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NUTRITIONAL COMPOSITION OF ANTHESISING MALE INFLORESCENCE OF OIL PALM *Elaeis guineensis* ESSENTIAL FOR DEVELOPMENT OF *Elaeidobius kamerunicus* Faust: FIRST REPORT

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ABSTRACT

Rearing of Elaeidobius kamerunicus*, the pollinator of oil palm, requires the anthesising male inflorescence of oil palm as the breeding and feeding ground. Various studies have reported on the weevils utilising the male inflorescence as feed to sustain its development. However, for routine rearing, the use of male inflorescence is inconvenient as it has to be continuously collected from the field. Thus, this pioneering study was conducted to determine the nutritional composition of anthesising male inflorescence of oil palm (*Elaeis guineensis *Jacq.) which is essential for* E. kamerunicus *life development. First, proximate analysis was performed to determine the nutritional composition of anthesising male inflorescence. Next, the nutritional profiles of amino acids, fatty acids and carbohydrates were determined and analysed separately. The average proximate analysis of anthesising male inflorescence of oil palm recorded a high moisture content of 75%, followed by carbohydrate, protein, fat and ash. The amino acid profile revealed the presence of 18 amino acids with lysine having the highest content. The spikelet also recorded a total of 32 fatty acids with oleic acid recording the highest value. Nevertheless, the carbohydrate profile was not determined as its presence was in trace amount only. This study provided new knowledge and reference standard on the nutritional composition of anthesising male inflorescence of oil palm that is essential for* E. kamerunicus.

Keywords: amino acid profile, carbohydrate profile, fatty acid profile, nutritional composition, proximate analysis.

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INTRODUCTION

Oil palm, *Elaeis guineensis* Jacq. is the main cultivated agriculture crop in Malaysia. In a recent report by Parveez *et al.* (2022), the total planting area in 2021 was approximately 5.74 million hectares as compared to 5.87 million hectares in 2020, reflecting a reduction of 2.2%. The Malaysian export revenue from oil palm products in 2021 was reported at RM108.52 billion (Parveez *et al.*, 2022).

Palms were pollinated by wind during the early cultivation of oil palm in Malaysia (Hardon and Turner, 1967). Nevertheless, Syed (1979) revealed that oil palm is an entomophilous plant, based on the research findings in Cameroon. In

1981 the main pollinator identified from Cameroon was imported to facilitate the low fruit set and high cost of assisted pollination in Malaysian oil palm cultivation (Syed *et al.*, 1982). *Elaeidobius kamerunicus* was selected and introduced based on its adaptability to the Malaysian climate (Syed, 1981). The introduction brings benefits to the industry and contributes to the increase in fresh fruit bunches (FFB) production.

Oil palm is a monoecious plant with the presence of both male and female inflorescences in a single palm tree (Kushairi *et al.*, 2019). Anthesising male inflorescence (AMI) of oil palm serves as the feeding and breeding site for *E. kamerunicus* (Syed, 1981). *Elaeidobius kamerunicus* host is specific to the *E. guineensis* male inflorescence, whereas the weevil reproduction and survival are reduced in closely related species such as *Elaeis oleifera* or hybrid oil palm (Kang and Karim, 1982; Syed, 1981). Syed (1981) documented that *E. kamerunicus* develops by feeding on the anther filament of the male inflorescence floret. The study also reported the whole immature stage development inside the male floret where the three-instar stage developed before emerging as an adult.

The study on the biology of the pollinator weevil in the laboratory or closed facility requires natural food sources to sustain the weevil population. Several documented studies depicted the sustenance of the weevil population using male inflorescence of oil palm in laboratory settings (Eko Prasetyo and Susanto, 2019; Ismail *et al.*, 2020; Zulkefli *et al*., 2022), but it is inconvenient to obtain continuously from the field and requires extensive management. Sarah Najihah *et al.* (2019) documented successful rearing of the immature stage *E. kamerunicus* using an artificial diet. Nevertheless, the diet required the incorporation of natural feed (male inflorescence). But its nutritional composition has not been investigated and reported. Thus, this study aimed to determine the nutritional composition of the AMI of oil palm that could play a key role in the survival of the *E. kamerunicus*.

MATERIALS AND METHOD

Collection and Preparation of Sampled Anthesising Male Inflorescence of Oil Palm

The oil palm cultivation area in Putra Agriculture Park (Universiti Putra Malaysia) at GPS: 2.988428, 101.722663 was utilised as the collection plot for the AMI of oil palm. The plot comprised of planting material *Dura* x *Pisifera* breed with oil palm of less than 10 years old. The nutritional composition analysis of the AMI samples was performed as described by Zulkefli *et al.* (2021). The first step was by bagging the pre-anthesising male inflorescence in the field using a muslin cloth, which was then left to occur naturally. The indicator for full anthesis of male inflorescence was the presence of yellowish pollen on the whole inflorescence spikelet and adult weevil swarming on the outside layer of the muslin bag. The whole inflorescence was cut down carefully and sent to the laboratory to be stored in the freezer at (-20°C) before being submitted for nutritional composition analysis. The collected spikelet was ensured to be free from insect and fungal infection.

