

## Fatty Acid, Amino Acid Composition and Effects of Soaking Temperatures on Physicochemical Qualities of *Hibiscus cannabinus* Seed Extract

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### ABSTRACT

Kenaf is a multipurpose herbaceous plant of economic significance. This study analysed the effects of different soaking temperatures of kenaf seed on the physicochemical qualities of the extracts. Kenaf seed extracts were extracted separately from kenaf seed soaked at temperatures of 25, 35, 45, 55 and 65 °C at their respective optimum soaking time of 10.27, 7.00, 5.00, 3.50 and 2.67 h. The extraction yield, proximate composition, anti-nutritional factors, colour profile and thermal stability of the extracts obtained at the different soaking temperatures and time were analysed and compared. Also, the fatty acids and amino acids compositions of kenaf seed meal and the extract were determined and compared. The extraction yield of the extracts significantly increased as the soaking temperatures of the seed increase from 25-65 °C. The different soaking temperatures had no significant effects on the proximate composition of the extracts except for the ash content which decreased as the soaking temperature of the seed increases. However, the soaking temperatures significantly affected the colour profile of the extracts. Soaking of kenaf seed significantly reduced the trypsin inhibitor activity, tannic and phytic acid of the extracts. This study also found that both the kenaf seed oil and kenaf seed extract oil were rich source of hexadecenoic acid (26.71 and 26.11 %), 9-octadecenoic acid (33.80 and 40.41 %) and 9,12-octadecadienic acid (30.59 and 28.62 %), respectively. Additionally, the total essential amino acids of the kenaf seed (462.80 mg/g) and freeze-dried kenaf seed extract (487.44 mg/g) were not significantly different. Soaking of kenaf seed at 25, 45 and 65 °C and their respective optimum soaking time of 10.27, 5.00 and 2.67 h are recommended for the extraction of kenaf seed extract.

**Keywords:** Anti-nutritional factors, Fatty acids, Kenaf seed, Soaking temperatures.

### INTRODUCTION

Kenaf (*Hibiscus cannabinus*) seed is a seasonal herbaceous plant that belongs to the Malvaceae family. Kenaf seed is more common as a raw material for kenaf stem fiber but, its use in food production is less common. The inspired interest of food researchers into the food uses of kenaf seed might have been due to its health-promoting potential. Researchers have revealed that kenaf seed components such as the oil and defatted seed meal have high antioxidant activities (Razmkhah *et al.*, 2013) and anticancer activities (Ghafar *et al.*, 2012; Yazan *et al.*, 2016).

Kenaf seed extract is a plant-based water extract of kenaf seed. It is a stable emulsion of protein, oil, water and other minor components. Kenaf seed extract may be accepted as an alternative plant beverage for human consumption (Karim *et al.*, 2020). Kenaf seed is a good source of protein (Kim *et al.*, 2018; Olawepo *et al.*, 2014), unsaturated fatty acids (Alexopoulou *et al.*, 2013) and phytochemical compounds (Ryu *et al.*, 2017). Extracting the component of the kenaf seed for use in food processing and preparation could serve as an innovative way of finding suitable uses to the seed as a source of nutrition. The common traditional method for extracting plant seed extract involves pre-soaking of the seed before cold milling to disrupt the cotyledon which allows the protein and other components to be extracted, then the slurry is hand-pressed to obtain the extract (Toda *et al.*, 2007). However, the effects of soaking the kenaf seed at different temperatures vis a vis the optimum soaking time may influence the physicochemical properties of the kenaf seed extract. This study therefore, aimed at

studying the effects of different soaking temperatures at given optimum soaking times, on the chemical composition, colour profile and thermal stability of kenaf seed extracts. Also, to compare the fatty acids and amino acids profiles of kenaf seed and kenaf seed extract.

### MATERIALS AND METHODS

#### Chemicals and Reagents

Concentrated sulphuric acid (95-98 % purity), boric acid (99.9 % purity), petroleum ether, acetic acid, tannic acid (> 65 % purity), iron (III) chloride (99 % purity), glycine (99 % purity), ethylene diamine tetra acetic acid disodium salt-dihydrate (EDTA; 99 % purity), isoamyl alcohol (≥ 98 % purity), hexane (95 % purity), sodium chloride (99.9 % purity) were purchased from Chemiz, Shah Alam, Malaysia. Kjeldahl catalyst, sodium hydroxide (98 % purity), N $\alpha$ -benzoyl-DL-arginine-p-nitroanilide hydrochloride (BAPNA) were procured from Fisher Scientific, Leicestershire, UK. Standard trypsin, 5-sulfosalicylic acid (99 % purity) and  $\alpha$ -aminobutyric acid (AABA) were purchased from Solarbio Life Science, Gaithersburg, USA. Tris (hydroxymethyl) Amino methane (99 % purity), concentrated hydrochloric acid (37 % purity) and methanol (99.8 % purity) were procured from R&M Chemicals, Essex, UK. Calcium chloride (96 % purity), vanillic aldehyde (99 % purity), sodium sulphate (99 % purity), sulphuric acid (90-91 % purity), boron trifluoride in methanol (14 %, w/v), anhydrous sodium sulphate (99 % purity) and acetonitrile (HPLC grade) were purchased from Sigma-Aldrich, St. Louis, USA. Amino acid hydrolysate standard, AccQ Flour Reagent diluent (2B), AccQ Flour Reagent Powder (2A), AccQ Flour Borate Buffer, AccQ-Tag Eluent A (acetate-

phosphate buffer) were procured from Waters, Milford, USA.

### Plant Material

Kenaf seed cultivar KB6 cultivated in Malaysia, Kelantan State, was a gift from the National Tobacco and Kenaf Board, Perlis, Malaysia. The seeds were manually cleaned and healthy unbroken seeds were used throughout the research.

### Soaking of Kenaf Seed

Exactly 100 g of healthy unbroken kenaf seeds were soaked in 500 mL of distilled water. The soaking was conducted at 25, 35, 45, 55 and 65 °C using a water bath (Memmert D-91126, Schwabach FRG, Germany) for a duration of 10.27, 7.00, 5.00, 3.50 and 2.67 h, respectively.

