

APPLICATION OF STABLE ISOTOPE RATIOS ANALYSIS FOR MALAYSIAN COCOA BEANS TRACEABILITY IN DIFFERENT GEOGRAPHICAL ORIGIN

*Mohd Zaini Nanyan¹, Ahmad Nazrul Abd Wahid⁶, Albert Ling Sheng Chang⁴, Ahmad Kamil Mohd Jaaffar⁴, Suzannah Sharif¹, Nurul Elma Sabri⁶, Mohd Noor Hidayat Adenan⁶, Nur Hafizati Halim⁶, Muhamad Taufik Abdullah⁶, Badrul Hisyam Zainudin¹, Nuraziawati Mat Yazik² and Aizat Japar⁵

¹Cocoa Innovation and Technology Center, Malaysian Cocoa Board, Lot 12621, Kawasan Perindustrian Nilai, 71800 Nilai, Negeri Sembilan

²Cocoa Research and Development Center, Bagan Datuk, Malaysian Cocoa Board, Peti Surat 30, Jalan Sg. Dulang, 36307 Sg. Sumun, Perak

³Cocoa Biotechnology Research Center, Malaysian Cocoa Board, Zon Komersial 1, KKIP Selatan, Jalan Norowot, 88460 Taman Perindustrian Kota Kinabalu, Sabah

⁴Malaysian Cocoa Board, Tingkat 5, 6 dan 7, Wisma SEDCO, Locked Bag 211, 88999 Kota Kinabalu, Sabah

⁵Cocoa Research and Development Center, Kota Samarahan, Lot 248, Blok 14, Daerah Muara Tuang, Bahagian Samarahan, Beg Berkunci 3131, 93450 Kuching Sarawak

⁶Malaysian Nuclear Agency, Bangi, 43000, Kajang, Selangor

*Correspondence author: mzaini@koko.gov.my

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ABSTRACT - This research investigates the use of stable isotope ratio analysis to trace the geographical origins of Malaysian cocoa beans by examining stable isotopic values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using an Elemental Analyzer – Isotope Ratio Mass Spectrometer (EA-IRMS). The study aims to determine whether these isotopic signatures can effectively differentiate Malaysian cocoa beans from those produced in other regions in Malaysia. Cocoa samples from diverse locations, including Sabah, Sarawak, and Peninsular areas, were analyzed to create baseline isotope profiles. The results revealed that isotopic values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were particularly useful in distinguishing cocoa beans from various geographic origins. The study demonstrates that stable isotope ratio analysis is a valuable tool for verifying the origin of cocoa beans, which can enhance product authenticity and quality control measures. The ability to differentiate Malaysian cocoa from beans of other origins underscores the potential for advancing traceability and highlighting the unique attributes of Malaysian cocoa in the global market. This work supports the development of more accurate traceability methods and enriches the understanding of how geographic factors influence cocoa bean composition.

Keywords: Stable isotope, cocoa beans, geographical origin, EA-IRMS, traceability, Malaysian cocoa beans

INTRODUCTION

Cocoa flavor is primarily developed through the processes of fermentation, roasting, and drying of cocoa beans. These beans, which are integral to chocolate production, contribute significantly to the flavor profile of cocoa and thus to the overall value and quality of chocolate products globally. The distinctiveness of cocoa flavor is a key factor in chocolate grading and quality assessment (Afoakwa *et al.*, 2008). Unique flavors in cocoa beans enhance their value and quality.

Determining the origin of cocoa beans presents a challenge since they are often sold in the form of chocolate, which complicates the application of authentication methods due to the blending of ingredients. Dark chocolate, the simplest form, contains only cocoa beans and sugar, whereas other chocolate varieties include additional components like milk. Research on tracing the origin

of cocoa beans has mostly focused on the beans themselves, with some studies extending to dark chocolate samples

Previous research has explored methods related to the metabolic characteristics of cocoa beans. For instance, Caligiani *et al.*, (2014) utilized ¹H NMR to analyze the metabolic profile of cocoa beans, finding that fermentation level significantly influenced the results. The origin of well-fermented beans was partially linked to the specific bean varieties cultivated in different regions

Jinap and Dimick (1990) categorized cocoa samples into three groups based on their acidic properties, such as pH, titratable acidity, and the presence of volatile and non-volatile acids, distinguishing between beans from Brazil and Far Eastern countries, Central and South America, and West Africa.

