Method validation and assessment of fatty acid content in variant types of durian (*Durio zibethinus* Murr) seeds in the West Bangka Area Using GC-MS (*Gas Chromatography-Mass Spectrometry*)

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Abstract. Durian seeds are often considered useless, but they contain fatty acids that are rich in nutrients. Therefore, this study aims to determine the validity of the test method for the content of fatty acids found in variant types of durian seeds (*Durio zibethinus* Murr) in the Bangka Belitung region using GC-MS. Validation of the GC-MS method was carried out with palmitic, stearic, and oleic acid standards by assessing different parameters, namely system suitability, linearity, selectivity, accuracy, and precision with the gas chromatography method. The derivatization using Soxhlet with n-hexane and BF3-Methanol. The method validation showed that the gas chromatography method met all the parameters for each fatty acid in the form of palmitic, oleic, and stearic acids with a selectivity Rs, linearity, LoD, LoQ, RSD, and K'. Additionally, the 3 types of durian seeds contain fatty acids in the form of palmitic, oleic, stearic, and linoleic acids. The composition of palmitic acid in Tembaga, Bangkok, and Sijantung seeds was 5.160 ± 0.080, 5.057 ± 0.132, and 4.216 ± 0.099 w/w. The stearic acid content in 3 variant types of Tembaga, Bangkok, and Sijantung durian seeds include 3.182 ± 0.027, 3.755 ± 0.098, and 2.670 ± 0.032 w/w. Keywords: Durian seed; fatty acid content; gas chromatography; validation.

1 Introduction

The Meth*od validation is an important step in the accreditation acceptance process. Furthermore, an analysis is often carried out in GLP (*Good Laboratory Practice*)/GMP (*Good Manufacturing Practice*), and all methods involved are then validated to ensure the results are accounted for properly with a high level of accuracy and precision [1, 2]. The characteristics of the analysis in method validation include system suitability, linearity, selectivity, accuracy, and precision. Each experiment must be conducted and validated to ensure that there is no possibility of deviation from the analytical data. The result of

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validation serves as a proof that the method or procedure used for fatty acid content test met the requirements for usage. GC–MS is the most frequently used method for fatty acid analysis [3].

Bangka Belitung is province which were located in west area of Indonesia, and it has several varieties of durian cultivated, including the Bangkok Durian, Tembaga, Semaulagi, Setiangkapal, Sepatu, Botak, Sijantung, and Sibawan [4]. This province is well known for its variety of fruit shapes with different smooth textures, yellow-yellow color, sweet taste, thick flesh, high productivity in the harvest season, and moderate to strong aroma.The seeds of durian are often wasted due to the increase in the production of the fruit, hence, a study is needed for their optimal usage. Furthermore, durian seeds have a relatively high starch content, which indicates that they have the potential to be an alternative to food ingredients [5, 6]. This component can also be used as a binding and disintegrating agent in paracetamol tablet formulations.

Several studies showed that durian seeds contain various fatty acids using the GC-MS method. Adeniyi et al. [7] revealed that they contain 26.8% palmitic acid, 8.4% palmitoleic acid, 3.3% stearic acid, 38.8% oleic acid, 5.9% linoleic acid, and 3% linolenic acid. Furthermore, Sidabutar *et al*. (2017) produced a more concentrated fatty acid content, namely 45.85% stearic acid, 26.75% palmitic acid, 14.95% oleic acid, and 12.45% linoleic [8].

Fatty acids contained in a material can be determined using a tool called *Gas Chromatography* (GC), which is often used to separate oil compounds by flowing a gas stream through the stationary phase [8]. The method coupled with a flame ionization detector is an appropriate technique for testing the content in durian seeds because it can separate organic or inorganic substances, which have volatile properties. The product often obtained for the extraction of durian seeds is fatty oil, which is an organic substance.

Our current study, validation of the GC-MS analysis technique was carried out on various types of durian seeds in the Bangka Belitung area. The result of this study is expected to serve as a validation for reliable methods that met the requirements for analysis. Furthermore, the differences in the fatty acid content of the various types of durian seeds were analyzed qualitatively using retention time and mass spectra as well as quantitatively with the relative % in the chromatogram report. Interestingly, our study in line with the Sustainable Development Goals (SDGs) industry, innovation and infrastructure (SDG 9).