### Extraction of Kenaf Seed Extract

The soaked kenaf seeds as described earlier were ground with 300 mL of distilled water using a Waring blender (Model 8011EG, China) at low speed for a duration of 2 min. The slurry obtained was manually hand pressed using double-layer muslin cloth. The extracts were then divided into two portions: one portion was kept at -20 °C for 24 h then freeze-dried to obtain powder extract while the other portion was used for physicochemical analyses.

### Determination of Extraction Yield, Total Soluble Solids and Colour Properties of Kenaf Seed Extract

The extraction yield of the extracts were calculated using equation 1. The total soluble solids were analyzed using a Refractometer. Briefly, 0.5 mL of the extract was loaded onto the Refractometer and the percentage soluble solids was read from the digital display. The colour properties of the extracts were determined following the method of Obatolu (2008). Exactly 50 mL of the extract was

measured into a container of 5 cm<sup>2</sup> surface area. The surface colour such as lightness (L), redness (a\*) and yellowness (b\*) of the extracts were determined using Hunter-lab chromometer (Konica Minolta, Japan). The chromometer was calibrated using white ceramic tiles prior to sample analyses.

$$\text{Extraction yield} = \frac{\text{Volume of extract}}{\text{Volume of slurry}} \times 100 \quad (1)$$

### Determination of Proximate Composition

The proximate analyses of kenaf seed extracts were determined on wet basis by the AOAC (2005) method, except for the crude lipid which was analyzed using the Gerber method described by Kleyn *et al.* (2001) with minor modifications. Ten (10) milliliters of conc. H<sub>2</sub>SO<sub>4</sub> was measured into butyrometer and 10.75 mL of the extract was added gradually to the side of the butyrometer. Then, 1 mL of isoamyl alcohol was added. The content in the butyrometer was vortex for 30 s and centrifuged at 1100 rpm for 4 min in a Gerber centrifuge. After the centrifugation, the butyrometer was kept in a hot water bath at 65 °C for 10 min and the reading was taken from the bottom of the lipid column to the lower border meniscus on the 10 % butyrometer scale.

### Determination of Anti-Nutrient Contents

The freeze-dried kenaf seed extracts was used for the analyses of trypsin inhibitor activity, tannic acid and phytic acid.

- a) The trypsin inhibitor activity was analyzed following the method of Kakade *et al.* (1974). Briefly, 1.00 g of the freeze-dried extract was mixed with 10 mL of 0.01 M NaOH and the pH was adjusted to 8.4 with 1 M HCl. The mixture was constantly stirred on an orbital shaker for 3 h. The extract was diluted to 50 mL with distilled water. Standard trypsin solution was prepared by dissolving 4 mg trypsin in 200 mL of 0.001 M HCl. Four test tubes were used for the analysis and the content of each tube is presented in Table 1.

Table 1: Sample preparation for determination of trypsin inhibitor activity

	Reagent Blank	Sample Blank	Standard	Sample
Water (mL)	2	1	2	1
Standard Trypsin Solution (mL)	-	-	2	2
Sample Extract (mL)	-	1	-	1

The content in all tubes were mixed and all tubes were incubated at 37 °C for 10 min. Then, 5 mL of prewarmed Na-benzoyl-DL-arginine-p-nitroanilide hydrochloride (BAPNA) solution (40 mg BAPNA in 100 mL of 0.05 M Tris buffer containing 50 mM CaCl<sub>2</sub>, pH 8.2) was added, and the mixture was vortexed for 10 s. The test tubes were then incubated in the water bath at 37 °C for another 10 min. The reaction was stopped by addition of 1 mL acetic acid solution (30 mL of glacial acetic acid in 70 mL water). The mixture in each tube was filtered using a 0.22 µm membrane filter. The absorbance of the filtrate was measured at 410 nm using a spectrophotometer

(model 4001-04 Thermo spectronic Genesys 20, USA) against the blank. The change in absorbance (AI) was determined using equation 2.

$$AI = (Ab - Aa) - (Ad - Ac) \quad (2)$$

where, AI = change in the absorbance of trypsin inhibition, Aa = trypsin inhibition activity in reagent blank tube, Ab = trypsin inhibition activity in standard tube, Ac = trypsin inhibition activity in sample tube, Ad = trypsin inhibition activity in sample blank tube.

The percentage inhibition (%) for each sample tube was calculated using equation 3.

$$\% I = 100 / (Ab - Aa) \quad (3)$$

The trypsin inhibitor activity for each sample (TIA) was calculated in terms of mg trypsin g<sup>-1</sup> sample following the expression in equation 4:

$$\text{TIA (mg/g protein)} = 2.632 * D * \text{AI/g sample} \quad (4)$$

where, D = dilution factor.

- b) Tannic acid was determined following the method of Price *et al.* (1978). The sample (0.2 g) was extracted with 10 mL of 1 % (2.703 mL conc. HCl in 97.30 mL methanol) concentrated hydrochloric acid in methanol for 20 min on a magnetic stirrer. Exactly 5 mL of vanillin reagent (0.5 %; w/v) was added to 1 mL of the extract, and the mixture was incubated at 30 °C for 20 min. The absorbance of the mixture was read at 500 nm. The concentration of tannic acid in the mixture was quantified using a prepared standard curve for tannic acid estimation in the range of 0.2 to 2 mg/mL.
- c) Phytic acid analysis was carried out by the titration method of Burgos-Lujánand (2015). Phytic acid was extracted from the sample by mixing 2.5 g of the sample with 10 mL of 0.4 M HCl (33.16 mL conc. HCl in 1 L Milli-Q water) and 10 mL of 5 % sodium sulphate solution (5 g Na<sub>2</sub>SO<sub>4</sub> in 100 mL Milli-Q water) in a test tube. The tube was closed and allowed to stand for 90 min at 25 °C with occasional vigorous shaking. The supernatant was decanted and 10 mL was mixed with 10 mL of 0.4 M HCl acid solution, 10 mL of 20 mM iron(III)chloride solution (5.406 g FeCl<sub>3</sub>·6H<sub>2</sub>O in 1 L Milli-Q water), and 10 mL of 20 % sulfosalicylic acid solution (20 g C<sub>7</sub>H<sub>6</sub>O<sub>6</sub>S in 100 mL Milli-Q water). The mixture was left for 24 h at 25 °C to form phytic acid-iron complex after which the mixture was vortexed for a minute and submerged in a boiling water bath for 15 min. The mixture was allowed to cool before centrifuged at 3500 rpm for 20 min. The supernatant (10 mL) was made-up to 100 mL with Milli-Q water and the pH of the solution was adjusted to 2.5 ± 0.5 using glycine. The solution was then heated to 70 – 80 °C and was immediately titrated with 5 mM EDTA solution (1.861 g in 1 L Milli-Q water) until the endpoint where the purple colour (iron-sulfosalicylic acid complex) changed to yellow colour (iron-EDTA complex).