Proximate Analysis of the Anthesising Male Inflorescence of Oil Palm

Determination of protein. Protein, carbohydrate and lipid acids are combined physically and chemically in food (Pomeranz and Meloan, 2000). Proteins functions as the sources of amino acids. The determination of protein was performed according to Kjeldahl's method based on the total nitrogen in the food sample. The method of determination was developed and reported by AOAC (981.10) was employed (AOAC, 2012). Meanwhile, the Kjeldahl method was utilised to determine the percentage protein using titration with acid and calculated using the Equation (1) as stated below:

$$\% \text{ Protein} = \frac{(\text{mL sample} - \text{mL blank}) \times \text{Conc HCl} \times 1.4007 \times \text{Factor}}{\text{Sample weight (g)}} \quad (1)$$

where, protein factor is 6.25

The procedure for protein determination began by digesting the sample in the fume hood. Around 1 g of well-mixed and ground sample was transferred into a 250 mL digestion tube, followed by adding two catalyst tablets along with 12 mL sulphuric acid in the tube. Thereafter, digestion tube was placed in a tube rack. The digestion was started until a temperature of 420°C was achieved for 45 min or until the solution became clear. The sample was allowed to cool to room temperature. To determine the protein content, the digested sample in the tube was attached to the distillation unit, ensuring that approximately 50 to 75 mL of distilled water and 50 mL of NaOH at 40% were distributed first. The receiver flask contained 25 mL H_3BO_3 and the probe was allowed to sink. Subsequently, the steam distillation was evaluated while the titration commenced automatically following changes in the pH for Vap 50. The amount of 0.1 N HCl required was recorded and the protein determination was first performed for the blank sample.

Determination of moisture content. Moisture content is among the significant attribute and is widely measured in food testing and processing. The amount of dry matter in food is inversely proportional to the moisture content, whereas the stability and quality of the food are affected by the moisture content (Pomeranz and Meloan, 2000). The method applied in determining the moisture content was the AOAC (950.46) technique reported by AOAC (2012).

The sample was first ground into smaller particles in a blender and mixed evenly. Then, an aluminium dish together with a cover was dried in an oven at 100°C (± 3°C) for 3 hr. Subsequently, the dish was cooled in a desiccator and weighed as soon as it reached the room temperature. After that, around 2 to 5 g of the prepared sample was placed inside an aluminium dish and dried in an oven overnight without a lid at 100°C (± 3°C). The lid was then placed while the dish was inside the oven before removing the dish. The dish was cooled in a desiccator and weighed as soon as it reached room temperature. This process was repeated until a constant weight was achieved or a 4% reduction in weight or less than 0.5 mg. Equation (2) for the moisture determination is as follows:

$$\% \text{ moisture} = \frac{(W1 - W2) \times 100\%}{Ws} \quad (2)$$

where, W1 is weight of sample and dish before drying, W2 is weight of sample and dish after drying and Ws is weight of the sample.

Determination of total fat. Lipid is a known class of food that is soluble in water and solvent of organic fat based (Belitz *et al.*, 2009; Pomeranz and Meloan, 2000). The determination of total fat was calculated and analysed using the method developed by AOAC (991.36) and reported by AOAC (2012). Total fat was determined using the hydrolysis method with a strong acid as described below.

First, the sample was prepared into particle sizes and around 2 to 5 g was weighed and transferred directly into a beaker to perform the hydrolysis using the Weibull-Stoldt method. Then, the sample was boiled in 100 mL 3M HCl using refluxed for an hour and allowed to cool until there was less vapour. The sample was filtered using a moist filter paper to prevent loss of fat and then rinsed with boiled water to become acid-free. No residue was left on the filter wall and the latter was dried for an hour at 100°C ± 3°C inside an oven with no lid on the watch glass.

The hydrolysed sample was placed into an extraction thimble and sealed using a cotton wool plug before placing it in an extraction chamber. Before that, an extraction cup of 250 mL along with the glass bead was heated and weighed. The solvent was added and the beaker was placed in an extraction system. The system was switched on for around 10 min and the sample was extracted while the side lever or discharging system was left open. The heating was continued for another 10 min until all the solvent had evaporated from the beaker into the receiver. The beaker along with the fat was dried in a forced air-drying oven for 30 min at 100°C ± 3°C and finally cooled and weighed. The total fat was calculated using the Equation (3) below:

$$\% \text{ Fat} = \frac{(B - A) \times 100}{C} \quad (3)$$

where, A is weight of extraction cup prior to extraction (g), B is weight of extraction cup after drying (g) and C is sample weight.

Determination of total ash. Ash is the remaining inorganic material after the organic matter has been burnt. The total ash value and composition depends on the food material and ashing method (Pomeranz and Meloan, 2000). The total ash is widely used in removing unwanted substance in food such as sugar and wheat flour. The total ash was determined following the AOAC (923.03) method (AOAC, 2012).

First, the ashing dish was ignited and it was allowed to cool in a desiccator and immediately weighed upon achieving room temperature (W2). The test sample was mixed evenly and around 3 to 5 g (Ws) was weighed. Thereafter, the sample was partially burnt on a hotplate. Then, the sample was ignited in a furnace to reach approximately 550°C (dull red) until light grey ash was obtained. Next, the dish was cooled in a desiccator and immediately weighed upon achieving room temperature (W1).

The determination of total ash was by the destruction of organic matter through heating on a furnace at a temperature of 550°C to a constant mass, which was then calculated using the following Equation (4):

$$\%\text{Total ash} = \frac{(W1 - W2) \times 100\%}{Ws} \quad (4)$$

where, W1 is weight of ash and ashing dish, W2 is weight of ashing dish and Ws is weight of sample.