2 Material and Methods

2.1 Materials

The samples used in this study were durian seeds obtained from the Delis Tani Durian Plantation located in the Air Belo area, Mentok, West Bangka, Bangka Belitung Islands. Indonesia. Furthermore, they were obtained from Tembaga, Sijantung, and Bangkok species fruit. Furthermore, the materials pro analysis (pa) were 96% ethanol, aquadest, n-hexane, BF3- methanol; pharmaceutical anhydrous Na_2SO4, as well as standard palmitic, oleic, and stearic acids. The tools used include Soxhlet, glassware, scissors, analytical balances, gas chromatography, and MS detectors.

2.2 Procedure

2.2.1 Material preparation

The seeds of 3 types of durians, namely Tembaga, Bangkok, and Sijantung were taken and washed entirely. They were then cut into several pieces and dried in the sun with a black cloth cover. Subsequently, the seeds were dry crushed/mashed using a blender, and the refined or powdered form was stored in a dry and air-tight container.

2.2.2 Preparation of standard fatty acid solutions

A total of 50 mg palmitic acid, 100 mg oleic acid, and 100 mg stearic acid were dissolved in 10 mL of n-hexane. The standard solution was then diluted with a concentration of 0.5 mg/mL – 2.0 mg/mL [10].

2.2.3 Validation of analysis methods

a. Selectivity determination

A total of 1.0 µL of the esterified solution of durian seeds samples was injected. After the chromatogram was obtained, the selectivity factor (a) and degree of separation (Rs) of the fatty acid peaks were calculated [11].

b. Linearity determination

A total of 1.0 µL of the esterification solution was injected into a standard solution of a mixture of fatty acids with various concentrations that had been prepared, namely 0.5 mg/mL – 2.0 mg/mL. From the chromatogram obtained, the correlation coefficient (r), the coefficient of variation of the function (Vxo), and the smallest concentration (Xp) were calculated through the regression line equation between the Area vs Concentration of each standard solution [11].

c. Determination of LoD and LoQ

Determination of LoD and LoQ was carried out by measuring the profile standard curve chromatogram with 4 concentrations namely 0.5, 1.0, 1.5, and 2.0 mg/mL. From the results, the value of standard deviation and limit of detection as well as the quantitation limit was calculated [12].

d. Precision determination

Precision was determined by calculating the value of the coefficient of variation (KV) of the chromatograms produced at the accuracy stage, while that of the instrument was assessed by injecting 1.0 µL of one of the sample solutions 3 times. After the chromatogram was generated, the KV value was then calculated [11].

e. System compliance test

The system suitability test was carried out by injecting the sample with a concentration of 1 mg/mL into the GC-MS 3 times. The number of theoretical plates, asymmetry, coefficient of variation of retention time, and peak area was then determined [12].

2.2.4 Esterification of fatty acids

Approximately 70 grams of finely ground powder was weighed and mixed with 5 grams of anhydrous Na_2SO_4 placed in a paper sleeve. They were then transferred into a Soxhlet flask, which was assembled, and the condenser was given a stream of cold water, followed by n-hexane 2 to 3 times [8].

Straining was carried out for 2 hours at a bath temperature of 65 ^{0}C or until the color of the solvent changed from that of the sample. Furthermore, n-hexane containing fatty extracts and fatty oils was dried using anhydrous Na_2SO_4 and then evaporated with a rotary evaporator at 35^{o}C. The fatty oils obtained were then collected and weighed for further analysis [8].

2.2.5 Fatty acid derivatization

The process of derivatization of fatty acids was carried out to obtain Fatty Acid Methyl Ester (FAME) from volatile samples. A total of 100 mg of Tembaga, Bangkok, and Sijantung seeds were weighed and placed into a test tube, followed by the addition of 5 mL of BF 3 solution in 20% methanol. Subsequently, the tube was heated in a water bath at 500C for 10 minutes

before used in the transesterification reaction and this facilitated the FAME heating reaction from glyceride to fatty acid methyl esters, followed by cooling for 15 minutes at room temperature. A total of 1.0 mL of n-hexane was added to the samples that have become esters using a micropipette to look for fatty acid esters. The gas chromatograph was then injected with 1.0 mL of the n-hexane phase [8, 12].