#### Determination of Thermal Stability

The thermal stability of kenaf seed extracts was determined against kenaf seed meal (control) using a differential scanning calorimetry as described by Mariod *et al.* (2010) with minor modifications. About 10-15 mg of kenaf seed extract/kenaf seed meal were measured into a 40 µL aluminium pan. The pan was hermetically sealed and subjected to scanning from 30 to 140 °C at a constant heating rate of 10 °C/min. The onset temperature of denaturation (T<sub>o</sub>), the peak/denaturation temperature (T<sub>d</sub>) and enthalpy of denaturation (ΔH) were computed automatically from the thermogram.

#### Fatty Acids Analysis of Kenaf Seed and Kenaf Seed Extract

Prior to fatty acids profile analysis, the oils from both kenaf seed meal and freeze-dried kenaf seed extract were extracted using petroleum ether at 40-60 °C for 4 h in a Soxhlet extractor (AOAC, 2005). The oil was recovered using rotary evaporator (Model N-1, Eyela, Tokyo Rikakikal Co., Ltd., Japan) set at 60 °C. The extracted oils from kenaf seed and kenaf seed extract were used for fatty acids analysis following the method of Nyam *et al.* (2009) with little modifications. To 1 mL of extracted oil, 0.5 mL of boron tri- fluoride in methanol (14 %, w/v) was added then incubated at 65 °C for 30 min. After cooling to room temperature, 1 mL each of hexane and H<sub>2</sub>O saturated with NaCl were added to the mixture, vortexed for 1 min then centrifuged at 3000 g for 5 min. The hexane layer containing the fatty acid methyl ester (FAME) was aspirated and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The aliquot was then analyzed using gas chromatography (GC) equipped with a flame ionization detector (FID) and mass spectrometer detector (MSD) at a flow rate of 1.0 mL/min and column temperature of 100 °C. The injector and detector temperatures were set at 250 °C each, pressure of 73 kPa was applied and Helium was used as a carrier gas. FAME were identified by comparison of retention time with standard FAME. Fatty acid concentrations were calculated as relative area percentage of total fatty acids.

#### Determination of Amino Acid Profiles of Kenaf Seed Meal and Kenaf Seed Extract

The amino acid profiles of kenaf seed meal and freeze-dried kenaf seed extract were analyzed following the method of Waters AccQ.Tag (1993) with little modifications. Following the method, 0.5 g of the sample was mixed with 5 mL 6 N HCl (51 mL HCl in 49 mL D.H<sub>2</sub>O) in an airtight Pyrex glass tube. The content of the tube was hydrolyzed in an oven (Gallenkamp, Model OV-160, England) at 110 °C for 24 h. After hydrolysis, 4 mL of 2.5 mM α-aminobutyric acid (6.45 mg AABA in 25 mL of 0.1 N HCl (4.25 mL conc. HCl in 500 mL D.H<sub>2</sub>O)) was added and the volume was made-up to 100 mL with D. H<sub>2</sub>O. The aliquot was filtered using a 0.22 µm membrane filter into a vial. The calibration standard with an internal standard was prepared by combining 40 µL of 2.5 mM AABA (internal standard solution), 400 µL of 2.5 mM Waters Amino Acid Hydrolysate Standard mixture (external standard solution) and 560 µL D. H<sub>2</sub>O into a sampler vial. AccQ-Tag Flour Reagent Powder (2A) was reconstituted by adding 1.0 mL of AccQ Flour Reagent diluent (2B) into 2A. The reconstituted powder was vortexed for 10 s and incubated at 55 °C for 10 min for all the powder to completely dissolved. The calibration standard and the hydrolyzed sample were derivatized by measuring 10 µL of each into a vial, and 70 µL of AccQ Flour Borate Buffer (BF) was added. While to the blank vial, 80 µL of BF was dispensed into it. To all the vials, 20 µL of reconstituted powder was added. The aliquot in each vial was vortexed for 10 s and incubated at 55 °C for 10 min, and ready for injection into a High

Performance Liquid Chromatography (HPLC) unit for amino acids analysis. The mobile phases used for running the column were: mobile phase A; 9:1 Milli-Q water and AccQ-Tag acetate-phosphate buffer, respectively. While mobile phase B; 6:4 HPLC grade acetonitrile and Milli-Q water, respectively. The derivatized calibration standard and samples were then injected against the blank into the HPLC (Agilent Technologies, German) and the separation of the amino acid derivatives were done on AccQ-Tag column (Nova-Pak™ C18, 4 μm) at a stable flow rate of 1 mL/min. A fluorescent detector (Agilent Technologies, German) was used with an excitation wavelength of 250 nm, emission wavelength of 395 nm.

