APPLICABILITY OF AAS, UV-VIS, GC-MS AND FT IR METHODS FOR THE ANALYSIS OF MYANMAR COFFEE PRODUCTS

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Abstract

Spectroscopic methods and modern techniques were applied to study the mineral composition and caffeine contents of five brands of Myanmar coffee products (Shwe Pu Zun, Nan Myaing, Suu, Premier and Mr. Coffee). Measurements of preliminary chemical composition of Myanmar coffee products such as moisture and ash were performed by conventional method. The relative abundance of elements in the coffee products was determined by EDXRF. The total contents of some minerals; (Ca, Cu, K, Zn and Fe) in solid samples were determined by means of Atomic Absorption Spectroscopy (AAS). The content of calcium in those coffee products was found to be in the ranges of 232.50 –524.40 ppm, copper (0.04 – 1.00 ppm), potassium (425.80 – 832.80 ppm), zinc (46.80 – 75.80 ppm) and iron (0.10 – 0.93 ppm) respectively. Caffeine in Myanmar coffee products were extracted by solvent extraction method. By the extraction and recrystallization processes, the weight percentages of crude caffeine in those products (Shwe Pu Zun, Nan Myaing, Suu, Premier and Mr. Coffee) were 0.3268, 0.4012, 0.8704, 0.1128 and 0.7140 %. Those crude products were purified by acetone and the pure caffeine from those products was confirmed by UV-Vis, FT IR and GC-MS.

Keywords: Caffeine, Myanmar Coffee products, EDXRF, AAS, UV-Vis, FT IR, GC-MS

Introduction

Coffee is a complex mixture of chemicals that provides significant amounts of chlorogenic acid and caffeine. Today, coffee is recognized as one of the most popular and widely consumed nonalcoholic beverage. The popularity of coffee is generally involved with the presence of caffeine. The composition of coffee beans is rather complex, being a mixture of many chemical compounds. The mineral content of coffee is often measured for the purpose of discriminating coffee varieties and types or determining the coffee origin, mainly because elements are more stable in the coffee commodity as

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compared to various organic substances. Commonly, atomic absorption and emission spectrometry methods are used to determine concentrations of various elements in coffee products. Mineral compositions of instant coffee samples were studied and analyzed the metal contents of roasted coffee samples of different geographical origins (Belitz *et al*., 2009).

Unfiltered coffee is a significant source of cafestol and kahweol, which are diterpenes that have been implicated in the cholesterol raising effects of coffee. The results of epidemiological research suggest that coffee consumption may help prevent several chronic diseases, including type 2 diabetes mellitus, Parkinson's disease and liver disease. However, coffee consumption is associated with increases in several cardiovascular disease risk factors, including blood pressure and plasma homocysteine. At present, there is little evidence that coffee consumption increases the risk of cancer. For adults consuming moderate amounts of coffee (3–4 cups/d providing 300–400 mg/d of caffeine), there is little evidence of health risks and some evidence of health benefits. However, some groups, including people with hypertension, children, adolescents, and the elderly, may be more vulnerable to the adverse effects of caffeine (Gerald *et al*., 2014).

An elemental analysis method to determine the geographical growing regions of coffee beans was also presented. Mineral profiles of coffee beans and instant coffee brands were evaluated. A few studies have been devoted to the organic composition of coffee by exploring Fourier transform infrared spectroscopy (FT IR). The roasted coffee beans are examined by FT IR in the carbonyl absorption region at wave number 1800–1680 cm^{-1} (Semen *et al*., 2017).

Caffeine was first isolated in 1821 by the French chemist Pierre Jean Robiquet, from coffee. In its pure state it is an intensely bitter white powder. Caffeine acts as a stimulant of the central nervous system, cardiac muscle and respiratory system as well as a diuretic. As such, it is found to delay fatigue. Caffeine is thought to act on the brain by blocking adenosine receptors (Andrzejewski *et al*., 2004).