Chaiseri and Dimick (1989) examined triacylglycerol compositions, which impact the hardness of cocoa butters, and found that climatic conditions contributed to three distinct groups, such as South American, Asian and Oceanian, North American and African cocoa butters

Using a multi-elemental fingerprinting method, Bertoldi *et al.*, (2016) successfully differentiated cocoa samples from five subcontinental regions: West Africa, East Africa, Asia, and Central and South America. Their leave-one-out cross-validation test, which included samples from countries with multiple entries, achieved a 93% correct classification rate for subcontinental origins.

Perini *et al.*, (2016) discovered that $\delta^{13}\text{C}$ values could distinguish between samples from the Northern and Southern Hemispheres, and even within East Africa, distinguishing Tanzanian from Ugandan beans. By analyzing isotope ratios of C, N, H, O, and S, Perini *et al.*, (2016) aimed to trace the origins of cocoa beans, showing that isotope profiles could reveal geographic distinctions.

Torres-Moreno *et al.*, (2015) identified three predominant fatty acids in cocoa beans, with only two showing significant differences between countries. The reliance on fatty acids present in low concentrations poses challenges for discrimination, making quality control and comparative analysis more difficult when the variables are minimal.

Overall, these studies indicated that cocoa bean samples exhibit regional differences, but the analysis is often limited to dark chocolate and does not account for additional ingredients in other chocolate varieties. Furthermore, there is a lack of data on the mineral and trace element contents of Malaysian cocoa beans, which could be crucial for promoting their nutritional benefits and medicinal properties. Enhancing the quality and uniqueness of Malaysian cocoa beans could revitalize the country's cocoa industry, which currently faces challenges due to lower flavor profiles and production levels.

The main objective of this study is to identify the geographical origin of Malaysian cocoa beans grown in different regions in Malaysia based on the different contents of stable isotope such as carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$).

MATERIALS AND METHODS

Eleven (11) fermented and dried cocoa beans samples listed in the Cocoa Excellence (CoEx) were

provided by Malaysian Cocoa Board. As shown in Figure 1, the 11 cocoa samples comprise of 5 samples from Sabah, 3 from Sarawak, 3 from Peninsular areas and one sample is labelled as organic reference.

The sample preparation for analyzing cocoa beans involves several precise steps to ensure uniformity and accuracy. Initially, dried cocoa beans are ground using a mortar and pestle to break them into smaller pieces, which begins the process of achieving a consistent texture. This ground material is then further refined in a blender to produce a fine, uniform powder. To ensure homogeneity and remove larger particles, the powder is sifted through a 125 μm mesh using a Retsch Test Sieve, with the process repeated twice for 5 minutes at 80 amplitudes. This meticulous sieving is crucial for obtaining a sample that is free of inconsistencies. Finally, the prepared cocoa powder is stored in a desiccator, which maintains a controlled, dry environment to prevent any moisture absorption that could affect the sample's integrity before it undergoes further analysis.

In the isotopic analysis phase, a precise 2.0 mg portion of the homogenized cocoa powder is carefully weighed and enclosed in a tin capsule to ensure accuracy in measurements. This sample is then analyzed using an EA-IRMS configured for CNS mode, which specializes in measuring stable isotope ratios. Specifically, the $\delta^{13}\text{C}$ analysis determines the ratio of Carbon-13 ($\delta^{13}\text{C}$) to Carbon-12 ($\delta^{12}\text{C}$), providing valuable information about the cocoa beans' photosynthetic pathways and the environmental conditions of their cultivation. Meanwhile, $\delta^{15}\text{N}$ analysis assesses the ratio of Nitrogen-15 ($\delta^{15}\text{N}$) to Nitrogen-14 ($\delta^{14}\text{N}$), offering insights into the types of nitrogen fertilizers used and the soil management practices, thereby reflecting the agricultural techniques employed in growing the cocoa beans. Together, these isotopic measurements help trace the geographical origin and production practices of the cocoa. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for all samples are tabulated in Table 1.