Determination of total carbohydrate. Carbohydrate is largely found in the earth as an organic compound, serving as a basic nutrition and a vital source of

energy (Belitz *et al.*, 2009). The carbohydrate content in food is known as the total carbohydrate, which is calculated as Equation (5) below (Pomeranz and Meloan, 2000).

$$\% \text{ Carbohydrates} = \begin{matrix} 100 - (\% \text{ Protein} + \% \text{ Fat} \\ + \% \text{ Ash} + \% \text{ Moisture}) \end{matrix} \quad (5)$$

Calculation of energy. The energy (kcal) in the sample was calculated using the Atwater conversion factor, which is recommended and utilised widely for the analysis. The conversion factor is estimated at 4 for carbohydrate, 4 for protein and 9 for fat (Pearson, 1976). The determination of energy in (kJ) is calculated based on the conversion of one calorie is equivalent to 4.1855 joule (Pearson, 1976). Equation (6) for energy calculation is described below:

$$\text{Energy (kcal)} = \begin{matrix} 4(\%\text{Carbohydrate}) + \\ 4(\% \text{ Protein}) + 9(\%\text{Fat}) \end{matrix}$$

$$\text{Energy (kJ)} = \text{Energy (kcal)} \times 4.2 \quad (6)$$

Nutritional Composition of the Anthesising Male Inflorescence of Oil Palm

Amino acid profile

a) Acid hydrolysis

Protein hydrolysis was utilised to determine the fraction of amino acid present in the test sample. An acid was used as the solution for the hydrolysis of the sample. The water AccQ Tag method was developed and utilised for the hydrolysate to enable faster determination of 18 amino acids subjected to liquid chromatography (LC) analysis. The acid hydrolysis was conducted using the method reported in previous studies (Cohen and De Antonis, 1994; Liu *et al.*, 1995; Ma *et al.*, 2015).

The hydrolysis of the test sample was conducted by weighing 100 mg of the samples and placing them into a 16 x 25 mm test tube. Then, 5 mL of 6 N HCl was added into the tube. Thereafter, the tube was flushed thoroughly with nitrogen or argon gas. The tube was then capped and placed into an oven at 112°C for 22 hr. After hydrolysis, the internal standard of 10 mL of 2.5 mM a-aminobutyric acid was added to the precooled hydrolysate mixture. Then, it was transferred into a 250 mL volumetric flask before adding purified water to the meniscus level. Finally, around 0.5 mL of the solution was filtered using a 0.45 mm acid compatible filter.

The derivatisation of the sample was performed using 10 µL of the filtered sample in a recovery autosampler vial, followed by adding 70 µL of AccQ-Fluor borate buffer. Then, the solution was vortex for a moment and 20 µL of reconstituted AccQ Fluor reagent was added. It was then vortex again for several seconds and heated using a heating block for 10 min at 55°C. The standard amino acid solution was prepared for threonine (Thr), alanine (Ala), proline (Pro), tyrosine (Tyr), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), aspartic acid (Asp), glutamic acid (Glu), serine (Ser), glycine (Gly), histidine (His), arginine (Arg), and lysine (Lys) by adding the amino acid to 0.1 M HCl. The concentration of standard solution was at 25 nM. The standard was mixed to create a mixed amino acid solution with 25 pmol concentration for each amino acid. A similar derivatisation was conducted for the mixed amino acid solutions.

High-performance liquid chromatography (HPLC) analysis was performed for approximately 5 to 10 mL of the prepared sample equipped with 470 scanning fluorescence detector, 486 variable-wavelength detector and 717 plus autosampler with heater or chiller accessory. The column used for the LC analysis was 150 X 4.6 mm AccQ - Tag C_{18} reversed-phase column with a column temperature of 37°C, flow rate of 1 mL/min, and three eluents (A= AccQ. Taq Eluent A, B = acetonitrile and C = water)

b) Alkaline hydrolysis

Tryptophan analysis was conducted using the hydrolysis method with alkali, comprising either 4.3 N lithium hydroxide or NaOH. The analysis was conducted using HPLC coupled with a florescence detector. The determination of the protein (tryptophan) present in the test sample was carried out using the method developed in previous studies (Nielsen and Hurrell, 1985; Slump *et al.*, 1991).

The test sample was prepared by weighing 0.2 g of the sample and placing it into a screw capped tube. The 4.3 N lithium hydroxide ($LiOH.H_2O$) was prepared by weighing 36.0856 g $LiOH.H_2O$ and placing into a 250 mL granule bottle. Then, 200 mL of water was added into the bottle. The prepared 15 mL of 4.3 N $LiOH.H_2O$ was also added into the sample tube, which was then flushed with nitrogen or vacuum sealed. The tube was heated for 16 hr at 120°C using an oven. Later, the hydrolysed sample was transferred into a beaker before adding water and 9 mL of 6 N HCl. The total volume in the beaker was controlled not to exceed 100 mL at a pH of 4.5. The latter was achieved by diluting the sample with HCl. The prepared hydrolysed sample was diluted to 100 mL using water in a volumetric flask. Subsequently, the diluted sample was filtered

using a filter paper and the aliquot was filtered with 0.2 mm cellulose acetate membrane. Around 10 mL of the aliquot was injected for LC analysis.

