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DETERMINATION OF ELEMENT CONTENTS IN COMMERCIAL TEAS MARKETED IN MALAYSIA & ITS INFUSION RESIDUE USING NEUTRON ACTIVATION ANALYSIS (NAA)

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ABSTRACT

Fifteen elements in black tea, rose flavoured tea and fruit tea commercially sold in Malaysian market were determined using neutron activation analysis (NAA). The elements analyzed include Al, Ba, Br, Ce, Cl, Eu, Fe, K, La, Mg, Mn, Na, Rb, Sc, and Zn. The concentration of these elements varied according to the tea types in the range of 2.2% K to 0.04 mg/kg Sc. The quality of analysis was assured using certified standard reference material. Infusion study of the tea residue was also performed to analyse the possible consumption of these elements by the population. Ce, Cl, Eu, K and Rb were found to be easily infused in all three types of teas. Kruskal-Wallis test found significant differences ($p < 0.05$) between elemental composition and types of teas. Principal component analysis (PCA) showed clustering based on types of teas.

Keywords: infusion, elemental, neutron activation analysis, principal component analysis, tea

INTRODUCTION

Tea (*Camellia sinensis*) is a very popular beverage in the world due to its pleasant aroma and refreshing taste (Zhao et al., 2017a). Many cultures around the world have their own practice of drinking tea which relates to its customs and rituals (Martin, 2011). Moreover, drinking tea is associated with its therapeutic and beneficial anti-inflammatory and antioxidant properties (Peluso & Serafini, 2017). There are several types of tea available in the market which can be categorized by the fermentation level. Black tea is fully fermented, green tea is unfermented while oolong tea is partially fermented (Lin et al., 1996). In Malaysia, tea plantation area covers 2271 hectares which is predominantly cultivated in Pahang and Sabah. It is estimated that 7523 tons of tea products come from that area (Jabatan Pertanian Semenanjung Malaysia, 2022). Many of these tea products are sold as loose powder or in bags. Black tea is widely consumed in Malaysia, apart from green tea and flavoured tea.

Tea contains proteins, amino acids, lipids, sugars, vitamins, fiber and minerals (Ramdani et al., 2013; Lim et al., 2021). The availability of minerals in tea leaves varies depending on the origin of tea, tea variety, geochemistry of the soil and the use of pesticides and fertilisers (Lagad et al., 2011; Islam & Ebihara, 2017; Lim et al., 2021). Tea is known to contain essential minerals such as manganese. Manganese is known to be involved in many physiological functions of the human body such as the metabolism of glucose and lipids as well as aids in protection against metabolic diseases such as obesity and diabetes mellitus (Li & Yang, 2018). Nonetheless, the consumption of minerals upon drinking teas is related to the solubility of the elements and the infusion times taken.

Due to the health benefits offered by drinking tea, various studies have been carried out to study the elemental compositions of tea as well as its infusion. Many of this elemental analysis of tea was done using spectroscopic techniques such as inductively coupled plasma mass spectrometry (Han et al., 2014), atomic absorption spectrometry (Brzezicha-Cirocka et al., 2016) and X-ray fluorescence spectroscopy (Lim et al., 2021). The use of neutron activation analysis (NAA) for elemental characterization of food products including tea has been growing increasingly in recent years (Chajduk, 2009; Zhang et al., 2011; Lagad et al., 2011; Mahani & Maragheh, 2011; Jonah & Williams, 2000; Islam & Ebihara, 2017). NAA is an analytical instrument capable of analysing various elements simultaneously with high precision and accuracy. As a non-destructive technique, it also offers other advantages such as much simpler preparation, does not require complex chemical digestion and therefore free of contamination from acid digestion.

This study aims to investigate the concentration of multi-element in tea leaves sold in Malaysian market using NAA and its leaching efficiency upon brewing. Statistical and classification technique are also used to find the relationship between elemental profile and type of teas.

MATERIALS AND METHODS

10 different tea samples in loose form and tea bags were purchased from local markets around Bangi, Selangor. The characteristics of tea samples used in this study are summarized in Table 1. The origin of the tea samples was determined solely from the packaging. The procedure described by Islam & Ebihara (2017) was followed for the preparation of the samples for elemental concentration determination. Each sample was dried in the oven at 50^{o}C overnight and then ground to a small size using a household blender.