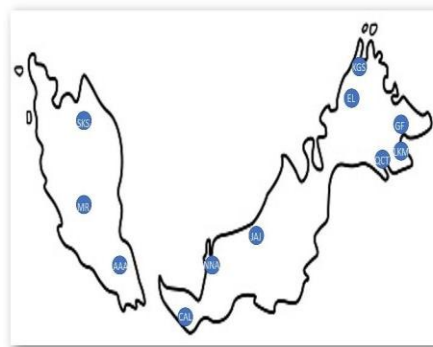


Figure 1. Location of Malaysian cocoa beans

RESULTS AND DISCUSSIONS

The values were expressed according to the International Union of Pure and Applied Chemistry (IUPAC) protocol which follows the formula:

$$\delta = (R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}$$

where R is the ratio between the heavier isotope and the lighter one, against international standards (Vienna-Pee Dee Belemnite - VPDB) for $\delta^{13}\text{C}$ and Air for $\delta^{15}\text{N}$. For $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, the isotopic values were calculated and calibrated against international reference materials; L-glutamic acid USGS (United States Geological Survey) 40 and USGS 41a for $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$.

For 11 Malaysian cocoa beans, $\delta^{15}\text{N}$ ranged from 3.29 ‰ to 7.78 ‰. Perini *et al.*, (2016) found that stable isotope analysis of carbon and nitrogen can effectively distinguish cocoa beans from different geographic origins with the $\delta^{15}\text{N}$ values in their study were 2.6 ‰ to 7.2 ‰. By measuring these isotope ratios through mass spectrometry, the study identified unique isotope profiles linked to various regions. This technique proves to be a reliable method for tracing the origin of cocoa beans, thereby supporting improved quality control, authenticity checks, and traceability in the cocoa industry.

Variation in $\delta^{15}\text{N}$ found between cocoa-producing areas are likely the result of different agricultural practices (Bindereif *et al.*, 2019). The study found that integrating these analytical methods provides a thorough characterization of cocoa beans, greatly improving the differentiation between beans from various regions.

By utilizing detailed molecular and isotopic profiles and employing advanced data analysis, this method enhances the verification of cocoa origin and authenticity, thereby offering a more reliable tool for quality control in the cocoa industry (Bindereif *et al.*, 2019).

The isotopic values of ^{13}C isotope are between - 31.05 ‰ to - 29.61 ‰ for the cocoa bean samples. The results are comparable with Acierno *et al.*, (2020) which found the isotopic values of $\delta^{13}\text{C}$ values ranging from -32.2‰ to -27.7‰, persist through the chocolate-making process.

The research highlights the effectiveness of these techniques for tracking the origin and verifying the authenticity of cocoa beans in chocolate, thereby improving quality control and traceability.

As illustrated in Figure 2, all samples could be clustered into six different groups based on the C

and N isotopic values. Groups labelled I, II, and III belong to Gofri Liang, Sik Kim Soon, and QC Tawau, respectively. Ahmad Abdul Aziz, Cyril Anak Langin, and Eson Lupan were grouped together and labelled as IV, whereas Mispari Ramli, KG Goshen, and LKM Madai were grouped together and labelled as group V. Meanwhile, the last group VI consists of Nasaruddin Ngu Abdullah and Janda Ak Jelian.

QC Tawau is recognized as a certified agricultural establishment that cultivates organic cocoa beans by Malaysian Cocoa Board. This certification indicates that the farmer uses organic fertilizers in their cultivation practices.

Based on Figure 2, QC Tawau is near the point x = 6. As stated above, variation in N isotopic values might be the result of different agricultural practices. Thus, the nearest to the point x = 6 is Mispari Ramli, which we could say that this farmer's agricultural practice is almost like the QC Tawau.

Linking the use of stable isotopes, specifically carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$), with traceability in identifying the geographical origin of cocoa is essential for ensuring product authenticity and quality.

The $\delta^{13}\text{C}$ isotope ratio offers insights into the type of photosynthesis utilized by cocoa plants, which varies by region. Cocoa plants using C3 photosynthesis, common in temperate zones, display different $\delta^{13}\text{C}$ ratios compared to those using C4 photosynthesis found in tropical regions.

These differences in $\delta^{13}\text{C}$ reflect variations in carbon dioxide levels and carbon sources. Analyzing $\delta^{13}\text{C}$ in cocoa can help pinpoint the geographic origin of the cocoa plants, enhancing traceability by linking cocoa products to specific regions based on their isotopic signatures (Bindereif *et al.*, 2019).

The $\delta^{15}\text{N}$ isotope ratio provides information about the types of fertilizers and soil conditions affecting cocoa cultivation. $\delta^{15}\text{N}$ values are influenced by the nitrogen sources absorbed by the plants and the agricultural practices used, such as organic versus synthetic fertilizers. Variations in $\delta^{15}\text{N}$ can indicate differences in farming practices and soil quality across different cocoa-growing regions.