The tryptophan standard was prepared by weighing 0.05 g of tryptophan and placing it into a 50 mL volumetric flask. Then, 0.1 N HCl was added followed by ultra-sonic treatment to dissolve the solute. Then, a 50 mL of water was added to dilute the concentration of tryptophan into 1000 mg/mL, followed by placing 10 mL of the solution into a vial of 10 mL with a mobile phase (0.0085 M Sodium acetate at pH 4.0 and Methanol at the ratio of 86.7:13.3) of 5 mg/mL concentration. Next, 10 mL tryptophan standard was injected into the HPLC system. The sample was analysed using HPLC coupled with a fluorescence detector (Ex λ at 285 nm, Em λ at 345 nm, gain at 10 and filter at 1.5 s). The column used was Phenomenex C18, 250 x 4.6 mm, 5 µm, mobile phase was 0.0085 M sodium acetate at pH 4.0: methanol (86.7:13.3), at a flow rate of 1 to 1.5 mL/min and ambient column temperature.

Fatty acid profile. Fatty acid profile was analysed to determine the faction of fatty acid present in the test sample in order to understand the functional compound and determine its role in the insect rearing. The fatty acid profile was conducted using the method developed by AOAC (2012), specifically the hydrolytic gas chromatography method (AOAC method, 996.06) for fat (namely total, saturated and unsaturated fat).

First, the sample was weighed and prepared, well-mixed and pulverised into smaller particles (the sample should have approximately 100 to 200 mg of fat). The sample was then forced into a Mojonnier flask as deep as possible, followed by adding 100 mg pyrogallic acid, 2 mL triglyceride internal standard solution and several boiling granules. Then, 2 mL of ethanol was added and mixed thoroughly until the sample turned into a solution. Next, 10 mL of 8.3 M HCl was added and mixed thoroughly. The flask was then placed into a basket at a slow agitation speed in a water bath at a temperature ranging from 70°C to 80°C. This process was executed for 40 min. The flask was vortex every 10 min to mix the contents that stuck to the sides of the flask. When the digestion was completed, the flask was removed from the water bath and cooled to room temperature (20°C to 25°C). Ethanol was added into the flask to fill the bottom reservoir and mixed thoroughly.

The extraction of fat was performed by adding 25 mL of diethyl ether into the flask before placing a flask stopper. The flask was then placed into a centrifuge basket and placed onto a wrist action shaker. Rubber tubing was used to secure the flask in the shaker and shook for 5 min. The residue on the stopper was rinsed with diethyl ether-petroleum ether mixture (ether mixture at 1:1 v/v ratio) into the flask. The flask was then added with 25 mL petroleum ether and the stopper was placed and shook again for 5 min. Later, the flask was centrifuged at 600 rpm for 5 min. The stopper was rinsed again with the ether mixture and the top layer (containing ether) was transferred into a 150 mL beaker. The tip of the flask was cautiously rinsed with ether mixture into the beaker. Evaporation of ether was conducted slowly on a steam bath using nitrogen gas. The residue inside the beaker was the extracted fat.

The procedure was continued with methylation to convert the fat into fatty acid for gas chromatography determination. The extracted fat obtained was dissolved in 2 to 3 mL of chloroform and 2 to 3 mL of diethyl ether. The mixture was separated into three-dram glass vial and sent for evaporation under nitrogen gas at 40°C in a water bath until dried. Then, 1 mL toluene and 2 mL 7% boron trifluoride reagent in methanol (BF_3) were added and the glass vial was sealed using a silicon septum on the screwcap. Thereafter, the vial was sent for heating in an oven at 100°C for 45 min. The vial was shaken carefully every 10 min. Later, the vial was cooled to room temperature (20°C to 25°C). Following that, 1 mL of hexane, approximately 1 g sodium anhydrous Na_2SO_4 and 5 mL H_2O were added into the solution. The vial was sealed and shaken for 1 min. Finally, the separation was allowed to occur and the top layer containing the fatty acid methyl ester ($FAME_s$) was transferred into another vial containing approximately 1 g of Na_2SO_4. The FAME was subjected to gas chromatography (GC) analysis.

The individual FAME standard solution was utilised to determine the relative retention time and the response of individual FAME, whereas the mixed FAME standard solution was employed in optimising the chromatographic response before injecting the test sample. Approximately 2 mL of each individual FAME standard and mixed FAME standard was used for chromatography analysis. The FAME fraction analysis was conducted using the GC coupled with hydrogen flame ionization detector (FID), split mode injector and capillary column of SP2560 100 m x 0.25 mm with 0.20 mm film. The temperature conditions for operation were at 225°C for injector and 285°C for detector. The initial temperature was at 100°C (4 min holding temperature), ramped at 3°C/min and final temperature at 240°C (15 min hold). The flow rate was at 0.75 mL/min with a carrier gas using helium, split ratio of 200:1 and 18 cm/sec of linear velocity.

Carbohydrate profile. Carbohydrate profile determination was utilised to elucidate the faction of the carbohydrate present in the tested sample. The carbohydrate profile comprising glucose, fructose, sucrose and maltose was determined using

the method developed by AOAC (2012). The liquid chromatography method of AOAC (982.14) was employed to determine the carbohydrate profile.

The sample was first submitted for fat extraction. Around 2 to 10 g of the test sample was finely ground and inserted into a 100 mL centrifuged bottle. Then, 50 mL of petroleum ether was added and centrifuged at 2000 rpm for 10 min. After that, the petroleum ether was removed by drawing the fluid without removing the residue material. The extraction was repeated and the residue of the petroleum ether was evaporated using N_2 stream gas. The solid material was then crushed using glass rod.

The process was continued with sugar extraction, which was followed by adding alcohol and water at a 1:1 ratio for approximately 100 mL mixture. Then, the whole mixture was weighed and transferred to a water bath at 80°C to 85°C for 25 min. The mixture was occasionally stirred. Thereafter, the sample was cooled to room temperature before adding alcohol until the original recorded weight was attained. Next, the extract was filtered using a nylon syringe filter at 0.45 mm. For the extracts that were cloudy, they were centrifuged at more than 2000 rpm for 10 min, and the process was repeated if the extract remained unclear. Finally, the extract was subjected to liquid chromatography analysis.