Caffeine is a purine alkaloid that occurs naturally in coffee beans (Figure 1). Some physiological effects associated with caffeine administration include central nervous system stimulation; acute elevation of blood pressure

and increased metabolic rate. Caffeine is rapidly and almost completely absorbed in the stomach and small intestine and distributed to all tissues, including the brain. Caffeine (3, 7-dihydro-1, 3, 7-trimethyl-1H-purine-2, 6-dione or 1, 3, 7-trimethylxanthine) is a biologically active xanthine alkaloid naturally occurring in coffee, tea, cacao, and guarana plant part (Danhelova *et al*., 2012).
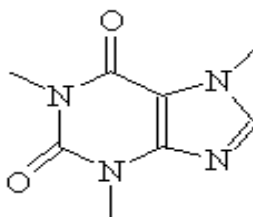

Figure 1: Structure of caffeine

Caffeine concentrations in coffee beverages can be quite variable. A standard cup of coffee is often assumed to provide 100 mg of caffeine, but a recent analysis of 14 different specialty coffees purchased at coffee shops in the US found that the amount of caffeine in   8 oz of brewed coffee ranged from 72–130 mg. Caffeine in espresso coffees ranged from   58–76 mg in a single shot. Interestingly, the caffeine content of the same type of coffee purchased from the same store on six separate days varied from 130 to 282 mg per 8-oz serving (Gerald *et al*., 2014).

The goal of the present work was to evaluate and compare the composition of ground, instant, and chicory coffee products available in the Polish market by spectroscopic and other physico-chemical methods. The applicability of FT IR spectroscopy in the investigation of coffee was demonstrated. Differences in composition of coffee types were studied based on their caffeine, oxalate, and minerals contents (Maja *et al*., 2013).

Materials and Methods

Reagents

All the chemicals were of analytical grade. Working standard solutions were prepared by dilution of a multi-elemental standard (Merck, Germany). The following solutions of Merck and POCh reagents were used: sodium hydroxide, nitric, hydrochloric, and sulphuric acids, hydrogen

peroxide, ethyl acetate, potassium permanganate, and calcium chloride. Deionized water was used in all experiments.

Samples

Five brands of Myanmar coffee products, Shwe Pu Zun, Nan Myaing, Suu, Premier and Mr. Coffee were analyzed.

Preliminary Analysis of Myanmar Coffee Products

Ash content was evaluated gravimetrically, based on the weight of the sample after burning at 550 ℃ in a muffle furnace. The moisture content was determined by evaluating weight loss after drying at 103 ℃ until a constant weight was obtained.

Determination of Minerals in Myanmar Coffee Products

The relative abundance of elements in the coffee products was determined by EDXRF. Minerals (major, minor, and trace elements) in five brands of Myanmar coffee products were measured by AAS after wet digestion. About 0.5 g of the coffee portions were weighed and decomposed on the hot plate with concentrated HNO_3. Residual sample solutions were quantitatively transferred into 25 mL volumetric flasks and made up with de-ionized water to the volume.

Extraction of Crude Caffeine

Pre-weighed coffee powder (50 g) was added to 100 mL of boiling deionized water in a large beaker. The mixture was allowed to sit for 10 min stirring periodically. The solution must be cooled to room temperature using the ice-water bath before it is used in the extraction step. To perform the extraction procedure, 20 mL of ethyl acetate solvent was used and this procedure was repeated by carrying twice more using fresh 20 mL portions of ethyl acetate and these two ethyl acetate layers were combined with the first. The combined ethyl acetate organic phases returned to the separatory funnel and washed with 20 mL of 6 M aqueous NaOH by adding the NaOH solution to the separatory funnel, shaking and then allowing the mixture to settle. After performing the extraction procedures, the crude caffeine may be purified either by re-crystallization or by sublimation (Rajković *et al*. 2004).

Re-crystallization

The crude caffeine was dissolved in the minimum amount (< 2.5 mL) of hot acetone on a steam bath and quickly transferred it to a crucible. The solution was cooled in an ice bath and needle shape crystals should start to form.