**Data Analysis**

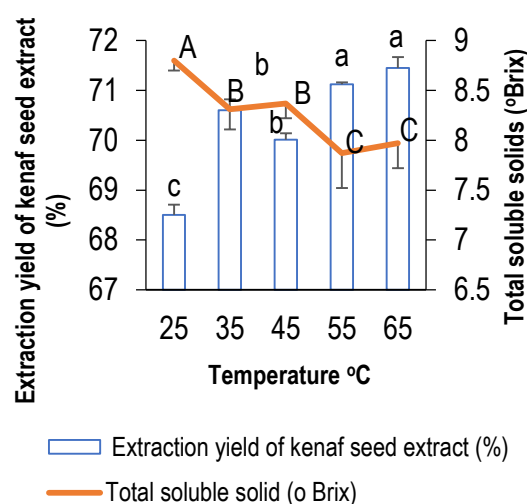
All analyses were conducted in triplicate and results were presented as mean ± standard deviation (SD). Minitab version 17.0 (Lead Technologies, Inc., PA, USA) was used for statistical data analysis and 2-samples t-test was used for amino acid and fatty acid analyses. While Analysis of variance (ANOVA) was used to determine whether significant differences existed among different groups and Tukey’s multiple comparison test was used to separate different groups at 5 % significant level.

**RESULTS AND DISCUSSION**

**Extraction Yield and Total Soluble Solids**

Figure 1 presents the effects of soaking temperatures of kenaf seed at their optimum soaking time on the extraction yield and total soluble solids of kenaf seed extracts. There was a significant increase in the extraction yield of the extracts as the soaking temperatures increase from 25-65 °C. The extracts obtained from the seeds soaked at 55 and 65 °C had the highest extraction yield of 71.12 and 71.45 %, respectively. However, the seed soaked at 25 °C had lower extraction yield of 68.50 % and extracts obtained from the seeds soaked at 35 and 45 °C had the extraction yield of 70.60 and 70.01 %, respectively. These might be because optimum moisture content is often achieved between temperature 47 °C to 67 °C as observed in our earlier studies (Ibrahim *et al.*, 2022). Likewise, higher temperature has been observed to damage cell membrane of seeds, causing the seeds to edge and rapidly absorb water.

Total soluble solids content is a vital parameter for beverage assessment in the food industry, and it is a measure of chemical character of a food (Ma *et al.*, 2015). The total soluble solids content of the extracts significantly decreased as the soaking temperatures of the seeds increase. The seed soaked at 25 °C had significantly higher soluble solids content of 8.80 °Brix and extracts obtained from seeds soaked at 55 and 65 °C had the least soluble solids content of 7.87 and 7.97 °Brix, respectively. These values were lower than the total soluble solids content (10.40 °Brix) for soybean extract (Ifediba and Nwabueze, 2018). The total soluble solids content of the extracts negatively correlates ( $r = -0.77$ ) with the extraction yield of the extracts.



**Figure 1:** Effects of soaking temperatures at the optimum soaking time on the extraction yield and total soluble solids of kenaf seed extracts.

The lowercase letters are for the extraction yield of the kenaf seed extracts and the bars with the same lowercase letters are not significantly different  $P \geq 0.05$ . While the uppercase letters are for the total soluble solids of the extracts and the data points with the same uppercase letter are not significantly different  $p \geq 0.05$ .

**Colour Profile**

The effects of soaking temperatures at the optimum soaking time on the colour properties of kenaf seed extracts are shown in Table 2. The lightness (L) for the extracts for the seeds soaked at 25, 55 and 65 °C (71.11, 71.07 and 71.46, respectively) were significantly lower than the extracts of the seeds soaked at 35 and 45 °C (72.76 and 72.63, respectively). The redness ( $a^*$ ) and yellowness ( $b^*$ ) of the extracts also significantly differs; the seed soaked at the 65 °C had significantly lower  $a^*$  and  $b^*$ . The significant decreased in the  $a^*$  and  $b^*$  values of the extract of the kenaf seed soaked at 65 °C might be due to higher rate of water absorption by kenaf seed at higher temperature which might affect the brownish surface colour of the seed. But the L predominates over the  $a^*$  and the  $b^*$ .

**Table 2:** Effects of soaking temperatures at the optimum soaking time on the colour profile of kenaf seed extracts

Soaking Temperature (°C)	L	$a^*$	$b^*$
25	71.11±0.04 <sup>b</sup>	1.22±0.09 <sup>ab</sup>	14.02±0.23 <sup>bc</sup>
35	72.76±0.07 <sup>a</sup>	1.34±0.02 <sup>ab</sup>	14.62±0.27 <sup>a</sup>
45	72.63±0.22 <sup>a</sup>	1.16±0.02 <sup>bc</sup>	14.60±0.15 <sup>ab</sup>
55	71.07±0.12 <sup>b</sup>	1.42±0.17 <sup>a</sup>	14.56±0.21 <sup>ab</sup>
65	71.46±0.71 <sup>b</sup>	0.98±0.01 <sup>c</sup>	13.73±0.19 <sup>c</sup>

The values are mean±SD of three replicate determinations. Values with the same superscript within a column are not significantly different ( $p \geq 0.05$ ). L =lightness;  $a^*$ = redness  $b^*$ = yellowness

### Proximate and Antinutritional Composition

The effects of soaking temperatures at their optimum soaking time on the proximate composition of kenaf seed extracts are presented in Table 3. There are no significant ( $p \geq 0.05$ ) differences in the moisture, crude protein, crude lipid and carbohydrate content of the extracts for the seeds soaked at different soaking temperatures. These findings concurred with the findings of Toda *et al.* (2007) for the effect of extraction temperatures of soybean extracts which were reported not to be significant on the protein, fat and carbohydrate contents of the soybean extracts. The moisture content for the extract range from 91.11-91.73 %, these values do not agree with the range of moisture content (85.30-89.60 %) found for different vegetable extracts (Ifediba and Nwabueze, 2018; Ukwuru and Ogbodo, 2001). Moisture content depends on the seed-to-water ratio during grinding and extraction.

