result for the type of recipe used for the preparation of coffee liqueur

		Subset for $\alpha = 0.05$	
Recipe	N	1	2
Tukey HSD[a]			
C	12	2730.1750	
B	12	2755.1583	2755.1583
A	12		2762.6750
Sig.		0.124	0.814

[a]Uses harmonic mean sample size = 12

with Recipe A with the lowest composition ratio of the roasted coffee bean powder (28%). It may allude there is no effect of the composition ratio of the powder and bean form of the roasted coffee for the extraction efficiency of caffeine during the preparation of coffee liqueur.

Caffeine intake via home-made coffee liqueur: The caffeine level in the home-made coffee liqueurs are from 2.65-2.85 mg/mL those are 74.2~79.8 mg/ounce. If one ounce size whisky cup contain about 28 mL of coffee liqueur, taking 5 cups (1 ounce size) of coffee liqueur let an adult intake almost 400 mg a day that is the MRDI amount of caffeine in Korea.

CONCLUSION

Caffeine concentration (2.65~2.85 mg/mL) quantitated in home-made coffee liqueur was slightly higher than the caffeine level in an espresso shot (1.01~2.26 mg/mL). The variety of coffee bean, roasting condition, ethanol percentage and many other factors might affect the caffeine amount in the final coffee liqueur. Because the lack of information about the adverse effect if caffeine and alcohol consumed together, reduction of caffeine consumption might be required for the preparation of home-made coffee liqueur by selecting of liquor with the different ethanol percentages and infusion periods in the future research.

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