Analysis of Caffeine by Spectroscopic Methods

The FT IR spectrum of extracted caffeine in the region 4000–400 cm^{-1} was measured on a Spectrum Two FT IR spectrophotometer. Purified coffee was confirmed by UV spectrophotometry (UV-Vis Spectrophotometer) between 190-400 nm and GC-MS (Sereshti and Samadi, 2014).

Results and Discussion

Chemical Analysis of Myanmar Coffee Products

The moisture contents of Myanmar coffee products were determined by the method of drying in the vacuum dryer; the weight % of moisture content in those samples were in the ranges of 0.82 – 4.78 % (Table 1). These ranges were consistent to the literature value < 5%. The ash contents of Myanmar coffee products were determined by the method of direct burning; the weight percent of ash content in those samples were in the ranges of 2.28 – 5.21 %. These ranges were consistent to the literature value < 6% (Gerald *et al*., 2014).

Mineral and Caffeine Contents in Different Myanmar Coffee Products

Relative abundance of minerals in Myanmar coffee products as determined by EDXRF were shown in Figure 2 and Table 2. These mineral contents (in ppm) were also determined by AAS and the data were shown in Table 3. It was found that potassium, calcium and zinc were the major minerals in all of the samples. The most abundant traces were Fe and Cu.

Pure caffeine content in the Myanmar coffee products was ranged from 0.02 % to 0.16 % (Table 4). Those ranges were lesser to those for other brands of coffee (0.32–2.95%) reported in the literature (Rajković *et al*. 2004).

Table 1: Preliminary Chemical Composition of Myanmar Coffee Product

Sr. No.	Samples	Wt. of samples (g)	Moisture		Ash	
			Wt. (g)	% by Wt.	Wt. (g)	% by Wt.
1	Shwe Pu Zun	2.0092	0.0320	1.59	0.1002	4.99
2	Nan Myaing	2.0079	0.0959	4.78	0.0878	4.37
3	Suu	2.0051	0.0889	4.43	0.1045	5.21
4	Premier	2.0003	0.0165	0.82	0.0478	2.39
5	Mr. Coffee	2.0021	0.0656	3.28	0.0456	2.28

Table 2: Relative Abundance of Minerals in Myanmar Coffee Products by EDXRF

Sr. No.	Samples	Ca (%)	Cu (%)	K (%)	Zn (%)	Fe (%)
1	Shwe Pu Zun	0.082	0.001	0.988	0.002	0.003
2	Nan Myaing	0.146	0.001	1.674	0.001	0.004
3	Suu	0.172	0.002	2.140	0.001	0.001
4	Premier	-	-	0.988	0.001	0.007
5	Mr. Coffee	0.253	0.001	2.556	0.002	0.002

West Yangon University
Chemistry Department

EDX Report   Report No.

Sample Information	
Sample Name	Nan Myaing
Meas. Date	2017/05/26 15:10:04
Comment	with mylar film
Group	powder CH balance
Operator	Dr Cherry Ohn

Measurement Condition					Collimator	10mm	Atmos.	Air
Channel	kV	uA	Filter	Acq.	Analysis	Time	DT%	
Na-U	50	76-Auto	----	0 - 40	0.00-40.00	Live- 60		30

Quantitative Result

Analyte	Result		Std.Dev.	Calc.Proc	Line	Intensity
K	1.674	%	[0.011]	Quan-FP	K Ka	50.8659
S	0.160	%	[0.009]	Quan-FP	S Ka	1.0773
Ca	0.146	%	[0.002]	Quan-FP	CaKa	7.5070
P	0.128	%	[0.022]	Quan-FP	P Ka	0.2547
Fe	0.004	%	[0.000]	Quan-FP	FeKa	2.8158
Mn	0.003	%	[0.000]	Quan-FP	MnKa	1.3178
Cu	0.001	%	[0.000]	Quan-FP	CuKa	1.9843
Rb	0.001	%	[0.000]	Quan-FP	RbKa	2.6747
Zn	0.001	%	[0.000]	Quan-FP	ZnKa	1.0431
COH	97.882	%	[------]	Balance	--------	--------

Profile

Figure 2: EDXRF spectrum of one of the Myanmar coffee products (Nan Myaing)