Table 1 Characteristics of analysed teas

Tea samples	Type of Tea	Origin	Confection
BT 1	Black tea	Ranau, Malaysia	Bags
BT 2	Black tea	Indefinite origin	Bags
BT 3	Black tea	Cameron, Malaysia	Loose
BT 4	Black tea	Cameron, Malaysia	Bags
RT 1	Rose flavoured tea	Malaysia	Loose
RT 2	Rose flavoured tea	Indefinite origin	Loose
FT 1	Fruit tea	East Africa	Bags
FT 2	Fruit tea	East Africa	Bags
FT 3	Fruit tea	East Africa	Bags
FT 4	Fruit tea	East Africa	Bags

Irradiation and counting

Samples were transferred into a polyethylene tube and heat-sealed. The weight of the samples used varies according to the intended radionuclides. For short-lived radionuclides approximately 0.07g of samples were weighed in, while for long-lived radionuclides, around 0.1g of samples were used. Samples and standard reference materials (SRMs) were co-irradiated in the same batch performed at the TRIGA Mark II reactor at Malaysian Nuclear Agency with thermal neutron flux approximately at $6.8 \times 10^{12} n \cdot cm^{-2} s^{-1}$. For short-lived radionuclides (Al, Cl, K, Mg, Mn, Na) samples and standard reference materials were irradiated for 1 minute using a pneumatic transfer system followed by cooling (decay period) for 8 minutes and then counted for 5 minutes using HPGe Gamma Spectrometer (EG&ORTEC, USA) and GammaVission software. As for long-lived radionuclides (Ba, Br, Ce, Eu, Fe, La, Rb, Sc, Zn) samples were irradiated for 4 hours followed by cooling (decay period) for 3 days for first counting and 21 days for second counting. Samples were then counted for 1 hour each. Standard reference material from the National Institute of Standards and Technology (NIST) 1515-Apple Leaves was chosen as quality control materials due to its similarity in matrix as tea leaves. The concentration of the tea samples as well as the SRM was calculated using Eq. (1) (Joel et al., 2018; Elias et al., 2018):

$$C_{EL} = \frac{A_{smp}}{A_{std}} \times \frac{W_{std}}{W_{smp}} \times C_{std} \quad (1)$$

Where
A_{smp} = net count of the selected peak area of an interested element in a sample
A_{std} = net count of the selected peak area of an interested element in a standard
W_{smp} = Weight of sample used (g)
W_{std} = Weight of standard used (g)
C_{std} = Concentration of interested element in standard (mg/kg)
C_{EL} = Concentration of interested element in sample (mg/kg)

As for the infusion study, the following method by Islam & Ebihara (2017) is used. Around 2.0g of tea samples were infused in 200 ml boiling DI water for 6 minutes and then filtered. The residual tea leaves were then collected and underwent the same procedure as the non-infused tea leaves.

Statistical Analysis

Statistical analysis was performed using PAST3 (Hammer, Harper, & Ryan, 2001) software. Normality test was done using the Shapiro-Wilk test followed by a non-parametric test as the data appeared to be a non-normal distribution. Kruskal-Wallis test was performed to find a significant difference followed by Dunn's post-hoc test. Principal component analysis (PCA) was also employed for the visualisation purpose.

RESULT & DISCUSSION

Quality of analysis

The quality of the data obtained was evaluated by measuring the elemental contents of standard reference material NIST 1515 Apple Leaves which act as the quality control material in this study. The energies of the gamma-ray used, mean concentration results, recovery percentage, percentage of RSD and *Z*-score are given in Table 2. This method shows good accuracy with a relatively high recovery percentage ranging between 89% to 118%. The obtained results were in good agreement with the certified values. Majority of the data shows high precision with %RSD < 10% except for K and Fe with slightly higher %RSD at 10.25 and 11.20% respectively. All of the data are within the 95% confidence limit as suggested by the *Z*-score below 3 (Lagad et al., 2011).

