



ESSENTIAL MINERALS AND HEAVY METALS ANALYSIS OF PENANG ASSAM LAKSA USING ATOMIC ABSORPTION SPECTROMETRY, FLOW INJECTION MERCURY SYSTEM, AND INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY

(Analisis Mineral Penting dan Logam Berat Asam Laksa Pulau Pinang Menggunakan Spektrometri Penyerapan Atom dan Spektrometri Pancaran Optik Induktif Gabungan Plasma)

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Abstract

Penang Assam Laksa is a sour, fish-based soup popular in Malaysian street food. The content of heavy metals (Cd, Hg, and Pb) as well as essential minerals (Fe, Ca, Na, and K) in ready-to-eat Penang Assam Laksa food samples collected from five stalls over three different days was determined using flame atomic absorption spectrometry (FAAS), a Flow Injection Mercury System (FIMS), and inductively coupled plasma optical emission spectrometry (ICP-OES) after a wet-digestion procedure. Statistical analyses such as Student's *t*-test and an inter-day precision study were conducted on the obtained results. The wet-digestion procedure was found to be effective, with recoveries above 85%. Meanwhile, an inconsistency in element content was observed for food samples collected on different days. Based on Student's *t*-test, both FAAS and ICP-OES were suitable for detecting and quantifying heavy metals and essential minerals in the food samples.

Keywords: Penang Assam Laksa, heavy metal, essential minerals, street food

Abstrak

Asam Laksa Pulau Pinang adalah sup masam yang berasaskan ikan. Kandungan logam berat (Cd, Hg, Pb) serta mineral penting (Fe, Ca, Na, K) dalam Asam Laksa Pulau Pinang (makanan sedia dimakan) yang dikumpul dari lima gerai terpilih pada tiga hari berbeza telah ditentukan menggunakan FAAS (spektrometri penyerapan atom nyalaan), FIMS (sistem merkuri suntikan aliran) dan ICP-OES (spektrometri pancaran optik induktif gabungan plasma) selepas prosedur pencernaan basah. Analisis statistik seperti *Ujian-t* pelajar dan kajian ketepatan antara hari telah dijalankan ke atas keputusan yang diperolehi. Prosedur pencernaan

basah didapati merupakan kaedah yang berkesan dalam mencerna sampel makanan dengan pemulihan lebih daripada 85%. Sementara itu, ketidakselarasan kandungan unsur diperhatikan daripada sampel makanan yang dikumpul pada hari yang berbeza. Berdasarkan *Ujian-t* pelajar, kedua-dua FAAS dan ICP-OES sesuai untuk pengesanan dan penentuan logam berat dan mineral penting dalam sampel makanan.

Kata kunci: Asam Laksa Pulau Pinang, logam berat, mineral penting, makanan jalanan

Introduction

Food is a fundamental requirement for sustaining life. The rapid acceleration of the human population has led to the creation of various kinds of foods, including street foods, which are defined as ready-to-eat foods and beverages that are prepared and/or sold by street vendors [1]. Generally, street foods are rich in macro- and micro-nutrients. Macro-nutrients such as carbohydrates, fats, and proteins are needed in relatively large amounts, as they provide energy to the human body, while micro-nutrients in food comprise vitamins and inorganic minerals such as calcium (Ca), potassium (K), and iron (Fe). Essential minerals are present in small quantities but play significant roles in human biology, especially as catalysts for enzymatic activities [2-5]. For example, sodium (Na) and K are common electrolytes that drive the transmission of signals along nerves as well as maintain water balance, while Fe is a principal component in hemoglobin production, which is responsible for oxygen transportation [6-8] in blood. Either inadequate or excessive intake of essential minerals will adversely affect human health. Hypocalcemia, also known as low blood calcium levels may result in tetany, whereas hypercalcemia may lead to kidney-stone formation (nephrolithiasis) [7].

Street foods can readily become contaminated with heavy metals such as cadmium (Cd), lead (Pb), and mercury (Hg) due to environmental pollution, which is all the more common in modern times [9]. Heavy metals are substances that occur naturally and are often present in the environment at trace levels [10]. Heavy metals have caused widespread concern due to their tendency to accumulate in selective human tissues (liver, brain, and kidney) and not decay over time. These characteristics will eventually lead to various types of disorders, such as peripheral neuropathy and different cancers. Previous studies have shown that

humans are exposed to heavy metals via the ingestion of contaminated food and beverages [3, 9, 10]. Hence, it has become necessary to perform food analysis in order to assess food quality and safety.