Table 3: Mineral Contents in Myanmar Coffee Products by Atomic Absorption Spectrometry

Sr. No.	Samples	Ca (ppm)	Cu (ppm)	K (ppm)	Zn (ppm)	Fe (ppm)
1	Shwe Pu Zun	371.90	0.19	435.30	53.70	0.78
2	Nan Myaing	395.10	0.71	776.40	59.90	0.20
3	Suu	330.40	1.00	734.30	60.40	0.10
4	Premier	232.50	0.04	425.80	46.80	0.93
5	Mr. Coffee	524.40	0.72	832.80	75.80	0.39

Table 4: Caffeine Contents in Myanmar Coffee Products

Sr. No.	Samples	Wt. of samples (g)	Crude caffeine		Purified caffeine	
			Wt. (g)	% by Wt.	Wt. (g)	% by Wt.
1	Shwe Pu Zun	50.0	0.1634	0.3268	0.0310	0.0620
2	Nan Myaing	50.0	0.2006	0.4012	0.0395	0.0790
3	Suu	50.0	0.4352	0.8704	0.0800	0.1600
4	Premier	50.0	0.0564	0.1128	0.0100	0.0200
5	Mr. Coffee	50.0	0.3570	0.7140	0.0697	0.1394

Characterization of Caffeine in Coffee Powder (Nan Myaing) by Spectroscopic Methods

Caffeine was determined by UV spectrophotometry (Shimadzu UV-Vis Spectrophotometer) at 273 nm after extraction into ethyl acetate from an aqueous solution at a pH about 12 and the peak at 205 nm could be oxalate from the extracted caffeine (Gerald *et al*., 2014). By this method, the caffeine and oxalate content in Myanmar coffee products could be rechecked (Figure 3).
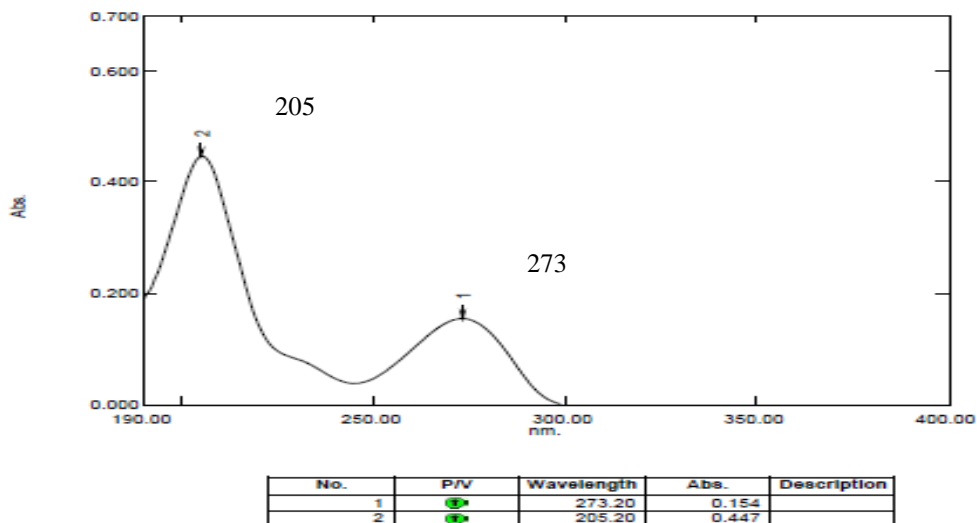
No.	P/V	Wavelength	Abs.	Description
1	P	273.20	0.154	
2	P	205.20	0.447	

Figure 3: UV Spectrum of one of the Myanmar coffee products (Nan Myaing)

Extracted caffeine from one of the Myanmar coffee product (Nan Myaing) was analyzed by IR spectroscopy and the spectrum was shown in Figure 4. The IR spectrum of coffee product is complex due to strong overlapping of peaks originating from many species. The band of 3113 cm^{-1} can be assigned to O-H stretching of hydroxyl groups and water, C-H stretching attached with double bond as well as N-H stretching of amine. The bands at 2925 and 2854 cm^{-1} were due to C-H stretching vibration of aliphatic groups. The band at 1706 cm^{-1} may be due to the presence of carbonyl group of aromatic or aliphatic carboxylic aids, aldehydes, and ketones. According to literature, the peaks around 1706, 1652 and 1599 cm^{-1} originated from the caffeine molecule (Garrigues *et al.*, 2000).