2.2.6 Fatty acid analysis

A total of 1 mL of the solution obtained from the derivatization of the n-hexane phase was injected into the gas chromatograph. This chromatography was equipped with a stationary phase of 95% diphenyl polysiloxane. The temperature of the injection site was 215 ^{0}C, while that of the column was 185^{0}C. The detector (Flame ionization) was at 210^{0}C, and the flow rate of the mobile phase, namely helium gas was 40 mL per minute [8].

a. Fatty acid qualitative analysis

   Qualitative analysis was carried out by analyzing the composition of fatty acids in 3 types of durian seeds. To identify the components in the samples, the retention time of fatty oil was equated with that of the standard fatty acids, namely stearic, oleic, and palmitic acids.

b. Quantitative analysis of fatty acids

   The results of the analysis were obtained by reading the relative percent peak area on the GC-MS chromatogram, which was then entered into the standard curve equation to ensure that the levels of the components in the compounds being analyzed were obtained.

2.3 Data analysis

Data analysis was carried out after all the data in this study were obtained. This was preceded by the validation of the analytical method to find out if the legal or invalid requirements were met by proving the validity of the parameters, which includes selectivity, linearity, accuracy, precision, and system suitability test.

The GC-MS results were analyzed qualitatively using retention time and mass spectrum as well as quantitatively. The results of the analysis of the sample were in the form of peaks with a mass spectrum, which became the basis for estimating the compound at a certain time when compared with that of the Mass Spectrometry (MS) database with a high similarity index value. The data were analyzed quantitatively by reading the relative percent peak area on the GC-MS chromatogram, which was then entered into the standard curve equation to ensure that the levels of components of the compound being analyzed were obtained.

3 Results and Discussion

3.1 Materials validation of analysis methods

Validation of an analytical method is an important factor because each analytical technique must be valid to ensure that it can be accounted for and used as a basis. The process was carried out because there were differences between the solvents used in previous studies and this study, namely petroleum ether and n-hexane solvent, respectively. Based on these two solvents, it is important to prove the effectiveness of the gas chromatography system with an n-hexane solvent. The validation carried out involves the determination of fatty acids in durian seeds using GC-MS with various parameters as follows:

3.1.1 Selectivity determination

The results show the injection chromatogram of standard fatty acid solutions containing palmitic acid, oleic acid, and stearic acid as well as the values of α and Rs for the peak components of these components.

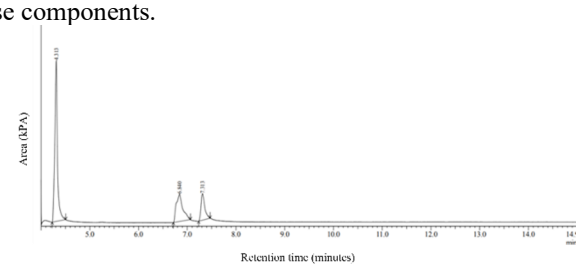

Fig. 1. Fatty acid selectivity graph

Figure 1 shows the existence of a chromatogram profile of the separation of fatty acids, with the first (mobile phase), second (palmitic acid), third (oleic acid), and fourth (stearic acid) peaks. The chromatogram obtained in this study is presented in Table 1.

According to Chiu and Kuo [3], a good selectivity result is characterized by a value of α ≥ 1 and Rs ≥ 1.5. Based on the data, the peaks of the fatty acid components in the sample are well separated except for palmitic acid where the separation was not complete because the solvent was still remaining.

3.1.2 Linearity determination

A mixture of standard fatty acid solutions of various concentrations that have been esterified into methyl esters was injected into the gas chromatography system based on the conditions in the table. The results of the injection and calculation of the ratio of the area to the 4 kinds of concentrations of fatty acids are presented in Table 2.

Based on the results of determining the linearity of Table 2, the value of r = 0.9969 was obtained and CV = 2.428%. According to Gumustas et al. [13], the acceptance criterion for linearity in the validation of the analytical method was r ≥ 0.98, while Yuwono and Indrayanto [11] reported CV < 5%. The parameters show that there was a linear relationship between the concentration of palmitic acid and its area from a standard solution of a mixture of fatty acid with a concentration of 0.5 to 2.0 mg/mL. The linearity image is presented in Figure 2.

Based on the results of determining linearity in Table 2, the value of r = 0.993 and CV = 4.757%. According to Gumustas et al. [13], the acceptance criterion for linearity in the validation of the analytical method was r ≥ 0.98, while Yuwono and Indrayanto [11] reported CV < 5%. The parameters show that there is a linear relationship between the concentration of oleic acid and its area from a standard solution of a mixture of fatty acids with a concentration of 0.5 to 2.0 mg/mL. The linearity image is presented in Figure 2.