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Table 2 Analytical results of elemental contents of the standard reference material NIST 1515 Apple Leaves

Element	Energy (keV)	Certified value (mg/kg)	This work (mg/kg)	Recovery (%)	%RSD	*Z*-Score
Al	1779	284.5 ± 5.8	278.8 ± 15	96.6	5.46	0.61
Ba	496	48.8 ± 2.3	46.3 ± 1.3	94.8	2.78	0.96
Br	554	(1.8)	1.70 ± 0.07	94.3	4.41	-
Ce	145	(3)	3.01 ± 0.25	100.3	8.33	-
Cl	2168	582 ± 15	567 ± 28	97.4	5.00	0.46
Eu	1408	(0.2)	0.23 ± 0.003	112.5	1.35	-
Fe	1099	82.7 ± 2.6	85.4 ± 9.7	103.3	11.20	-0.27
K	1524	16080 ± 210	15125 ± 155	94.1	10.25	0.61
La	1596	(20)	18.61 ± 1.59	93.0	8.57	-
Mg	1014	2710 ± 120	2965 ± 204	109.4	6.90	-1.08
Mn	1811	54.1 ± 1.1	56.2 ± 4.0	103.9	7.05	-0.51
Na	2754	24.4 ± 2.1	22.0 ± 1.3	89.9	6.02	0.99
Rb	1077	10.2 ± 1.6	9.2 ± 0.35	90.4	3.80	0.60
Sc	889	(0.03)	0.03 ± 0.001	101.7	0.21	-
Zn	1116	12.45 ± 0.43	14.74 ± 0.91	118.4	6.19	-2.27

Concentrations are mentioned as mean ± SD
Values given in parentheses ( ) are information values
Z-score = $\frac{x-\mu}{\sigma}$ where x = observed mean value, μ = certified value and σ = standard deviation of certified value

Elemental composition of tea leaves

The analytical result of the elemental contents in tea samples is shown in Table 3. A total of 15 elements can be determined from this analysis. Generally, K, Mg, Al, Mn, Cl, and Fe are the primary elements that can be found in tea leaves. Black tea leaves from Ranau, Sabah have the most abundant amount of K (20873 mg/kg) as compared with the other Malaysian-grown black tea leaves. High concentration of K in tea leaves sold in other Asian countries was also reported (Lagad et al., 2011; Huang et al., 2016; Lim et al., 2021). Comparison of elemental levels in tea leaves from various origins is shown in Table 4. Mg concentration is the highest in black tea leaves (3030 – 3572 mg/kg). It is twofold higher than Mg concentration in black tea leaves from selected region in China (Ma et al., 2019). Interestingly, high concentration of Al is observed in all tea samples at around 1224 - 1932 mg/kg. Alzheimer’s disease (AD) is reportedly linked to over-exposure to heavy metals such as aluminium (Deng et al., 2000; de Oliveira et al., 2018). Being a popular drink worldwide, tea is known as a major dietary source of Al, hence, heavy consumption of tea might increase risk of the disease. Mn concentration reported here is between 460 – 1748 mg/kg with fruit teas has considerably higher Mn content than black teas and rose flavoured teas. The average Mn concentration of tea leaves in this study is considerably higher than Mn concentration of European black tea and green tea (8 – 709 mg/kg) (Girolametti et al., 2023). Cl concentration is relatively high in all tea samples (653 – 1175 mg/kg). The value reported here is slightly higher than Cl concentration of tea leaves sold in Nigeria (Jonah & Williams, 2000) but relatively similar to black tea leaves sold in Poland (Chajduk, 2009) as well as Japanese green tea leaves (Islam & Ebihara, 2017) (Table 4). Fe concentration is between 126 – 290 mg/kg. Generally, fruit tea has the highest concentration of Fe among the tea samples.

Table 3 Elemental concentration of tea samples sold in Malaysian market (mg/kg)