The standard sugar solution of individual sugars, namely, fructose, maltose, sucrose and glucose were prepared by drying at 60°C for 12 hr under vacuum. Then, the solution was dissolved in alcohol and water solution at a 1:1 ratio to obtain fructose, glucose and maltose at a concentration of 3 mg/mL, whereas that of sucrose was at 15 mg/mL concentration. The determination of the carbohydrate profile was performed using HPLC, which was detected using an evaporative light scattering detector (ELSD). Approximately 10 to 50 mL of the test sample was injected into the column (250 mm x 4.6 mm, Miscrosorb 5 mm amino column) with a flow rate of the mobile phase (CH_3CN -LC grade and purified H_2O at ratio 80:20) at 1.5 to 2.5 mL/min. A similar volume of the sugar standard was injected. The determination of the test sample was based on the comparisons of the peak response of the sugar standard.

Statistical Analysis

The AMI of oil palm was submitted for nutritional analysis. The analysis was conducted using six different samples of AMI to represent six replications (N=6) of the nutritional profile, and which were then used to obtain the average result of the nutritional profile. The descriptive results of the samples were obtained using SAS 9.4 version software and Microsoft Excel 2018. One sample t-test was performed using AMI sample data against standard data reference from chemical composition of date palm *Phoenix dactylifera* L. pollen study reported by Hassan (2011) to determine the significant different between both data. Since none of the standard was reported for male inflorescence of oil palm, date palm was selected as the reference for this study with the same family as oil palm.

RESULTS

Proximate Analysis of the Anthesising Male Inflorescence (AMI) of Oil Palm

The average proximate analysis that recorded the highest content within the AMI of oil palm was the moisture content *(Table 1)*. The value of the moisture content in the spikelet was 73.5 ± 0.643 g/100 g. This significant finding revealed that the moisture in the spikelet was approaching 75% of the total content inside the anthesising spikelet. Whereas for nutrient content, the highest value was recorded by carbohydrates at 19.1 ± 1.191 g/100 g, followed by protein, ash and fat. The spikelet was also noted to supply energy value at 105.5 ± 3.667 kcal/100 g. The AMI proximate analysis was significantly different from the standard reference data *P. dactylifera* pollen grain (Hassan, 2011) for all the five parameters as reported in *Table 1*.

TABLE 1. THE AVERAGE VALUE OF PROXIMATE ANALYSIS FOR ANTHESISING MALE INFLORESCENCE OF OIL PALM AND COMPARISON TO *Phoenix dactylifera* POLLEN GRAIN (Hassan, 2011)

Proximate analysis	N	T-value	P-value	Mean	±	Standard Error	*Phoenix dactylifera* pollen grain*
Protein, g/100 g	6	-180.08	<.0001	3.7	±	0.152	31.11
Total fat, g/100 g	6	-23.50	<.0001	1.6	±	0.815	20.74
Total carbohydrate, g/100 g	6	4.78	0.0050	19.1	±	1.191	13.41
Ash, g/100g	6	-21.31	<.0001	2.1	±	0.117	4.57
Moisture, g/100 g	6	69.53	<.0001	73.5	±	0.643	28.80
Energy, kcal/100 g	6	-	-	105.5	±	3.667	-
Energy, kJ	6	-	-	443.0	±	15.343	-

Source: *Hassan (2011).

Nutritional Composition of the Anthesising Male Inflorescence of Oil Palm

Amino acid profile. The AMI of oil palm was recorded with an accumulation of 18 amino acids. The individual AMIs ranged from 16 to 18 of amino acid fractions. Based on the average value of the amino acid (*Table 2)*, the highest amino acid recorded in the AMI was lysine at 0.407 g/100 g followed by glutamic acid and aspartic acid. Whereas that of arginine, serine, alanine, leucine, proline, threonine, glycine, hydroxyproline, valine, phenylalanine, tyrosine, isoleucine and histidine ranged from 0.1 to 0.2 g/100 g. The lowest amino acid for the AMI was recorded for tryptophan and methionine. Out of the six replications of the AMI sampled, hydroxyproline was recorded in three out of six replications and histidine in four of the six replications. The remaining amino acids were recorded in all samples. The difference in the concentration between the amino acids elucidates their possible function towards the growth of *E. kamerunicus.* The one sample t-test indicated that the AMI amino acid analysis was significantly different compared to the standard reference data *P. dactylifera* pollen grain (Hassan, 2011) for all the 18 amino acids as shown in *Table 2*.

Fatty acid profile. The value of the fatty acid was lower *(Table 3)* compared to that of amino acid. Despite the total fatty acid recorded was at 32, the individual AMI did not accumulate for all the fatty acids, thus reflecting a variational difference. The overall total fat revealed that the saturated, monounsaturated and polyunsaturated fat was 304.13 mg/100 g, 703.08 mg/100 g and 572.79 mg/100 g, respectively. The highest recorded fatty acid in the AMI was oleic at 673.230 mg/100 g, followed by linoleic (cis) and palmitic. Other fatty acids recorded less than 20.000 mg/100 g and the lowest fatty acid was tridecanoic at 0.035 mg/100 g. The high fatty acid was present in all the tested AMI, whereas the lowest fatty acid was only present in two of the six samples tested. This finding reflects the importance of the analysed fatty acids and their potential interaction in the development of *E. kamerunicus*. The AMI fatty acid profile was significantly different from the standard reference data *P. dactylifera* pollen grain (Hassan, 2011) in eight of the 13 fatty acids reported except for Capric, Lauric, Stearic, Linoleic (Cis) and a-Linolenic as shown in *Table 3*.