Food analysis is a challenging task in analytical chemistry; the presence of heavy metals and essential minerals in trace quantities, as well as the complex nature of food matrices, have only increased its difficulty. Proper and efficient sample preparation followed by a highly sensitive instrumental analysis is often required to obtain quality element-content data in food [11]. There are numerous types of sample digestion methods that have been successfully employed in determining element content in diverse food samples. Dry-ashing and wet-digestion procedures are well established methods, and their efficiency has been reported in previous studies. Dry ashing is known to be effective in digesting organic materials in food samples, but it is time-consuming and prone to losses due to volatilization and/or retention problems [9, 12, 13]. Wet digestion is relatively simple, rapid, and inexpensive compared to dry ashing. This procedure benefits from small sample quantities and greater flexibility, as the sample weight and digestion conditions can be changed accordingly.

In recent years, numerous analytical techniques have been developed for determining element content in food samples. These techniques include neutron activation analysis (NAA), voltammetry, atomic absorption spectrometry (AAS), inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS) [4, 9, 10, 14, 15, 16]. Among these techniques, AAS (including flame atomic absorption spectrometry, FAAS, and graphite furnace atomic absorption spectrometry, GFAAS), ICP-OES, and ICP-MS [4, 15] are well established as mainstays in food analysis

because of their rapidity and effectiveness in detecting and quantifying elements in samples at extremely low concentrations [4, 10, 11, 16]. In addition, flow injection analysis (FIA) using devices such as the Flow Injection Mercury System (FIMS) is also employed, as it combines the advantages of flow injection and atomic absorption measurement into a compact Hg analyzer [17].

In Malaysia, Penang is well known as a food paradise due to its wide variety of street foods. These foods reflect the multicultural makeup of the state, which has citizens of Chinese, Malay, and Indian descent. One of the most famous Penang street foods is Penang Assam Laksa, which is a broth made of shredded fish (*Kembung* fish or mackerel) and finely sliced vegetables [cucumber, onions, red chilies, pineapples, lettuce, fresh mint, and torch ginger (in Malay: *bunga kantan*)] [18]. An increased interest in street foods has led to public concern about their safety and quality, owing to their socioeconomic importance as well as nutritional value. However, there is a paucity of proper studies and data on this kind of fresh, ready-to-eat food, as most studies focus on raw, uncooked foods or commercialized food products. Thus, there is a need to perform a proper scientific study to obtain a general picture of the nutritional content of Penang Assam Laksa. Such information can help prove the safety of the food and increase confidence among consumers.

This study mainly aimed to detect the essential minerals (Ca, Fe, Na, and K) and heavy metals (Hg, Cd, and Pb) in Penang Assam Laksa by using FAAS, FIMS, and ICP-OES. The safety of the food was further assessed by comparing the element content obtained from collected food samples to the permissible levels stated in Malaysia Food Act 1983 and Food Regulations 1985 [19], as well as the recommended dietary allowance (RDA) from U.S. Dietary Guideline 2010 [18]. Finally, a simple comparative study was performed to evaluate the agreement between the results provided by these two different groups of techniques.

Materials and Methods

Chemicals and Standards

Concentrated nitric acid (HNO_3 , 65%, Merck, USA) and hydrogen peroxide (H_2O_2 , 35%, Hmbg, India) were purchased from local chemical suppliers. Commercial 1000-ppm standard stock solutions (Perkin Elmer Laboratory preparation, USA) containing elements of interest were used to prepare working standard solutions, all of which were freshly prepared prior to analysis. Ultrapure water was produced by a Milli-Q System (Millipore, Bedford, MA, USA) and used for solution preparation as well as sample digestion.

Sample Collection and Handling

Fifteen food samples (Penang Assam Laksa) were purchased from five selected areas throughout Penang Island on three different days, all of which were cooked and ready-to-eat. Upon purchasing, the solid ingredients (sliced vegetables and noodles) and the broth of the samples were packed separately with leak-proof nylon bags. During sample preparation, the solid ingredients were soaked in the broth and left for 20 min. After that, the mixture was filtered, and the broth was collected for further treatment.

Wet Digestion of Samples

The wet-digestion procedure performed in this work was adopted from a study by Salau and Hassan [5]. Three 5-mL aliquots of previously filtered broth samples were transferred to three conical flasks. Ten mL of HNO_3 was added to the sample and left overnight (24 h) in a fume hood. The following day, further digestion was carried out by adding a mixture of HNO_3 and H_2O_2 . Details about the procedure and its steps are summarized in Table 1. The solutions were filtered with white filter papers (WHATMAN, qualitative filter papers, 125-mm diameter) and then re-filtered with a syringe filter. Then, the filtered solutions were diluted with ultrapure water to 100 mL and transferred into polyethylene sample bottles and kept in a refrigerator.