The kenaf seed extracts for the seeds soaked at 25 and 35 °C had significantly higher ash content (0.59 and 0.54 %, respectively) than the kenaf seed extracts of the seeds soaked at 45, 55 and 65 °C. This variation is due to the slower rate of water absorption at lower temperature as observed in our earlier studies (Ibrahim *et al.*, 2022). The ash content (2.46 % and 1.88 %) reported for corn milk and breadfruit extracts, respectively (Ifediba and Nwabueze, 2018) were higher than the ash content found for the kenaf seed extracts for the different soaking temperatures of the seed. High mineral content in vegetable extracts has been attributed to a high ash content (Odu *et al.*, 2012).

The crude protein content of the extract ranged from 3.08-3.33 %. These values are like the protein content of soybean extract (3.2 %) reported by Onweluzo and Nwakalor (2009). However, the values were higher than the protein content of 2.66-2.81 % reported for soybean extract by Odu *et al.* (2012). But, lower than 3.91 % for corn seed extract, 4.76 % for breadfruit extract (Ifediba

and Nwabueze, 2018). Soaking is known to enhance the softness of plant seed, reduce the slurry particle size and improved protein solubility (Ifediba and Nwabueze, 2018). These might have been the reason for the observed protein content of the kenaf seed extract. Also, the mild water soaking temperatures (25-65 °C) might have probably prevent protein denaturation.

The crude lipid of the kenaf seed extracts ranged from 3.18-3.31 %, these values agreed with 3.40 % found for breadfruit extract (Onweluzo and Nwakalor, 2009). But, higher than the range of values (0.45-1.70 %) obtained for different vegetable beverages (Ifediba and Nwabueze, 2018; Odu *et al.*, 2012). These differences might be because of variation in processing methods employed. Different soaking conditions such as temperature and time were known to affect the permeability of the cotyledon and lipid extractability into the soaking medium at a varied rate (Ifediba and Nwabueze, 2018). The lipid content of the kenaf seed extracts were higher than the minimum (3 %) level required by codex Alimentarius Standard (Ifediba and Nwabueze, 2018). But, the high lipid content of the extracts might converse lipid-stability disadvantage. it might reduce the shelf life of the extracts as the prospect of rancidity occurring is very high.

The total carbohydrate of the kenaf seed extracts varied from 1.35-2.03 %, these values agreed with the lower range values of 1.99-3.40 % reported by Kolapo and Oladimeji (2008); Odu *et al.* (2012) for soybean extract. But, lower than the carbohydrate content (8.27 and 7.61 %) for corn and bread fruit extracts, respectively (Ifediba and Nwabueze, 2018). The decreased in the carbohydrate contents of kenaf seed extracts might be due to the extraction process rather than the soaking temperature. Some of the carbohydrate might have been lost into the residue (fiber) during extraction. Because, the soaking temperatures have been reported to significantly increased the carbohydrate content of the soaked seeds (Ibrahim *et al.*, 2020).

**Table 3:** Effects of soaking temperatures at optimum soaking time on the proximate composition of kenaf seed extracts

Soaking Temperature (°C)	Moisture	Crude Protein	Crude Lipid	Ash	Total Carbohydrate
	%				
25	91.73±0.14 <sup>a</sup>	3.13±0.03 <sup>a</sup>	3.20±0.02 <sup>a</sup>	0.59±0.05 <sup>a</sup>	1.35±0.34 <sup>a</sup>
35	91.55±0.11 <sup>a</sup>	3.08±0.05 <sup>a</sup>	3.30±0.06 <sup>a</sup>	0.54±0.02 <sup>a</sup>	1.53±0.34 <sup>a</sup>
45	91.17±0.64 <sup>a</sup>	3.24±0.10 <sup>a</sup>	3.18±0.01 <sup>a</sup>	0.47±0.01 <sup>b</sup>	1.94±1.08 <sup>a</sup>
55	91.11±0.47 <sup>a</sup>	3.11±0.07 <sup>a</sup>	3.31±0.09 <sup>a</sup>	0.44±0.04 <sup>b</sup>	2.03±0.95 <sup>a</sup>
65	91.42±0.33 <sup>a</sup>	3.33±0.12 <sup>a</sup>	3.21±0.04 <sup>a</sup>	0.49±0.06 <sup>b</sup>	1.55±0.78 <sup>a</sup>

Values are mean±SD of three replicate determinations. Values with the same superscript letter down a column are statistically not significant ( $p \geq 0.05$ ).

The levels of phytic acid, tannic acid and trypsin inhibitor activity of kenaf seed extract as affected by the soaking temperatures 25-65 °C at the optimum soaking time are presented in Table 4. The phytic acid for the extracts ranged from 1.94-2.34 %, the tannic acid ranged from  $3.05 \times 10^{-2}$ - $5.16 \times 10^{-2}$  mg/mL and the trypsin inhibitor activity ranged from 9.24-10.34 mg/g. These values were significantly lower than the phytic acid (2.74 %), tannic acid ( $8.20 \times 10^{-2}$  mg/mL) and trypsin inhibitor activity (11.18 mg/g) for the raw kenaf seed (control). These indicate that the process of soaking, grinding and removal of kenaf seed residue reduced the level of the anti-nutrients. The reduction in the anti-nutrient of the extract agreed with the findings of El-Adawy *et al.* (2000) for tannic acid, phytic acid and trypsin inhibitor activity in soybean, lupin and bean seed which were reported to be probably due to the internal process of leaching. The different soaking temperatures at their respective optimum soaking time affected the level of the anti-nutrients in a different way. The phytic acid content for the extracts for the seed soaked at 25, 35 and 65 °C at their optimum soaking time (10.27, 7.00 and 2.67 h, respectively) were significantly lower than the extracts for the kenaf seed soaked at 45 and 55 °C at the optimum soaking time of 5.00 and 3.50 h, respectively. However, the tannic acid for the extracts of the seeds soaked at 25, 45 and 65 °C were significantly lower than that of the