Element	BT 1	BT 2	BT 3	BT 4	RT 1	RT 2	FT 1	FT 2	FT 3	FT 4
Al	1690 ± 33	1444 ± 134	1684 ± 85	1809 ± 6	1932± 130	1589 ± 44	1328 ± 42	1365 ± 18	1224 ± 27	1371 ± 47
Ba	105 ± 12.8	42.2 ± 2.9	53.9 ± 0.9	30.7 ± 1.8	88.3 ± 1.9	78.3 ± 6.6	47.7 ± 6.4	29.9 ± 3.7	43.1 ± 0.5	42.8 ± 0.8
Br	2.82 ± 0.20	4.37 ± 0.15	2.49 ± 0.02	3.11 ± 0.08	2.38 ± 0.07	2.42 ± 0.01	5.43 ± 0.43	4.93 ± 0.10	4.68 ± 0.13	4.61 ± 0.03
Ce	0.29 ± 0.05	0.19 ± 0.24	0.13 ± 0.17	0.11 ± 0.15	0.27 ± 0.09	0.17 ± 0.06	0.40 ± 0.51	0.39 ± 0.05	0.39 ± 0.01	0.42 ± 0.05
Cl	1075 ± 16.7	761 ± 35.5	954 ± 55.1	1047± 18.1	1175 ± 7.0	1013 ± 76.9	857 ± 115	860 ± 13.8	728 ± 7.4	653 ± 8.6
Eu	0.07 ± 0.008	0.06 ± 0.001	0.06 ± 0.009	0.04 ± 0.004	0.06 ± 0.001	0.07 ± 0.002	0.09 ± 0.04	0.06 ± 0.009	0.07 ± 0.002	0.07 ± 0.006
Fe	126 ± 0.7	243 ± 16.5	132 ± 5.1	224 ± 26.8	290 ± 8.7	190 ± 8.0	233 ± 13.7	267 ± 12.7	242 ± 2.7	223 ± 35.2
K	20828 ± 366	20090 ± 767	16513 ± 248	17458 ± 222	13569 ± 535	22331 ± 242	20750 ± 222	22001 ± 86	20873 ± 84	19865 ± 474
La	0.67 ± 0.06	1.24 ± 0.05	0.49 ± 0.05	0.28 ± 0.05	0.77 ± 0.18	0.54 ± 0.08	1.56 ± 0.01	1.72 ± 0.02	1.51 ± 0.01	1.74 ± 0.03
Mg	3176 ± 328	3572 ± 816	3030 ± 707	3326 ± 292	3037 ± 304	2718 ± 204	2877 ± 764	2392 ± 392	3353 ± 514	2885 ± 650
Mn	1133 ± 12.5	1570 ± 90.9	553 ± 18.4	460 ± 23.8	903 ± 54.9	951 ± 33.5	1644 ± 63.6	1748 ± 48.0	1574 ± 9.4	1694 ± 25.8
Na	22.2 ± 0.74	35.24 ± 3.07	21.41 ± 1.05	34.75 ± 0.73	36.55 ± 1.96	35.12 ± 2.52	52.65 ± 0.56	61.06 ± 3.85	49.58 ± 1.66	52.56 ± 6.47
Rb	107 ± 0.9	122 ± 3.2	62.5± 3.0	57.3 ± 1.2	66.9 ± 0.8	81.5 ± 5.6	101 ± 0.2	99.6 ± 1.5	95.4 ± 1.8	101 ± 4.0
Sc	0.05 ± 0.002	0.07 ± 0.001	0.05 ± 0.005	0.05 ± 0.002	0.08 ± 0.01	0.05 ± 0.001	0.04 ± 0.004	0.04 ± 0.001	0.04 ± 0.002	0.04 ± 0.001
Zn	25.77 ± 2.49	25.92 ± 1.62	26.65 ± 2.93	37.27 ± 4.02	32.20 ± 7.96	25.25 ± 3.61	20.36 ± 0.86	20.33 ± 1.30	21.43 ± 0.97	19.76 ± 0.36

e-JSNM

Table 4 Comparison of concentration value of elements in tea leaves from various origins