Table 1. Wet digestion procedure for samples

Step	Ultrapure water added (mL)	HNO ₃ added (mL)	H ₂ O ₂ added (mL)	Heating (°C)	Time (min)
1	-	10	-	-	Overnight
2	-	5	2	100	30
3	-	10	4	100	30
4	30	5	2	100	30
5	30	10	2	100	20

Recovery Study

Standard solutions containing the elements of interest were prepared at three different concentrations: 6.00, 8.00, and 10.00 ppm, except for those of Hg, which were prepared at 0.10, 0.15, and 0.20 ppm. Five-mL samples of the prepared food were spiked with 0.5 mL of the standard solutions. The spiked samples were prepared in triplicate at each concentration. Simultaneously, unspiked blank solutions containing 5 mL of food sample and 0.5 mL of ultrapure water were prepared as well. These spiked and unspiked samples then went through the digestion procedure as described above before subsequent instrumental analysis.

Instrumental Analysis

A Perkin Elmer AAnalyst 400, a Perkin Elmer FIMS 100, and a Perkin Elmer Optima 8000 were used for FAAS, FIMS, and ICP-OES analyses of the target elements, respectively. All the instruments were equipped with WinLab 32 computer software for data acquisition. For every target element, calibration was performed prior to instrumental analysis. The calibration curves for all elements were plotted as absorbance intensity against concentration in ppm (with the exception of ppb for Hg analysis) using Microsoft Excel, and correlation coefficients, R^2 , for all curves were determined. Working standard solutions of certain concentrations were prepared accordingly by diluting the 1000-ppm standard stock solutions. The standard stock solutions were drawn by using micropipette. Samples collected on Days 1 and 2 were run on instruments at the same time, while samples

collected on Day 3 were run separately with a new calibration cycle. The concentrations of the working standards for each element remained unchanged for the first and second runs.

Statistical Analysis

Student's *t*-test was performed by means of Excel in order to determine whether the two methods (FAAS/FIMS and ICP-OES) gave significantly different results. Meanwhile, an inter-day precision study was conducted for the samples collected on different days by expressing the repeatability in terms of relative standard deviation (RSD).

Results and Discussion

Recovery and Efficiency of Wet Digestion Procedure

The accuracy and efficiency of the wet digestion procedure performed on the spiked samples at three different concentration levels were evaluated in triplicate. The average recovery (%) of all target elements is presented in Table 2, which shows that the recoveries for all the target elements were satisfactory - more than 85% of the elements were recovered from the spiked samples. In FAAS, the highest digestion efficiency was achieved for Cd at 96.1%, whereas Pb exhibited the lowest efficiency at 86.3%. In contrast, Hg exhibited the highest average recovery percentage in ICP-OES (94.5%). Based on this recovery assay, the efficiency of the digestion procedure used in this work was confirmed.

Table 2. Average recovery for all targeted elements

Elements	Average Recovery (%)	
	FAAS/FIMS	ICP-OES
Hg	91.6	94.5
Cd	96.1	85.8
Pb	86.3	91.7
Fe	94.0	90.3
Ca	91.7	91.1
Na	93.5	93.5
K	94.1	86.9

Instrumental Analysis of Studied Foods

Linearity of Analysis: Every elemental analysis carried out via FAAS, FIMS, and ICP-OES consisted of two calibration cycles, as the food samples were run in two batches. All the calibration curves exhibited linearity, with R^2 values greater than 0.99, except for Hg via ICP-OES, as well as K and Na via FAAS, which gave slightly lower R^2 values. As an overall observation, all the calibration curves demonstrated a linear response within a particular range. It was noted that both calibration cycles for the elements generated different regression equations and R^2 values, but these differences were quite small and negligible, as they did not possess any significant meaning.

Detection of Essential Minerals in Studied Food Samples: The mineral-content ranges of the food samples were compared to the RDA for minerals according to U.S. Dietary Guideline 2010, as shown in Table 3. Since the RDA values stated in the guideline are in units of mg/day, for ease of comparison, an assumption was made wherein the mineral content presented in the Tables 3 and 4 under this section is normalized as that in 250-mL samples. Adequate Fe intake is crucial to prevent anaemia [2]. It was noted that the order of Fe content for each sample collected at different locations showed the same pattern for the three different collection days. The highest detected Fe content was 1.98 mg/L by FAAS and 1.51 mg/L ICP-OES. According to U.S. Dietary Guideline 2010 [8], adult females and males are recommended to take

approximately 18.00 mg and 8.00 mg of Fe per day, respectively. In general, all the studied food samples were considered to have adequate levels of Fe for an adult male older than 19 years. However, the Fe content was insufficient for an adult female because they experience the Fe losses associated with physiological processes (menstruation) [8].