seeds soaked at 35 and 55 °C. The variations in the concentration of the trypsin inhibitor activity of kenaf seed extracts depend on the soaking temperature of the seeds. The extracts obtained from the seeds soaked at 45, 55 and 65 °C had significantly lower trypsin inhibitor activity. Whereas, the extracts of the seeds soaked at 25 and 35 °C had significantly higher trypsin inhibitor activity. The work of Nowshin *et al.* (2018) has highlighted the benefit of higher temperature towards significant reduction in the trypsin inhibitor activity of soybean extract. The authors reported that a significant reduction in the level of trypsin inhibitor activity of soybean extract under the condition of combined soaking, blanching and hot water grinding (100 °C) was higher than cold water grinding. However, the findings of Machado *et al.* (2008); Mendes *et al.* (2007) have pointed out that, for a significant reduction of trypsin inhibitor activity in soybean extract, that the soybean should be autoclave at 120 °C for 18 min. Likewise, Nowshin *et al.* (2018) suggested that steam injected hot grinding should be use for extraction of soybean extract, as it is effective in preserving nutrients and reducing the level of trypsin inhibitor activity. Similarly, to boost the rate of inactivation of trypsin inhibitor activity in beverages, soaking of plant seeds in a salt solution at 98 °C has been reported to be more effective than soaking in water (Giri and Mangaraj, 2012).

**Table 4:** Effects of soaking temperatures at the optimum soaking time on antinutritional content of kenaf seed extracts

Soaking Temperature (°C)	Phytic Acid (%)	Tannic Acid (mg/mL) x 10 <sup>-2</sup>	Trypsin Inhibitor mg/g
25	2.04±0.02 <sup>b</sup>	3.75±0.06 <sup>c</sup>	10.34±0.23 <sup>a</sup>
35	2.11±0.06 <sup>b</sup>	4.65±0.08 <sup>b</sup>	10.05±0.15 <sup>a</sup>
45	2.34±0.04 <sup>a</sup>	3.05±0.07 <sup>c</sup>	9.89±0.05 <sup>b</sup>
55	2.50±0.01 <sup>a</sup>	5.16±0.02 <sup>b</sup>	9.55±0.10 <sup>b</sup>
65	1.94±0.04 <sup>b</sup>	3.44±0.01 <sup>c</sup>	9.24±0.21 <sup>b</sup>
<b>Kenaf Seed Meal (Control)</b>	2.74±0.33 <sup>a</sup>	8.20±0.008 <sup>a</sup>	11.18±0.11 <sup>a</sup>

Values are mean±SD of three replicate determinations. Values with the same superscript down a column are statistically not significant ( $p > 0.05$ ).

### Thermal stability

The effects of soaking temperatures at the optimum soaking time on the thermal stability of kenaf seed extracts are presented in Table 5. There was no significant difference in the onset temperature ( $T_o$ ) of the kenaf seed extracts as compared with the different soaking temperatures of the seeds and against the control. These indicated that excessive denaturation of the protein was around 96.77-104.14 °C. Kenaf seed meal had significantly lower enthalpy of denaturation ( $\Delta H$ ) 0.29 J/g. All the extracts had significantly higher enthalpy of denaturation, indicated higher proportion of undenatured proteins in the kenaf seed extracts, possibly due to the presence of other macromolecules. The

extracts obtained from the seeds soaked at 25 and 45 °C had the highest enthalpy of denaturation (18.25 and 16.12 J/g, respectively). There were no significant effects of soaking temperatures of seeds on the peak of denaturation ( $T_d$ ) of the kenaf seed extracts. Both the kenaf seed meal and the kenaf seed extracts had no significant difference in the peak of denaturation. This indicated that the protein of kenaf seed can be completely un-fold at temperature of between 107.32-113.81 °C. The denaturation temperature of the kenaf seed meal and kenaf seed extracts were like that of 11S globulin of soybean and higher than the un-folding temperature of cowpea protein 85 to 88 °C (Horax *et al.*, 2004). The impacts of the soaking temperatures of the

seeds on the degree of thermal stability of the extracts will assist in the choice of appropriate cooking temperature for the use of kenaf seed in food processing and production.

### Fatty Acids Composition

The amount of oil extracted in kenaf seed extract (41.25 %) was significantly higher than that obtained from

kenaf seed meal (24.69 %), this was because sequential processing of kenaf seed such as soaking, grinding, removal of residue and freeze-drying of the extract increased the crude lipid content of the kenaf seed extract powder.

**Table 5:** Effects of soaking temperature at optimum soaking time on the thermal stability of kenaf seed extracts

Soaking Temperature (°C)	Enthalpy ( $\Delta H$ , J/g)	Onset ( $T_o$ , °C)	Peak ( $T_d$ , °C)
25	18.25 $\pm$ 1.15 <sup>a</sup>	99.49 $\pm$ 2.12 <sup>a</sup>	108.38 $\pm$ 1.15 <sup>a</sup>
35	13.02 $\pm$ 0.09 <sup>b</sup>	104.14 $\pm$ 1.02 <sup>a</sup>	113.73 $\pm$ 1.46 <sup>a</sup>
45	16.12 $\pm$ 2.01 <sup>a</sup>	103.76 $\pm$ 0.09 <sup>a</sup>	113.81 $\pm$ 1.63 <sup>a</sup>
55	12.97 $\pm$ 0.05 <sup>b</sup>	101.03 $\pm$ 1.11 <sup>a</sup>	110.55 $\pm$ 1.82 <sup>a</sup>
65	11.53 $\pm$ 0.07 <sup>c</sup>	100.94 $\pm$ 1.53 <sup>a</sup>	107.32 $\pm$ 1.99 <sup>a</sup>
<b>Kenaf Seed Meal (Control)</b>	0.29 $\pm$ 0.02 <sup>d</sup>	96.77 $\pm$ 2.76 <sup>a</sup>	117.22 $\pm$ 2.68 <sup>a</sup>

Values are mean $\pm$ SD of three replicate determination. The values with the same superscript letter within the same column are not significantly different ( $p \geq 0.05$ ).