Carbohydrate profile. The result of the carbohydrate analysis for the oil palm AMI are presented in *Table 4*. Accordingly, a lower value or trace amount was obtained for fructose, glucose, sucrose and maltose based on the individual analysis. The highest value recorded was fructose with less than 0.02 g/100 g but it was not documented for the replication analysis. The earlier proximate analysis showed that approximately 20% of the AMI was carbohydrate.

TABLE 2. THE AVERAGE VALUE OF INDIVIDUAL AMINO ACID IN THE ANTHESISING MALE INFLORESCENCE OF OIL PALM AND COMPARISON TO *Phoenix dactylifera* POLLEN GRAIN

Amino acid	N	T-value	P-value	Mean (g/100g)	±	eStandard Error	*Phoenix dactylifera* pollen grain (g/100 g)*
Lysine	6	-59.02	<.0001	0.407	±	0.043	2.95
Glutamic Acid	6	-15.20	<.0001	0.319	±	0.094	1.74
Aspartic Acid	6	-44.77	<.0001	0.245	±	0.074	3.55
Arginine	6	-22.53	<.0001	0.196	±	0.063	1.61
Serine	6	-42.57	<.0001	0.193	±	0.040	1.89
Alanine	6	-58.53	<.0001	0.182	±	0.041	2.61
Leucine	6	-77.43	<.0001	0.161	±	0.041	3.34
Proline	6	-3.00	0.0300	0.158	±	0.041	0.28
Threonine	6	-40.77	<.0001	0.150	±	0.039	1.72
Glycine	6	-45.15	<.0001	0.139	±	0.047	2.24
Hydroxyproline	6	-	-	0.124	±	0.056	-
Valine	6	-59.70	<.0001	0.117	±	0.028	1.81
Phenylalanine	6	-63.98	<.0001	0.110	±	0.024	1.63
Tyrosine	6	-83.03	<.0001	0.092	±	0.018	1.55
Isoleucine	6	-51.52	<.0001	0.089	±	0.027	1.49
Histidine	6	-40.03	<.0001	0.085	±	0.038	1.61
Tryptophan	6	-	-	0.035	±	0.001	-
Methionine	6	-8.75	0.0003	0.035	±	0.009	0.11

Source: *Hassan (2011).

TABLE 3. THE AVERAGE VALUE FAME INSIDE THE ANTHESISING MALE INFLORESCENCE OF OIL PALM AND COMPARISON TO *Phoenix dactylifera* POLLEN GRAIN

Structure		Fatty Acid Methyl Ester (FAME)	N	T-value	P-value	Mean (mg/100g)	±	Standard Error	Mean (%)	*Phoenix dactylifera* pollen grain (%)*
Saturated Fat	C 6	Caproic	6	-	-	0.21	±	0.131	0.08	-
	C 8	Caprylic	6	-	-	1.26	±	0.285	0.35	-
	C 10	Capric	6	-0.97	0.3788	0.75	±	0.357	0.30	0.46
	C 11	Undecanoic	6	-	-	0.10	±	0.082	0.01	-
	C 12	Lauric	6	-1.15	0.3008	8.39	±	3.251	3.01	4.82
	C 13	Tridecanoic	6	-	-	0.04	±	0.035	0.01	-
	C 14	Myristic	6	-11.25	<.0001	7.76	±	1.849	2.44	13.33
	C 15	Pentadecanoic	6	-	-	0.30	±	0.148	0.09	-
	C 16	Palmitic	6	-2.71	0.0421	242.68	±	100.189	24.22	34.45
	C 17	Heptadecanoic	6	-	-	1.04	±	0.346	0.12	-
	C 18	Stearic	6	1.68	0.1539	20.98	±	8.632	4.08	2.04
	C 20	Arachidic	6	-24.36	<.0001	7.38	±	3.054	0.62	7.32
	C 21	Henicosanoic	6	-	-	1.67	±	0.798	0.52	-
	C 22	Behenic	6	-	-	5.57	±	1.993	1.04	-
	C 23	Tricosanoic	6	-	-	1.57	±	0.753	0.24	-
	C 24	Lignoceric	6	-	-	4.45	±	1.493	0.69	-
	C 14:1	Myristoleic	6	-	-	0.84	±	0.277	0.17	-
	C 15:1	Cis-10-Pentadecenoic	6	-	-	0.11	±	0.11	0.05	-
	C 16:1	Palmitoleic	6	-47.2	<.0001	11.63	±	5.054	1.06	7.07
Monounsaturated Fat	C 17:1	Cis-10-Heptadecanoic	6	-	-	0.25	±	0.19	0.02	-
	C 18:1n9t	Elaidic (Trans)	6	-	-	9.03	±	9.027	0.21	-
	C 18:1n9c	Oleic	6	6.87	0.001	673.23	±	374.443	33.82	7.19
	C 20:1n9	Cis-11-Eicosenoic	6	-	-	3.22	±	1.504	0.22	-
	C 22:1n9	Erucic	6	-	-	1.36	±	0.672	0.09	-
	C 24:1	Nervonic	6	-	-	3.42	±	1.288	0.34	-
Polyunsaturate Fat	C 18:2n6c	Linoleic (Cis)	6	1.99	0.1032	551.58	±	318.336	23.62	14.24
	C 18:3n6	y-Linolenic	6	-22.81	<.0001	0.22	±	0.215	0.05	1.27
	C 18:3n3	a-Linolenic	6	2.4	0.0614	18.17	±	6.358	2.27	0.79
	C 20:2	Cis-11,14--Eicosadienoic	6	-	-	0.40	±	0.397	0.01	-
	C 20:4n6	Arachidonic	6	-32.44	<.0001	0.55	±	0.545	0.14	4.57
	C 20:5n3	Cis-5,8,11,14,17-eicosapentaenoic	6	-5.79	0.0022	1.61	±	1.306	0.10	0.52
	C 22:2	Cis-13,16--Docosadienoic	6	-	-	0.26	±	0.263	0.01	-

Source: *Hassan (2011).