Calcium is the most abundant cation in the human body. According to U.S. Dietary Guideline 2010 [8], an adult is recommended to take 1.00×10^3 mg of Ca daily as an upper intake level (UL). From Table 3, the highest detected Ca content was 3.25×10^2 mg/L (FAAS) and 2.61×10^2 mg/L (ICP-OES). The high Ca content in the studied food samples was most probably due to mackerel, the main ingredient of the food samples, since it was reported in a previous study that mackerel contains high levels of Ca, ranging from 1.60×10^3 to 1.80×10^3 mg/L [20]. All the studied food samples were considered to have adequate Ca levels for an adult older than 19 years.

For Na, adequate intake (AI) levels are used instead of RDA, since the latter could not be determined. The determined Na concentration of all the studied food samples approached the AI level and was less than the UL. The highest Na levels were 1.23×10^3 mg/L (FAAS) and 1.44×10^3 mg/L (ICP-OES). The Na content in the studied food samples was most likely due to salt (sodium chloride), which is added to enhance taste. Based on the obtained results, the

studied food samples were considered to have adequate Na levels. However, the consumption of Penang Assam Laksa is advised to be reduced in order to avoid excessive Na intake.

Potassium is important for building muscle as well as metabolizing proteins and carbohydrates [8]. The highest concentration of K was detected at 1.52×10^3

mg/L and 6.67×10^2 mg/L for FAAS and ICP-OES, respectively. The AI level for K established by U.S. Dietary Guideline 2010 is approximately 4.70×10^3 mg/day; all the studied samples were considered to have sufficient K, most likely because of the mackerel - the main ingredient of the broth [21].

Table 3. Ranges of Fe, Ca, Na, and K contents in the studied food samples detected by FAAS and ICP-OES (assuming 250 mL of broth per serving unit)

Element	Range of Concentration Detected (mg/L)		Recommended Dietary Allowance
	FAAS	ICP-OES	
Fe	5.48×10^{-1} to 1.98	5.57×10^{-1} to 1.51	18.00 mg per day (female above 19 y.o.) 8.00 mg per day (male above 19 y.o.)
Ca	3.96×10 to 3.25×10^2	1.18×10^2 to 2.61×10^2	1.00×10^3 mg per day (male and female above 19 y.o.) Adequate Intakes
Na	7.32×10^2 to 1.23×10^3	6.87×10^2 to 1.44×10^3	1.50×10^3 mg per day (male and female above 19 y.o.)
K	1.92×10^2 to 1.52×10^3	1.44×10^2 to 6.67×10^2	4.70×10^3 mg per day (male and female above 19 y.o.)

*All values are expressed as a mean in ppm or mg/L

* y.o. = years old

* Recommended dietary allowance and adequate intakes (Source: US DA & US DHS 2010)[8]

Detection of Heavy-Metal Contents in Studied Food Samples: The concentration of heavy metals detected in all food samples was tabulated and compared to the permissible levels stated in Malaysia Food Act 1983 and Food Regulations 1985 [19], as seen in Table 4.

Cadmium affects cell proliferation, differentiation, and apoptosis; it also inhibits both cellular respiration and oxidative phosphorylation at low concentrations [22]. In this study, Cd was not detectable via ICP-OES for all samples, while it was detectable via FAAS. This observation does not lead to the conclusion that the samples were free of Cd. Instead, the results only suggest that Cd was undetectable in ICP-OES, as FAAS was able to give readings. Basically, the Cd

content in the studied food samples ranged from 7.80×10^{-2} to 2.85×10^{-1} mg/L (in FAAS determination). This concentration level was relatively low and did not exceed the permissible level stated in Malaysia Food Act 1983 and Food Regulations 1985 [19], which is 1.00 mg/L.

The FIMS used in this study was a dedicated Hg system coupled with a high-sensitivity Hg detector [17]. This instrument was designed purposely for Hg quantification with an extremely low detection limit (as suggested by the user manual of the instrument) of less than 5.00×10^{-6} mg/L, as compared to the much higher 5.10×10^{-3} mg/L for ICP-OES. In other words, ICP-OES is not sensitive enough to detect Hg at trace

levels; thus, it yielded no readings or measurements in this study. The Hg detected in most of the food samples was present at trace amounts ranging from 9.00×10^{-4} to 8.10×10^{-3} mg/L, lower than the permissible level of 5.00×10^{-2} mg/L as stated in Malaysian Food Act 1985 [12]. Meanwhile, Hg was undetectable in a few samples.