The composition and amount of saturated and unsaturated fatty acids of kenaf seed oil (KSO) and kenaf seed extract oil (KSMEO) are presented in Table 6. There was no significant difference in the total saturated fatty acids of kenaf seed oil. The oil from the kenaf seed meal contained more of saturated fatty acids (30.51 %) than kenaf seed extract oil (29.93 %). The monounsaturated fatty acids content of kenaf seed extract oil (41.12 %) was higher than that of kenaf seed oil (34.75 %) although not significant. Likewise, there was no significant difference in the composition and amount of polyunsaturated fatty acids of both oils, KSO had 30.59 % and KSMEO had 28.62 %. These implied that processing conditions such as soaking of the seeds, grinding and removal of the residue as in the case of the oil obtained from the kenaf seed extract does not significantly affect the composition of its fatty acid profile. Both the oils contained more than 30 % of monounsaturated fatty acids and oil rich in monounsaturated fatty acids had been correlated to decrease risk of heart diseases (Corbett, 2003). These results showed that both KSO and KSMEO had higher amount of monounsaturated fatty acids (34.75 and 41.12 %, respectively) than peanut oil (13.00 %), soybean oil (25.00 %) and roselle seed oil (27.40 %). However, the polyunsaturated fatty acids of the oils; KSO (30.59 %) and KSMEO (28.62 %) were lower than that of peanut oil (78.00 %), soybean oil (62.00 %), roselle seed oil (39.70 %) but, similar to that of pumpkin oil (30.40 %) (Nyam *et al.*, 2009). These indicate that both kenaf seed

oil and kenaf seed extract oil could be used as an edible healthier oil, as the new trends shifted to vegetable oils that have more monounsaturated fatty acids (oleic acid).

The major saturated fatty acids of both oils were hexadecanoic (palmitic) acid and octadecanoic (stearic) acid. While the major unsaturated fatty acids were 9-octadecenoic (oleic) acid and 9,12-octadecadienoic (linoleic) acid. These results agreed with the types and amounts of saturated and unsaturated fatty acids of kenaf seed oil reported by Nyam *et al.* (2009). The amounts of linoleic acid content for both KSO and KSMEO 30.59 % and 28.62 %, respectively indicated that these oils might be used as a good source of essential omega 6 fatty acids. The total oleic acid and linoleic acid content for KSO and KSMEO oils were 64.39 % and 69.03 % respectively, these values were lower than the previously reported value for kenaf seed oil (74.8 %). These differences might be due to variation in the cultivar of the seed used, planting season, soil difference and geographical location. The high oleic and linoleic acid of both oils means that the oils could be used as a source of edible oil, salad dressing and in the production of margarine. The presence of high amount of oleic acid in the oils indicated that the oils could be suitable as a nutritionally high stable cooking oils. Although, the quantity of polyunsaturated fatty acids of the oils are substantially high and this means that the oils might easily oxidize when used for frying at higher temperature for longer time.



**Table 6:** Fatty acid composition of extracted oils from kenaf seed and kenaf seed extract

Chemical Name	Molecular Formula	KSO Area (%)	KSMEQ Area (%)
<b>Methyl tetradecanoate (C15:0)</b>	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	0.03±0.01 <sup>b</sup>	0.05±0.01 <sup>a</sup>
<b>Hexadecanoic Acid (C16:0)</b>	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	26.71±0.22 <sup>a</sup>	26.11±0.57 <sup>a</sup>
<b>9-Hexadecenoic Acid (C16:1)</b>	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	0.40±0.02 <sup>a</sup>	0.28±0.01 <sup>b</sup>
<b>Heptadecanoic Acid (C17:0)</b>	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	0.04±0.00 <sup>b</sup>	0.05±0.00 <sup>a</sup>
<b>Octadecanoic Acid (C18:0)</b>	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	3.17±0.47 <sup>a</sup>	2.94±0.05 <sup>a</sup>
<b>9-Octadecenoic Acid (C18:1)</b>	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	33.80±2.39 <sup>a</sup>	40.41±1.22 <sup>a</sup>
<b>9,12-Octadecadienoic Acid (C18:2)</b>	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	30.59±0.62 <sup>a</sup>	28.62±0.86 <sup>a</sup>
<b>10-Nonadecenoic Acid (C20:1)</b>	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	0.55±0.04 <sup>b</sup>	0.74±0.01 <sup>a</sup>
<b>Methyl 18-methylnonadecanoate (C21:0)</b>		0.31±0.01 <sup>a</sup>	0.46±0.18 <sup>a</sup>
<b>Docosanoic Acid (C22:0)</b>	C <sub>22</sub> H <sub>44</sub> O <sub>2</sub>	0.15±0.01 <sup>a</sup>	0.19±0.01 <sup>a</sup>
<b>Tetracosanoic Acid (C24:0)</b>	C <sub>24</sub> H <sub>48</sub> O <sub>2</sub>	0.10±0.01 <sup>a</sup>	0.13±0.02 <sup>a</sup>
<b>Saturated</b>		30.51±0.73 <sup>a</sup>	29.93±0.84 <sup>a</sup>
<b>Monounsaturated</b>		34.75±2.45 <sup>a</sup>	41.12±1.24 <sup>a</sup>
<b>Polyunsaturated</b>		30.59±0.62 <sup>a</sup>	28.62±0.86 <sup>a</sup>

Values are mean±SD of three replicate determinations. The values with the same superscript letter across the row are not significantly different  $P \geq 0.05$ . Note: KSO: kenaf seed oil, KSMEQ: kenaf seed extract oil.

It was reported that linoleic acid oxidizes 50 times higher than oleic acid (Brinkmann, 2000). Thus, significant consideration has been given to vegetable oils with higher amount of oleic acid to be used as edible cooking and deep-frying oil; due to its higher oxidative stability. Minor saturated fatty acids identified in the oils were methyl tetradecanoate (methyl myristate), heptadecanoic (margaric acid), methyl 18-methylnonadecanoate (18-methylnonadecanoic acid, methyl ester), docosanoic acid (behenic acid) and tetracosanoic acid (lignoceric acid). Most of these saturated fatty acids have been reported as recommended flavouring food additives (WHO, 2004).