The region below 1500 cm^{-1} was rather complex, but some bands can be assigned: the peak at 1486, 1457, 1400 and 1358 cm^{-1} attributed to the asymmetric C-H bending of aliphatic groups. The band in the 1188–973 cm^{-1} region was involved with C-O-C group of polysaccharides (e.g., C-O stretching at 1025 cm^{-1}). The FT IR method was found to be a suitable technique to demonstrate the presence of caffeine in Myanmar coffee products.
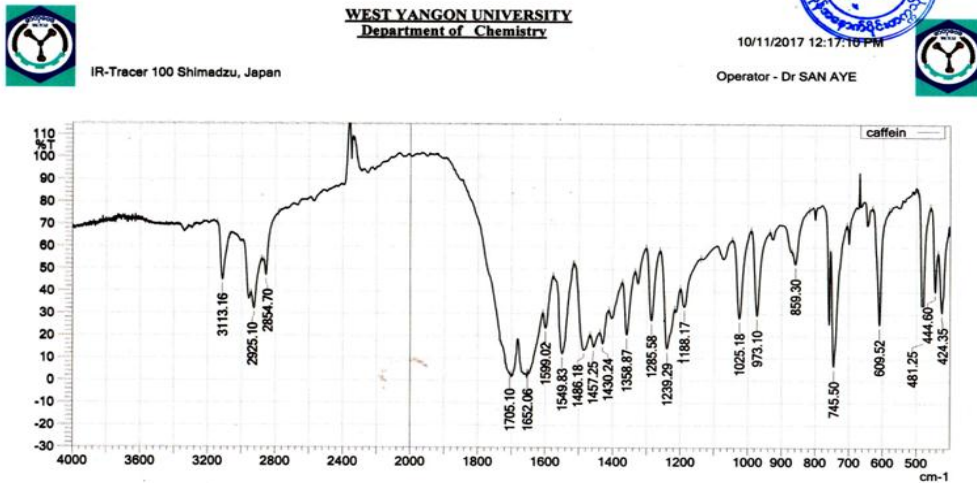
Figure 4: FT IR spectrum of extracted caffeine from one of the Myanmar coffee products (Nan Myaing)