The results of linearity determination as depicted in Table 2, the value of r = 0.995 and CV = 5.160 % was obtained. According to Gumustas et al. [13], the acceptance criterion for linearity in the validation of the analytical method was r ≥ 0.98, while Yuwono and Indrayanto [11] reported < 5%. The parameters indicate that there is a linear relationship between the concentration of stearic acid and its area from a standard solution of a mixture of fatty acids with a concentration of 0.5 to 2.0 mg/mL (Figure 2).

3.1.3 Determination LoD and LoQ

LoD (*Limit of Detection*) is the lowest analyte concentration in a sample that can still be detected but not always quantified. It can be determined with acceptable precision and accuracy under the operational conditions of the method used. Determination of LoD and LoQ in this study was carried out by measuring the standard curve chromatogram profile with 4 concentrations, namely 0.5; 1.0; 1.5; 2.0 mg/mL (Table 2). Based on Yuwono and Indrayanto [11], it can be concluded that the LoD and LoQ results show the sensitivity of the method as indicated by the values that fall within the optimum working range of the tool, namely 0.01 – 2 mg/mL.

3.1.4 Precision determination

The precision was determined by calculating the price of the coefficient of variation (KV) from the data generated in evaluating the concentration of the sample. The results obtained for palmitic, oleic, and stearic acids are presented in Table 3. The precision RSD of palmitic acid entered the acceptance criteria, namely 0.782%, while oleic and stearic acids were 1.000% and 1.321%, respectively. In validating the analytical method on precision parameters, 3 replications were used with the acceptance criterion of RSD < 2% [11].

3.1.5 Determination of system conformity test

A suitability test was carried out to ensure that the operating system is effective and appropriate by injecting a sample with a concentration of 1.0 mg/mL into the GC-MS apparatus 3 times. The values for the capacity factor, tailings factor, resolution, and theoretical plate number were then determined. The results of the suitability determination of the system test are presented in Table 3.

This experiment was carried out using palmitic, oleic, and stearic acids at a concentration of 1 mg/mL. With K' acceptance criteria ranging from 2 - 10, a good tf was = 1. If tf <1, then the chromatogram contains fronting and when it is > 1, it experiences tailing. The Rs value indicates a perfect compound separation process, namely Rs > 1.5. Based on the data, those that did not meet the acceptance criteria were the tf values of all fatty acids and the Rs for palmitic acid.

3.2 Qualitative and quantitative analysis

Qualitative and quantitative analyses were carried out on various types of durian seeds, namely Tembaga, Bangkok, and Sijantung. Before the analysis, the samples were extracted with n-hexane using the soxhlet method [14]. This was carried out to obtain the oil compounds contained in the durian seeds. The durian seeds oil obtained was derivatized using BF 3-Methanol by heating, followed by extraction with n-hexane to obtain methyl-ester compounds, which can evaporate to ensure they can be analyzed with a gas chromatography system.

3.2.1 Qualitative analysis

Qualitative analysis was carried out to determine the presence or absence of fatty acid content in the samples by comparing their retention time with that of the standard. The values obtained for standard palmitic, oleic, and stearic acids were 4.313 minutes, 6.840 minutes, and 7.313 minutes, respectively. The qualitative test was carried out three times for each sample. Table 4 shows the tr data for various fatty acids in the 3 types of durian seeds. Based

on the results, the durian seeds of the Tembaga, Bangkok, and Sijantung contain fatty acids in the form of palmitic, oleic, stearic, and linoleic acids.

3.2.2 Quantitative analysis

Quantitative analysis was carried out to determine the levels of fatty acids contained in the durian seeds samples. The determination was entered into the linear regression equation that has been obtained in the linearity validation parameter [15]. Durian seeds contain fatty acids in the form of palmitic, oleic, linolenic, and stearic acids, but only the levels of palmitic and stearic acids can be calculated. The peaks of oleic and linoleic acids did not produce good separation because there was an overlap between their concentrations, thereby making the calculation difficult, as shown in Figure 3**.** In this case, it is necessary to optimize the solvent to ensure it can separate the oleic and linoleic fatty acid components in the durian seeds sample. The data for the fatty acids contained in the sample are presented in Table 5.