The Pb levels in the food samples were significantly higher than those of Cd and Hg. According to Malaysia Food Act 1983 and Food Regulations 1985 [19], the

permissible level of Pb in food is 2.00 mg/L. Unfortunately, the majority of the samples contained excessive Pb levels, which possibly originated from the raw ingredients used to prepare the foods, such as fish, spices, and vegetables. This finding is of great concern, as Pb was classified as a “human carcinogen” by International Agency for Research on Cancer (IARC) in 1993 [23]. Excessive Pb intake can cause severe brain and kidney damage. Moreover, a high intake of (or exposure to) Pb may cause miscarriages in pregnant women [10, 24].

Table 4. Ranges of Cd, Hg, and Pb contents in the studied food samples detected by FAAS/FIMS and ICP-OES (assuming 250 mL of broth per serving unit)

Elements	Range of Concentration Detected (mg/L)		Permissible Level in Malaysia
	FAAS/FIMS	ICP-OES	
Cd	7.80×10^{-2} to 2.85×10^{-1}	n.d.	1.00 mg/L
Hg	9.00×10^{-4} to 8.10×10^{-3}	n.d.	5.00×10^{-2} mg/L
Pb	2.87 to 5.26	1.27 to 5.04	2.0 mg/L

*All values are expressed as a mean in ppm or mg/L

*n.d = non-detectable

*Permissible Level in Malaysia (Source: Malaysia Food Act 1983 and Food Regulations 1985, 2017 [19])

Statistical Testing

Student's *t*-test: The results provided (Table 5) by the two types of analytical instruments, FAAS/FIMS and ICP-OES, were compared using Student's *t*-test. There was no significant difference (at a confidence level of 95%) in the results of essential minerals as determined by FAAS and ICP-OES. However, there was a significant difference found in the heavy-metal results. This indicated that both FAAS and ICP-OES were suitable for the detection and quantification of essential

minerals in food samples. For heavy metals, both analytical instruments failed to show a good correlation between the obtained results. Perhaps this was associated with the trace levels of heavy metals in the food samples and the sensitivities of the instruments. Thus, the suitability of an instrument in detecting and quantifying heavy metals in food samples is dependent upon its detection limit.

Table 5. Summary of Student's *t*-test for all targeted elements

Elements	<i>t</i> -calculated	<i>t</i> -critical	Result
Fe	1.24	2.15	No Significant difference
Ca	1.67		No Significant difference
Na	1.91		No Significant difference
K	1.25		No Significant difference
Cd	11.2		Significant difference
Hg	3.18		Significant difference
Pb	8.11		Significant difference

*Student's *t*-test performed at a 95% confidence interval

Inter-day Precision Study

An inter-day precision study was performed in order to assess the consistency of the determined content of minerals and heavy metals in the studied food samples. Sample collection was repeated on three different days at selected locations. The precision of repeatability is expressed as %RSD. In general, the acceptable level for %RSD is less than 10%[8]. Overall, almost all the elements exhibited a %RSD value above 10%, which suggests the inconsistency of the content of heavy metals and minerals for samples collected on different days. The variability of the element content is most likely a result of the ingredients comprising the food samples. However, it is difficult to ascertain the exact cause for such variation, as there are a wide variety of factors involved.

Conclusion

The heavy metals (Cd, Hg, and Pb) and essential minerals (Fe, Ca, Na, and K) present in Penang Assam Laksa collected from different areas were successfully characterized by using both FAAS/FIMS and ICP-OES. Cadmium and mercury were detected in Penang Assam Laksa at trace levels, whereas lead was found at levels higher than those permitted by Malaysian Food Act 1983 and Food Regulations 1985. Most of the Penang Assam Laksa contained adequate levels of iron, calcium, potassium, and sodium. As determined by Student's *t*-test, both FAAS and ICP-OES can be satisfactorily used in the detection and quantification of minerals, while an inter-day precision test showed variability of the element content in food samples

collected on different days. Moreover, the wet-digestion procedure was efficient and suitable for digesting the food samples. In general, this work represents the first scientific study on the element content in Penang Assam Laksa, one of the most famous street foods in Penang. However, the findings stated above cannot be generalized to all Penang Assam Laksa samples, as this study just focused on five selected locations. Instead, this work serves as valuable direction for other researchers in the field. Further studies are needed in order to provide a full picture of the nutritional content in Penang Assam Laksa.

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