Analyzing $\delta^{15}\text{N}$ allows for the differentiation of production areas based on agricultural methods and helps assess their impact on cocoa quality. This strengthens the ability to trace cocoa's geographic origin, ensuring the product's

authenticity and adherence to quality standards (Acierno *et al.*, 2020).

Currently, we only gather the information of C and N isotopic values for eleven Malaysian cocoa beans. To develop a good model, bigger sample size (at least 150 samples) is necessary for database of Malaysian cocoa beans. To identify the geographical origin, soil and water samples of the same origin of cocoa beans are compulsory. Soil and water samples are required to establish the origin

which is the main objective for this project. Figure 2 illustrates the geographical origin model for eleven Malaysian cocoa beans, based on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values. From the figure, Borneo and Peninsular cannot be clustered appropriately due to insufficient data.

Table 1: $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for 11 Malaysian Cocoa Beans.

No	Farmer/ Place	Label	State	$\Delta^{15}\text{N}$ (AIR)	$\Delta^{13}\text{C}$ (VPDB)
1	Sik Kim Soon	SKS	Kelantan	7.78	-30.48
				7.74	-30.52
				7.75	-30.57
2	Kg Goshen	KGS	Sabah	5.61	-30.89
				5.7	-30.92
				5.61	-30.87
3	QC Tawau	QCT	Sabah	5.94	-30.15
				6.03	-30.16
				5.92	-30.13
4	Cyril Anak Langin	CAL	Sarawak	4.38	-30.24
				4.36	-30.28
				4.47	-30.27
5	LKM Madai	LKM	Sabah	5.58	-30.98
				5.4	-31.05
6	Ahmad Abdul Aziz	AAA	Johor	4.96	-30.1
				4.81	-30.15
				5.45	-30.1
7	Nasaruddin Ngu Abdullah	NNA	Sarawak	3.73	-31
				3.85	-31.02
				3.68	-31
8	Mispari Ramli	MR	Selangor	6.14	-30.9
				6.15	-30.85
				6.14	-30.86
9	Eson Lupan	EL	Sabah	4.67	-30.47
				4.88	-30.44
				4.81	-30.45
10	Janda Anak Jelian	JAJ	Sarawak	3.46	-30.85
				3.31	-30.87
				3.29	-30.87
11	Gofri Liang	GL	Sabah	7.22	-29.63
				7.17	-29.67
				7.07	-29.61