Origin	Iran	India	China	China	Europe	China	Nigeria	Japan	Poland	This study
Type of tea	Black tea	Black tea	Various	Puerh, green & black tea	Black & green tea	Various	Black tea	Green tea	Black tea	Black, fruit & rose flavoured tea
Al (mg/kg)			1397 – 1553	116 - 862	733 – 4865	217 – 607	47.8 – 1904	304 – 1100	622 – 1053	1224 – 1932
Ba (mg/kg)		11.9 - 144	3.1 – 3.4			14.7 – 37.9			10.4 – 33.4	29.9 – 105
Br (mg/kg)	4.20 – 7.07	0.9 – 1.3						2.78 – 4.44	2.02 – 9.81	2.38 – 5.43
Ce (mg/kg)		0.12 – 0.69							386 – 580	0.11 – 0.42
Cl (mg/kg)							422 – 921	560 - 1110	739 – 934	653 – 1175
Eu (mg/kg)									7.0 – 18.5	0.04 – 0.07
Fe (mg/kg)		71 – 397	114 – 150		77 – 99			57.8 – 105	82.8 – 207	126 – 290
K (wt. %)	1.20 – 2.10	0.98 – 1.96	1.56 – 1.57				1.54 – 2.08	1.54 – 2.19	1.87 – 2.17	1.36 – 2.23
La (mg/kg)		0.06 – 0.42						0.05 – 0.41	0.16 – 0.56	0.28 – 1.74
Mg (wt. %)			0.22 – 0.26	0.12 – 0.23		1.93 – 2.86	0.23 – 1.24	0.18 – 0.28	0.19 – 0.21	0.24 – 0.36
Mn (mg/kg)	150 – 500	163 - 1191	629 – 845	386 – 780	8 – 709		286 – 801	528 – 965	444 – 787	460 – 1748
Na (mg/kg)	18 – 120	11.9 – 95.2				252 – 551	15.1 – 492	5.16 – 53.6	30.8 – 93.8	21.4 – 61.1
Rb (mg/kg)		19.8 – 90.4	26.6 – 62.9			3.1 – 25.7		13.2 – 24.7	39.1 – 144	57.3 – 122
Sc (mg/kg)		0.01 – 0.02		0.04 – 0.24				0.004 – 0.02	0.05 – 0.08	0.04 – 0.08
Zn (mg/kg)		17.6 – 92.9	23.8 – 27.7	33.8 – 95.9	21.9 – 39.7			14.7 – 26.4	29.3 – 31.4	19.8 – 37.3
References	Mahani & Maragheh, 2011	Lagad et al., 2011	Lim et al., 2021	Ma et al., 2019	Girolametti et al., 2023	Zhao et al., 2017b	Jonah & Williams, 2000	Islam & Ebihara, 2017	Chajduk, 2009	

Some elements in tea samples can be categorized as microelements which includes Ba, Na, Rb and Zn (19.76 – 122 mg/kg). Rose flavoured tea has a relatively higher concentration of Ba than black tea and fruit tea. Black tea from Ranau has the highest concentration of Ba in this study. Na concentration in fruit tea is higher than black tea and rose flavoured tea. The concentration of Na reported here is very much less than Na concentration of multiple varieties of tea leaves from China analysed over different seasons (Zhao et al., 2017b) (Table 4). Rb concentration is between 57.3 – 122 mg/kg. Fruit tea has considerably higher content of Rb than other types of teas. Zn concentration is between 19.76 – 37.27 mg/kg. It is lower compared to black tea from China (33.8 – 95.9) (Ma et al., 2019) but quite similar with black tea in Poland (20.7 – 37.3 mg/kg) (Polechońska et al., 2015). Some elements in tea leaves were present in trace amounts. These include Br, Ce, Eu, La and Sc (0.04 – 5.43 mg/kg). As for Br, its concentration in black tea is almost similar to rose flavoured tea but lower than fruit tea. The concentration of Br is higher than those reported of black tea leaves of Indian origin (Lagad et al., 2011) but quite similar to tea leaves sold in Iranian market (Mahani & Maragheh, 2011) (Table 4). As for the lanthanides, the concentration is roughly in the following order: La>Ce>Eu=Sc. Fruit tea has the highest concentration of the lanthanides followed by rose flavoured tea and black tea.

Multi-element analysis of infusion tea leaves residue

The extraction efficiencies of the elements by infusion were also evaluated to assess the potential consumption of elements studied from drinking the tea. In order to show a better representation of the leaching efficiencies of the tea samples, they were separated based on types of teas; black tea, rose flavoured tea and fruit tea and their mean extraction efficiencies are compared. Figure 1 below shows the extraction efficiencies of the tea leaves among the three types of teas. Extraction efficiencies are calculated as the ratio between infusion residue and its corresponding tea leaves.