The three types of durian seeds had levels of palmitic and stearic acids. The highest content of palmitic acid in the durian seeds of the Tembaga type was 5.160 ± 0.080 %w/b, while the highest stearic acid was found in the Bangkok type, namely 3.775 ± 0.032 %w/b. Table 5 shows that palmitic acid (saturated fatty acid) has the highest levels in the durian seeds sample. Furthermore, it can reduce serum cholesterol (normolipemic) and also serve as a transmitter protein that plays a role in the process of blood clotting in the body. Stearic acid helps in increasing fat metabolism and converting it into energy as well as moisturizing the skin [16].

Aforementioned description indicated that on durian seeds contain fatty acids, which are very useful in the body [17, 18]. They are not only processed into wheat flour but also fish oil to improve digestion, increase appetite, and reduce cholesterol levels [5]. While the starch from durian seeds can be used as a binding and disintegrating agents in the formulation of paracetamol tablets [19, 20].

Table 1. Data on the results of determining (Rr, α, and Rs) fatty acid selectivity

Fatty acid	Rr	Selectivity (α)	Degree of separation (Rs)
Palmitic Acid	4.313	1.060	1.065
Oleic Acid	6.840	1.586	8.423
Stearic Acid	7.313	1.071	1.603

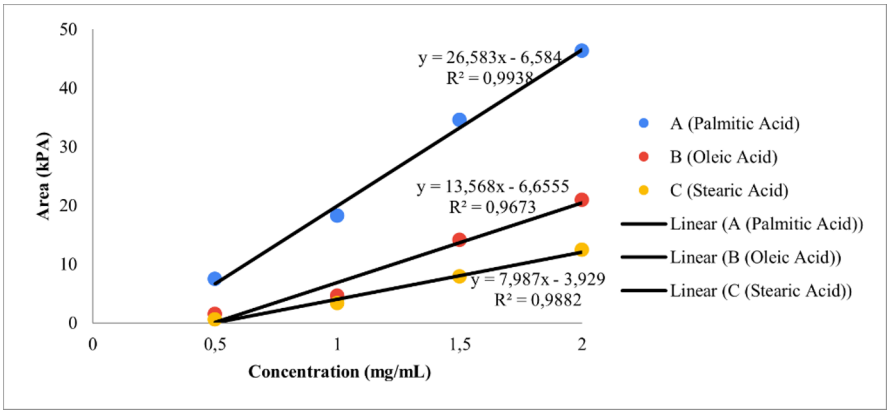

Fig. 2. The relationship between concentration and area of palmitic acid, oleic acid, and stearic acid. Comparing palmitic acid to oleic acid and stearic acid, palmitic acid shows the most sensitive results

Table 2. Data on the relationship between concentration and area of palmitic acid, oleic acid, and stearic acid

Content	Standard solution concentration (mg/mL)	AUC (%)	Regression equation
Palmitic acid	0.5	7.488	y = 26.582 x – 6.583; r = 0.996; CV= 2.428%
	1	18.201	
	1.5	34.544	
	2	46.345	
Oleic acid	0.5	1.535	y = 13.568 x – 6.655; r = 0.993; CV = 4.757%
	1	4.591	
	1.5	14.121	
	2	20.972	
Stearic acid	0.5	0.598	y = 7.986 x – 3.928; r = 0.995; CV = 5.160%
	1	3.348	
	1.5	7.871	
	2	12.402	

Table 3. LoD, LoQ, precision determination, and system suitability results

Fatty acid	LoD Value (% b/v g/100 mL)	LoQ Value (% b/v g/100 mL)	Content		System Suitability Test			
			Average ± SD ((%b/v g/100 mL))	RSD (%)	K' (Capacity factor)	tf (Tailings factor)	Rs (Resolution)	N (Number of plates)
Palmitic Acid	0.186	0.621	1.483 ± 0.011	0.782	2.059	0.830	0.963	2,876.901
Stearic Acid	0.255	0.861	1.333 ± 0.017	1.321	2.799	0.845	1.456	13,625.170

*Note: SD = Standard Deviation *RSD = Relative Standard Deviation

Table 4. Retention time data of variant types of Durian

Sample	tr Palmitic Acid	Steric Acid tr
Tembaga Durian Seeds	4,277	7,267
Bangkok Durian Seeds	4,269	7,241
Sijantung Durian Seeds	4,261	7,246