This finding contradicts the carbohydrate profile analysis, thereby indicating that the carbohydrate is in a complex form inside the spikelet and was not altered into short-chain as observed in the trace amount after analysis. In order to understand the carbohydrate function as a nutrient support for *E. kamerunicus,* it is important to perform a direct analysis of the insect pollinator for the type and rate of consumption for carbohydrate. This will enable correct prediction of the carbohydrate consumed for the weevil development.

TABLE 4. CARBOHYDRATE ANALYSIS IN THE ANTHESISING MALE INFLORESCENCES OF OIL PALM

Parameter, Unit	Weight (g/100 g)		
	1	2	3
Fructose	<0.001	0.007	0.019
Glucose	<0.001	0.003	0.020
Sucrose	<0.001	0.003	0.090
Maltose	<0.001	<0.001	<0.001

DISCUSSION

Protein is reported as a source of amino acids for insects through food ingestion (Nation, 2016). The amino acid requirement for insects are the 10 essential amino acids, which are similar to those of vertebrates (Cohen, 2015; Nation, 2016). The essential amino acids are isoleucine, leucine, lysine, methionine, threonine, tryptophan, valine, arginine, histidine and phenylalanine. In the present experiment, 10 essential amino acids and another eight non-essential amino acids were detected in the AMI of oil palm at varied concentrations. The protein is broken down and circulated to the insect cell where it would be used in insect body development (Cohen, 2015) and for juvenile hormone secretion (Nation, 2016). It is also reported by Cohen (2015) that protein supplies nitrogen for the insect. The deprivation of protein resulted in the disruption of insect growth development. This protein deprivation was documented by Nation (2016), which was unable to produce the juvenile hormone that is essential for female reproductive system (egg and ovary development).

Genc (2006) reported incomplete development for *Helicoverpa zea, Myzuz persicae, Pectinophora gossypiella, Apis mellifera* and *Tribolium confusum* when they lacked those essential amino acids. Diets lacking all essential amino acids or individual essential amino acids resulted in *Ceratitis capitate* larvae mortality (Chang, 2004), whereas the lack of non-essential amino acids resulted in larvae development at a slower pace, reduced larvae weight or reduced adult emergence. Lee (2007) documented that *Spodoptera littoralis* fed with diets containing low-quality protein caused a reduction in body weight and slow development of the caterpillar. *Drosophila melanogaster* growth development was reported to be affected by protein concentration. Lee (2015) documented that when *D. melanogaster* was fed with a protein-deficient diet, the lowest fecundity and shorter longevity was observed. In another study, insect sworn trade-off in development where *Nilaparvata lugens* that were reared on amino acid deficient medium had deformities in the wing, longer periods of larvae, longer pre-oviposition period, reduced egg production and short life span for female BPH compared to the control medium (Pan *et al*., 2014).

In this experiment, the highest amino acid in the AMI of oil palm was lysine at 14%. Lysine is considered an important amino acid throughout the development of insects as feed for human and reared animal. The function of lysine is for the synthesis of protein and it is currently reported that all cereal proteins have a low concentration of lysine (Tomé and Bos, 2007). Nevertheless, insects were documented as a good source of protein such as lysine. Insect meal consisting of *Spodoptera littoralis* was reported with a higher concentration of lysine as compared to meat and bone meal (Hatab *et al.,* 2020). Oibiokpa *et al*. (2018) reported on the comparison of various insect amino acid concentrations. It was observed that grasshoppers had the highest lysine concentration when compared to cricket, termite and moth. This indicated that insects could also have variations between amino acid concentrations.

Lipid is also the macronutrient available in natural food sources of herbivorous insects. Cohen (2015) summarised the function of lipids in the development of cell membranes, particularly for the transportation of nutrients through the cells, developing ecdysteriod hormone and juvenile hormone, energy sources and material for other structure development. Insect requires specific lipid in the feed including sterol and several species required polyunsaturated fatty acid (Chapman, 2013; Nation, 2016). Polyunsaturated fatty acid is another component in the lipid that contains essential compounds for the growth and development of several species. The effect of polyunsaturated fatty acids in coleopterans caused decreased development and reduced fecundity in adult beetles (Chapman, 2013; Nation, 2016). This effect of a diet deficient in polyunsaturated fatty acid resulted in the unsuccessful pupation or moulting of adult lepidopterans (Chapman, 2013; Nation, 2016) or successfully moulted adult lepidopterans with deformed wings and descaling of the body (Nation, 2016). Similar effects were observed in orthoptera (Chapman, 2013). Nation (2016) documented that diets deficient in fatty acids resulted in deformed adult short-horned grasshoppers. Polyunsaturated