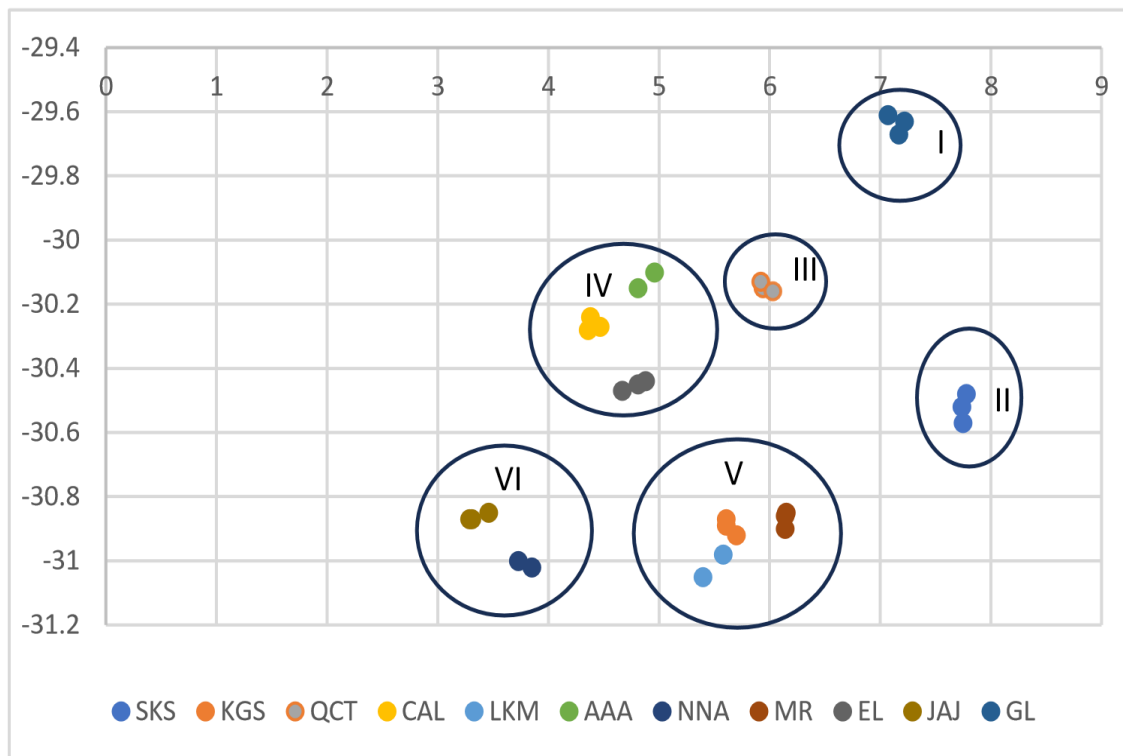


Figure 2: Data distribution of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for 11 Malaysian cocoa beans from different geographical origins.

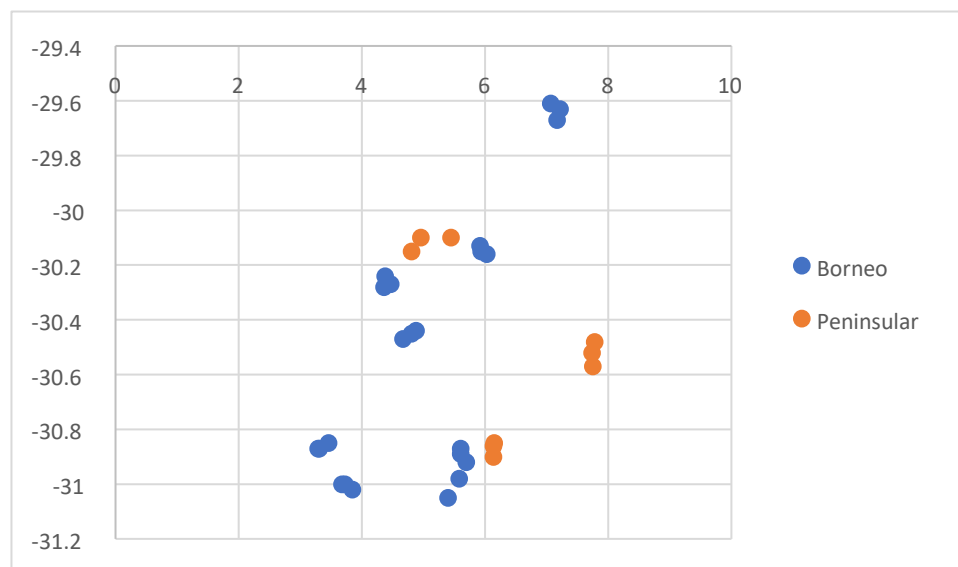


Figure 2: The geographical origin model for eleven Malaysian cocoa beans, based on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values.

CONCLUSIONS

Stable isotope analysis of carbon and nitrogen is crucial for determining the origin and production methods of cocoa beans, providing key insights into their geographical and agricultural context.

Carbon isotopes, notably $\delta^{13}\text{C}$, vary according to the type of photosynthesis used by plants and can be influenced by regional climate and soil conditions. This variation allows $\delta^{13}\text{C}$ to be an effective tool for differentiating cocoa beans from various locations, as each region imparts distinct isotopic signatures to the beans.

On the other hand, nitrogen isotopes, particularly $\delta^{15}\text{N}$, offer insights into agricultural practices by reflecting the types of fertilizers used and soil management strategies. Different fertilizers and soil conditions result in unique $\delta^{15}\text{N}$ values, which can indicate specific farming practices.

By examining both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, researchers can establish a detailed "fingerprint" for cocoa beans, aiding in verifying their origin, maintaining quality control, and confirming claims of sustainable or organic cultivation. This combined approach improves the ability to trace and authenticate cocoa products, thereby enhancing transparency and trust in the cocoa supply chain.

In summary, examining carbon and nitrogen isotopes aids in determining the geographical source of cocoa and provides a better understanding of environmental and agricultural influences on cocoa quality. This approach enhances traceability within the cocoa industry, supporting accurate origin verification and product authenticity.

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