#### Amino Acids Composition

The amino acids composition of kenaf seed meal and freeze-dried kenaf seed extract are presented in Table 7. Kenaf seed meal contained a significantly higher amount of hydrophilic amino acids (693.70 mg/g) than the freeze-dried kenaf seed extract (363.69 mg/g). Whereas, the hydrophobic amino acids composition for both the kenaf seed and kenaf seed extract (453.10 mg/g and 486.47 mg/g, respectively) were not significantly different. Balance between hydrophilic and hydrophobic amino acids of a substance determines its solubility, as solubility depends on the interaction of hydrophilic and hydrophobic amino acids with water molecules (Brishti *et al.*, 2017). The solubility of kenaf seed is expected to be higher than that of kenaf seed extract on the facts that protein solubility increases as the number of hydrophilic amino acids are high. But it has been reported that the distribution of both the hydrophilic and hydrophobic amino acids in a protein structure determines the extent of the protein solubility (Brishti *et al.*, 2017). There was no significant difference in the total essential amino acids of kenaf seed meal (462.80 mg/g) and freeze-dried kenaf seed extract (487.44 mg/g). These values were higher than the total essential amino acids (213 mg/g and 386

mg/g) for the defatted kenaf seed cultivar QP3 and V36, respectively (Mariod *et al.*, 2010).

Furthermore, the kenaf seed meal contained significantly higher amount of non-essential amino acids (684.00 mg/g) than the extract (362.72 mg/g). However, the value obtained in this study for the non-essential amino acids content of kenaf seed meal disagreed with the findings of Mariod *et al.* (2010) who reported a significantly lower value for defatted QP3 kenaf seed variant (418 mg/g) and a significantly higher value for defatted V36 kenaf seed variant (874 mg/g). These differences might not only depend on the cultivars, growing season but, also on the extent of the processing of the sample used. In the case of this current study whole kenaf seed meal and the freeze-dried kenaf seed extract were used against defatted kenaf seed meal of both QP3 and V36 cultivars that were used in the study of Mariod *et al.* (2010). Both the kenaf seed meal and the extract were high in aspartic acid, glutamic acid, arginine, lysine, phenylalanine, leucine and isoleucine but low in methionine. These were like the amino acid profiles of different pulses (which includes; pea, chickpea, lentil, soybean and bean seed) reported by Boye *et al.* (2010). Similarly, vegetable proteins high in branched chain amino acids have been associated with health promoting benefits (Oomah, 2001).

#### CONCLUSION

The different soaking temperatures have no significant effect on the proximate composition of the extracts. However, the trypsin inhibitor activity, phytic acid and tannic acid of the extracts significantly decreased as the soaking temperature increased.



**Table 7:** Amino acid profiles of kenaf seed meal and kenaf seed extract

Amino Acids	Kenaf Seed Meal (mg/g)	Freeze-dried Kenaf Seed Extract (mg/g)
Aspartic Acid	114.39±4.29 <sup>a</sup>	94.21±0.01 <sup>a</sup>
Serine	87.97±5.92 <sup>a</sup>	35.14±0.01 <sup>b</sup>
Glutamic Acid	124.37±2.87 <sup>a</sup>	58.77±0.01 <sup>c</sup>
Histidine	39.67±2.35 <sup>a</sup>	17.79±0.01 <sup>b</sup>
Arginine	178.72±0.89 <sup>a</sup>	70.79±1.61 <sup>b</sup>
Threonine	54.65±2.31 <sup>a</sup>	19.14±0.01 <sup>b</sup>
Tyrosine	34.15±0.46 <sup>a</sup>	11.04±0.00 <sup>b</sup>
Lysine	59.87±1.47 <sup>b</sup>	56.81±0.02 <sup>b</sup>
Hydrophilic	693.70±20.56 <sup>a</sup>	363.69±1.68 <sup>b</sup>
Glycine	62.21±2.25 <sup>a</sup>	23.79±0.01 <sup>b</sup>
Alanine	46.61±4.33 <sup>a</sup>	14.70±0.01 <sup>b</sup>
Proline	35.69±2.75 <sup>b</sup>	54.31±0.01 <sup>a</sup>
Valine	64.11±2.14 <sup>a</sup>	28.24±0.01 <sup>c</sup>
Methionine	28.09±0.79 <sup>a</sup>	12.00±0.00 <sup>b</sup>
Isoleucine	54.07±0.36 <sup>b</sup>	95.50±1.35 <sup>a</sup>
Leucine	91.32±0.62 <sup>b</sup>	129.59±0.04 <sup>a</sup>
Phenylalanine	71.01±0.51 <sup>b</sup>	128.34±0.04 <sup>a</sup>
Hydrophobic	453.10±13.75 <sup>a</sup>	486.47±1.47 <sup>a</sup>
Essential	462.80±11.01 <sup>a</sup>	487.44±1.48 <sup>a</sup>
Non-essential	684.00±23.76 <sup>a</sup>	362.75±1.61 <sup>b</sup>

Values are mean±SD of three replicate determinations. The values in the same row with different superscripts letters are significantly different ( $P < 0.05$ ).

Furthermore, the Td (107.32-113.81 °C) of the extracts was not significantly affected by the soaking temperatures. This study also found that both the kenaf seed oil and kenaf seed extract oil were rich sources of hexadecenoic acid, 9-octadecenoic acid and 9,12-octadecadienoic acid. Additionally, the total essential amino acids of the kenaf seed meal (462.80 mg/g) and kenaf seed extract (487.44 mg/g) were not significantly different. Moreover, both the kenaf seed meal and the kenaf seed extract were high in aspartic acid, glutamic acid, arginine, lysine, phenylalanine, leucine and isoleucine but low in methionine. Thus, this study concluded that soaking temperatures of 25, 45 and 65 °C at their respective optimum soaking time can be used for the soaking of kenaf seed for the extraction of kenaf seed extract for onward use in food production.

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