Extracted caffeine was also confirmed by GC-MS. Electron energy, 70 eV (EI mode). Mode of acquisition: selected ion monitoring (SIM), m/z 55, 67, 82, 109 and 194 for Caffeine (3, 7-dihydro-1, 3, 7-trimethyl-1H-purine-   2, 6,-dione or 1, 3, 7-trimethylxanthine) (Figure 5). The ions m/z 194.14 for (3, 7-dihydro-1, 3, 7-trimethyl-1H-purine-2, 6,-dione or 1, 3, 7-trimethylxanthine), m/z 194 for standard caffeine were used for quantification and the others for confirmation (Soares *et al*., 2006).
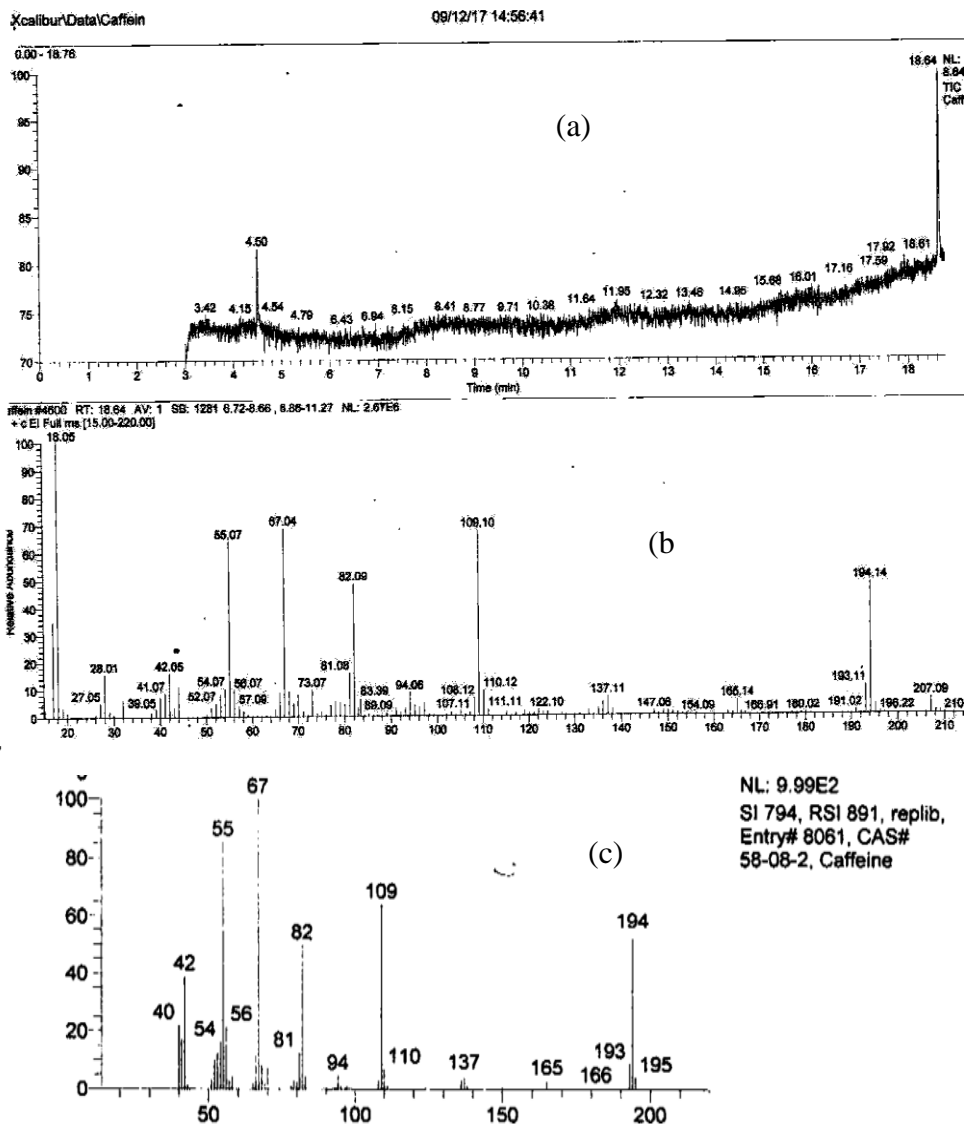
Figure 5: (a) Gas chromatogram of extracted caffeine from one of the Myanmar coffee products (Nan Myaing)

(b) Mass spectrum of extracted caffeine from one of the Myanmar coffee products (Nan Myaing)

(c) Library mass spectrum of the caffeine

Conclusion

The spectroscopic methods, that is, optical emission spectrometry, UV spectrometry, and FT-IR spectroscopy, applied here with chemical methods, were suitable tools to compare nutrient concentrations of five brands of Myanmar coffee products and to find differences in their compositions. Moisture and ash contents were found to be in the range of 0.82% to 4.78% and 2.28% to 5.21% respectively. The relative abundance of minerals in Myanmar coffee products were determined by EDXRF. From determination of mineral contents by AAS, it was found that calcium and potassium concentrations were higher in Myanmar coffee products by about 524.4 ppm and 832.8 ppm respectively, whereas zinc content was considerable amount in Myanmar coffee products. Copper and iron contents were lower amount in Myanmar coffee products. Pure caffeine content in the Myanmar coffee products was ranged from 0.02 % to 0.16 %. The caffeine content in Myanmar coffee products was confirmed by UV-Vis spectrometry, FT IR and GC-MS. These spectroscopic methods were found to be a suitable technique to investigate the organic matrix of Myanmar coffee products as well as prove the presence of caffeine.

Acknowledgements

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