Table 5. Data on fatty acid levels in variant types of Durian

Fatty acid	Sample	Average content (%b/b)	SD	CV (%)
Palmitic Acid	Tembaga Durian Seeds	5.16	0.08	1.46%
	Bangkok Durian Seeds	5.057	0.132	2.82%
	Sijantung Durian Seeds	4.216	0.099	2.62%
Stearic Acid	Tembaga Durian Seeds	3.182	0.027	1.04%
	Bangkok Durian Seeds	3.755	0.098	2.48%
	Sijantung Durian Seeds	2.67	0.032	0.44%

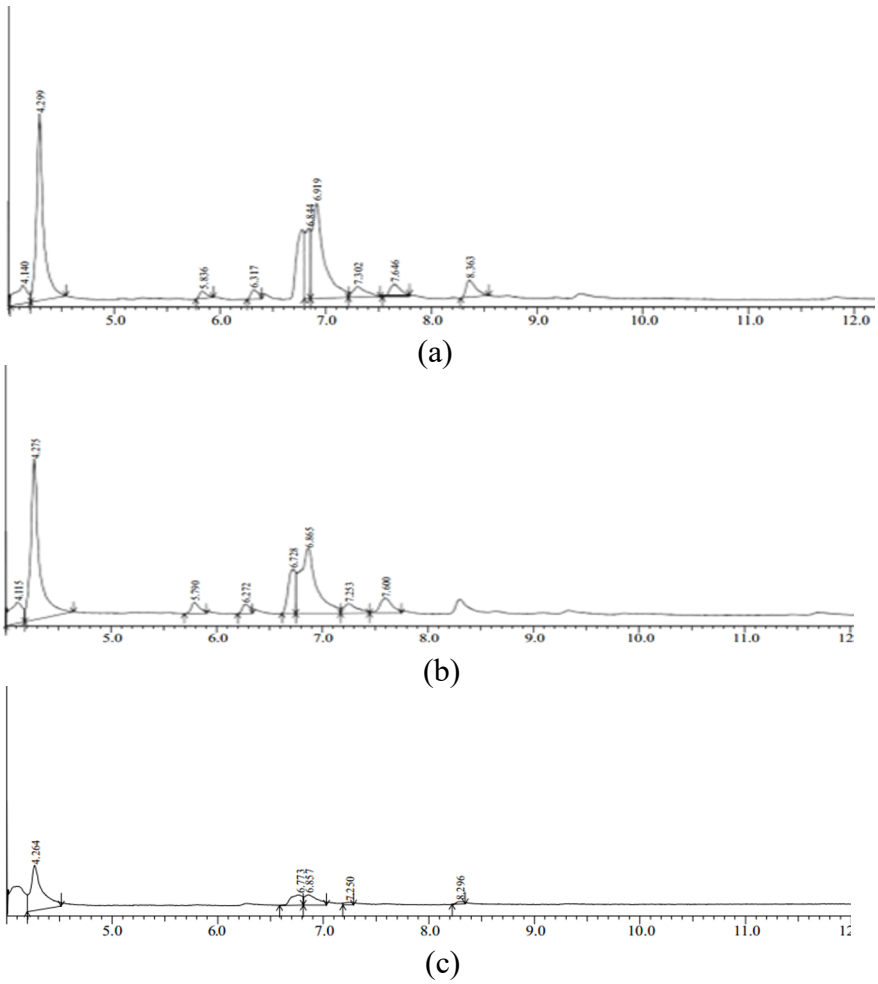

Fig. 3. Chromatogram profile of durian seeds samples for various species: (a) Tembaga durian seeds chromatogram (b) Bangkok durian seeds chromatogram (c) Sijantung durian seeds chromatogram

4 Conclusion

The validation results met the requirements, namely the parameters of selectivity, linearity, LoD and LoQ, and precision. Fatty acid content, namely palmitic, oleic, stearic, and linoleic acids in Tembaga, Sijantung, and Bangkok durian seeds in the Bangka Belitung area have no visible difference based on the analysis results.

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Mustofa contributed in drafting manuscript; Any Guntiarti and Laela Hayu Nurani contributed to conceptualization, methodology, resources, and supervision; Any Guntarti, Citra Ariani Edityaningrum and Laela Hayu Nurani were involved in critical revisions of manuscript and supervision; Ayu Marliana Agustine and Hayati Syarifa contributed to technical administration.

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