According to Matsuura et al., (2001), extraction efficiencies of tea leaves can be categorized into three groups: highly extractable (>55%), moderately extractable (20 – 55%), and poorly extractable (<20%). Figure 1 indicates that elements Ce, Cl, Eu, K and Rb are easily leached from tea leaves upon brewing in all three types of teas whereas some elements are poorly leached which include Al, Mn and Zn. Highly extractable element is highly ionic as suggested by Islam & Ebihara (2017). Thus, it is expected that the amounts of elements extracted into tea infusion depend strongly on its solubility in the solution. It is also postulated that chelation of those elements with tannic acid and tannins might cause such variations to occur (Brzezicha-Cirocka et al., 2016).

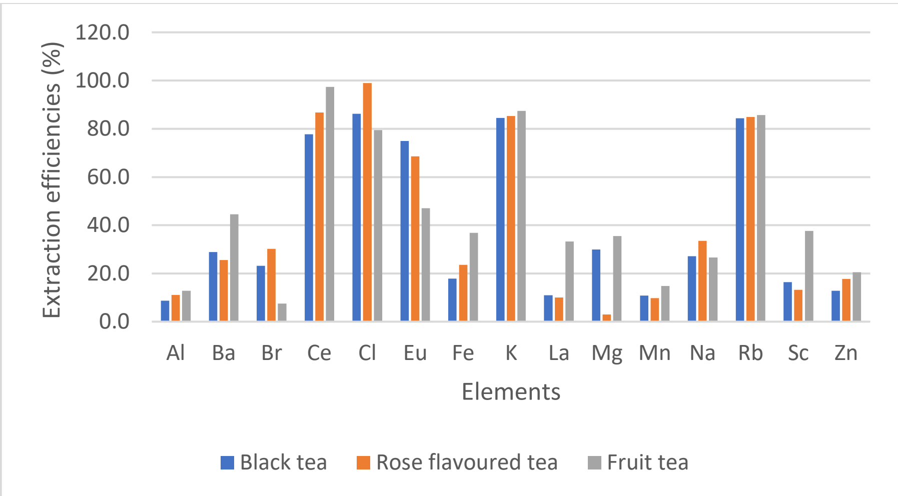

Figure 1 Extraction efficiencies between black tea, rose flavoured tea and fruit tea

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Kruskal-Wallis test

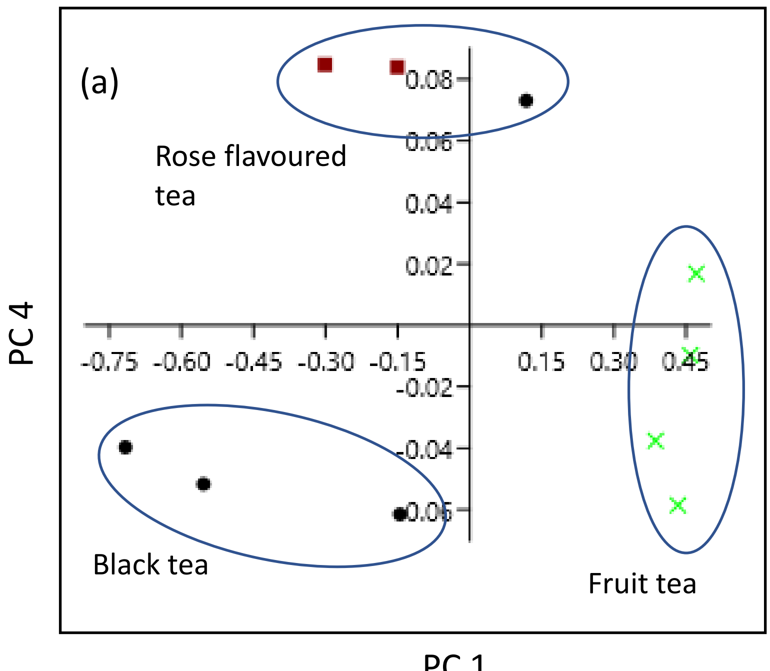
Kruskal-Wallis test was performed to determine whether or not there were statistically significant differences between elemental levels of different types of teas. The result showed that there exists such interdependence. Result of the Kruskal-Wallis test is as follows: Al ($H = 6.627$; $p = 0.036$), Br ($H = 7.855$; $p = 0.019$), Ce ($H = 6.627$; $p = 0.036$), La ($H = 6.627$; $p = 0.036$), Mn ($H = 6.545$; $p = 0.038$), Na ($H = 7.282$; $p = 0.024$), Zn ($H = 6.627$; $p = 0.036$). The ensuing Dunn's post-hoc test identified elements that are contributing to the differences between black tea and fruit tea as well as rose flavoured tea and fruit tea ($p < 0.05$) as shown in Table 5 below. Previous researchers have used elemental data to differentiate between types of tea or their origin (Fernández-Cáceres et al., 2001; Kara, 2009; Brzezicha-Cirocka et al., 2016; Ma et al., 2019; Lim et al., 2021).