fatty acids, linoleic and linolenic, also contributed to the development of insects. Wang *et al.* (2006) revealed that *Morpho peleides* larvae and adult butterflies solely fed on *Pterocarpus* leaves had high value of polyunsaturated fatty acids of linolenic and linoleic. Correspondingly, the leaves also contained high concentration of linolenic and linoleic acids. The high concentration of polyunsaturated fatty acid in the leaves or diet fed on by the butterfly contributes significantly to the insect's fatty acid production. The deficiency or low concentration of linolenic and linoleic caused unsuccessful moulting in some hymenopterans (Nation, 2016). The absolute requirement of the lipid is uncertain but the addition of the polyunsaturated fatty acids in diets boosts growth of some insects (Nation, 2016). High-fat diets induced higher mortality, reduced body mass and slower growth rate for the *Manduca sexta* caterpillar larvae, which was attributed to lesser consumption of feed for the larvae (Cambron *et al*., 2019).

The AMI of oil palm was analysed with the highest concentration of oleic acid. The diet could play a significant role in the fatty acid production of insects. Paul *et al.* (2017) analysed *Tenebrio molitor* larvae, *Acheta domesticus*, *Chorthippus parallelus* and *Conocephalus discolour* and found that the dominant fatty acid in the insect was oleic. The fatty acid profile in insects could be influenced by the diet, species, environment and insect life stage. The synthesis of various fatty acids for different life stage is as per the body's fatty acid utilisation (Paul *et al.*, 2017). Another report also documented the major fatty acid in superworms (late instar larva of the beetle *Zophobas mori*), waxworms (late instar larva of the moth *Galleria mellonela*) meal-worms (late instar larva of the beetle *Tenebrio molitor*) and Cricket (late instar *Acheta domestica* nymphs) was oleic acids (Finke, 2015). The comparison of both studies reported the highest oleic acid was in mealworm (Paul *et al*., 2017) and waxworms (Finke, 2015). Thus, indicating the diet fed to the reared insect could also influence the fatty acid production in the species.

The macronutrient of carbohydrates is essential as a sources of energy in insects (Cohen, 2015; Nation, 2016), building material (Cohen, 2015) and breathing energy for converting into lipids and synthesis of amino acids (Chapman, 2013). They are also vital for the insect cuticle through polysaccharide chitin (Chapman, 2013; Cohen, 2015). Nation (2016) also summarised that lipids and amino acids could be utilised as sources for carbohydrates. In addition, Chapman (2013) stated that some insects such as larvae of screw worm fly and wax moth could be reared on a carbohydrate deficient diet. Nevertheless, the carbohydrate was reported as the key for reaching maturity for *Tenebrio* sp., *Ephestia* sp. and *Oryzaephilus* sp. (Nation, 2016). In the study conducted by Filho *et al.* (2018), the biological development of sugarcane borer, *Diatraea saccharalis* was influenced by the concentration of sugar in the reared diet. Meanwhile, Filho *et al.* (2018) observed that sugar concentration between 26.26 and 52.52 g per litre of diet influenced the survivability of *D. saccharalis* development from egg to adult with viability of more than 75% for eight generations tested. The moderate sugar concentration also influenced shorter generation times and greater reproduction rates of sugarcane borers as compared to the other tested sugar concentration. Nevertheless, the sugar concentration does not influence the fecundity of *D. saccharalis* (Filho *et al.*, 2018). Higher sugar concentration facilitates higher production of ovaries in *A. mellifera*. Likewise, a high mixture of sugar between glucose and fructose impacts positively on ovaries production in vitro (Kaftanoglu *et al.*, 2010).

Both male and female inflorescences of oil palm were validated for the production of volatile compound which acts as the sources of attraction for *E. kamerunicus*. The volatile compound was first identified on the pollen of oil palm (Opute, 1975) and validated later on both inflorescences as Estragole (1-methoxy-4-(2-propenyl) benzene) (Lajis *et al*., 1985). The report by Muhamad Fahmi *et al.* (2016) also documented estragole as the major volatile compound in the inflorescence of oil palm despite different cultivated areas namely sandy soil, clay soil and peat soil. The compound character includes transparent liquid and anise-like scent (Budavari, 1989), which was documented in spices for instance fennel, anise, basil *etc*. (Bristol, 2011). It was used in the perfume industry as a flavouring for its anise's sweet taste (Arctander, 1960). In the study conducted, estragole was documented as the source of attraction on *E. kamerunicus* in all the tests performed (Hussein *et al.*, 1991; Hussein and Lajis, 1992; Lajis *et al.*, 1992). Another report in Indonesia documented that the highest volatile compound in male inflorescence was estragole, whereas that in female inflorescence was farnesol and the concentration accompanied the flowering stages of the inflorescence (Anggraeni *et al.*, 2013). Currently, estragole is the only documented compound for the inflorescence of oil palm and with no significant function towards the nutrition of weevils. Thus, this research is the first report on nutritional value for male inflorescence and may be applied in the rearing of *E. kamerunicus*.

CONCLUSION

The study provided the early nutritional value standard of anthesising male inflorescences of oil palm that served as the feeding and breeding

grounds for the development of *E. kamerunicus*. The standard can be applied for studies on developing artificial diet for weevils in closed facility as the present results can be used to compare the nutritional value required to sustain the weevil population. Furthermore, the nutritional value can be applied for research area such low fruit set to elucidate whether the nutritional value of AMI affect the weevil population and its effectiveness. Moreover, this reference can be explored to compare between palms cultivated in different soil types. Further studies are needed to determine the carbohydrate and mineral value of the AMI.

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