Table 5 Dunn's post-hoc test of the elemental data

	Black tea	Fruit tea
Black tea	-	Al, Ce, La, Mn, Na, Zn
Rose flavoured tea	-	Al, Br

Principal component analysis (PCA)

Principal component analysis (PCA) was attempted with elements that showed statistically significant differences ($p < 0.05$). PCA allows for simplification and provides with graphical visualisation of the data. The first five components with eigenvalue more than 1 were extracted which accounts for 98.9% variances of the data. The first component accounted for 85.9%, the second for 6.3%, the third for 3.9%, the fourth for 1.62% and the fifth for 1.1% of the total variation of the data. Figure 2 shows the PCA scatter plot of PC1 vs PC4 and its loading plot. It is obvious that there was a clear distinction of samples from different types of teas which supports our prior statistical finding. However, one of the black teas was clustered together with rose flavoured tea. Upon inspection, it was revealed that the black tea sample is BT2 which has an indefinite origin than the rest of Malaysian grown black tea. Hence, this might explain why such variation occurred. The first component was known to represent the maximum variation of the data set (Kara, 2009). Hence, from the loading plot Br, Ce, La, Mn and Na have the most discrimination power in the differentiation of types of tea.



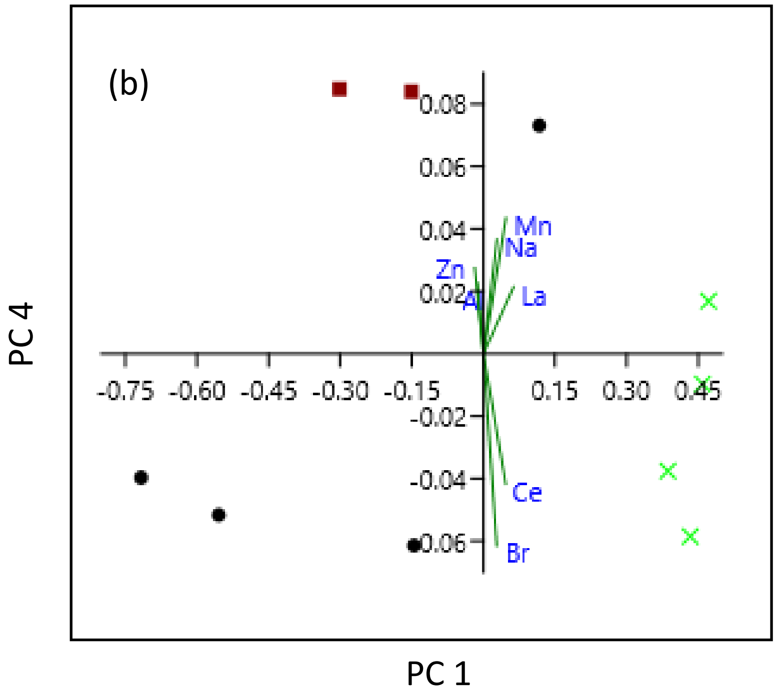


Figure 2 PCA scatter plot of PC1 vs PC4 (a) and its loading plot (b)

CONCLUSION

Analysis results indicate that black tea, rose flavoured tea and fruit tea sold in Malaysian market contain various essential and trace elements which vary according to the tea types. Data analysis showed that tea samples are a good source of essential elements such as K, Na, Mg and Mn. The infusion study showed that these elements might be transferred out into the solution at different rates upon brewing. In general, Ce, Cl, Eu, K and Rb are mostly leached out and can be consumed by the human body. Significant differences were found between black tea and fruit tea, as well as rose flavoured tea and fruit tea based on their elemental levels. The PCA showed that differentiation of types of teas is possible using its mineral profile. This study demonstrates that NAA is a useful tool to assess the quality and safety of teas.

COMPETING INTERESTS

The authors declare that they have no potential competing interests.

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