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Alternative Dietary Fiber Sources from Kenaf (*Hibiscus cannabinus* L.) Seeds and Their by-Products

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ABSTRACT

This study evaluates the macronutrients and Dietary Fiber (DF) of kenaf seeds and their secondary by-products to promote food sustainability and support the zero-waste concept. The first part concentrates on macronutrients and potential DF sources of kenaf seeds and their by-products, i.e., kenaf seed meals and dregs. Following this, the DF from the most probable source was fractionated to quantify its composition. The results showed that the macronutrients of kenaf seeds are comparable to other commercial oilseeds such as soybean, almond, and hemp seeds. Additionally, the secondary by-products could be reused as DF sources. It was found that the kenaf seed by-products had 20.63–35.08% DF contents which were comparable to soybean by-products. Moreover, the fractionation of DF from kenaf seed dregs showed that the DF comprised 1.86%, 1.01%, 6.33%, and 66.33% (dry basis) of acid-soluble pectin, calcium-bound pectin, alkali-soluble hemicellulose, and cellulose, respectively. The soluble (pectins and hemicelluloses) and insoluble (cellulose) fractions are related to the modulation of gut microbiota which have similar potential to conventional prebiotics and an excellent role in bodyweight management, respectively. These findings provide useful information for researchers and industries to venture into alternative DF sources from kenaf seeds as a value-add ingredient for functional food applications.

Keywords: by-products valorization, dietary fiber fractions, food sustainability, oilseeds, potential prebiotics

INTRODUCTION

Obesity and type 2 diabetes are epidemics that are causing global concern. Extrinsic factors, such as sedentary lifestyle and excessive caloric intake, are primary contributors to their emergence (Sharma *et al.* 2018). According to a recent study, more attention should be paid to the adequacy of fiber intake in diet-based weight loss programs aimed at improving body composition and metabolic health (Tremblay *et al.* 2020). Dietary Fiber (DF) is a crucial food component that is resistant to digestive enzymes in the small intestine and can be fermented partially or completely in the large intestine (Fuller *et al.* 2016). It has a unique role as a component of prebiotics, which is helpful for the growth of intestinal microflora (Kusharto 2006). DF exists in insoluble and soluble forms with varying physiological and physicochemical properties (Gupta & Premavalli 2011).

Over the last two decades, the research has thoroughly documented the health benefits of DF (Li & Komarek 2017). Besides diabetes and cardiovascular diseases, adequate DF consumption lowers the risk of gastrointestinal disorders and improves immune function (Hussain *et al.* 2020). There are three major trends in the development of food products using diverse DF sources: (1) utilization of the whole fiber-rich raw materials in conventional food production; (2) fortification of isolated or purified fiber in unconventional food; (3) utilization of food by-product as a source of DF and other bioactive compounds (Li & Komarek 2017).

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Studies on the fermentability of DF in the colon and its interactions as prebiotics with colonic bacteria were limited due to the misunderstanding of the initial definition of DF, which referred to the non-digestible food component in the upper Gastrointestinal (GI) tract (Li & Komarek 2017). Researches have correlated the health properties of DF to various functionalities of the upper GI tract (Mackie *et al.* 2016). Specifically, the increase of viscosity caused by soluble DF can alter gastric emptying, provide satiety sensations and result in the nutrient release and sensing in the duodenum. The interactions between insoluble DF and colonic microbiota, on the other hand, have been investigated using current DNA sequencing technologies (Simpson & Campbell 2015). It is proposed that DF fermentation releases varying amounts of Short-Chain Fatty Acids (SCFAs), which play a crucial role in the metabolic activity and diversity of the microbial community. This impacts gut health, the immune system, and the body's resistance to certain chronic diseases (Simpson & Campbell 2015).

The various health benefits of DF have fueled the interest of many researchers and food manufacturers to incorporate DF in developing novel food products (Dhingra *et al.* 2012). However, because DF contains a complex mixture of undigested polysaccharides, the exact mechanism by which DF components may exert their effect has not been well understood (Brownlee 2014). Thus, the fractionation of DF into its constituent is necessary to isolate and quantify the essential components, eliminate undesirable compounds and to better understand the function of DF. However, only limited research has been done in this area.

Kenaf (*Hibiscus cannabinus* L.) seed is one of the most commonly discarded kenaf plant by-products (Chan *et al.* 2013). However, due to their richness in essential nutrients, extensive studies of kenaf seed oil, protein concentrate, and kenaf seed-based food products (i.e. noodles, tofu, milk) have been conducted in recent years (Ibadullah *et al.* 2021; Karim *et al.* 2020; Mariod *et al.* 2017; Monti 2013; Zawawi *et al.* 2014). Defatted kenaf seed meals and kenaf seed dregs are the secondary by-products of kenaf seed oil and milk extractions. These by-products can serve as a potential source of bioactive compounds and subsequently reduce the amount of food processing waste which is beneficial for the environment. However, to date, no work has been reported on the composition of kenaf seed by-products as an alternative, low-cost DF source. Therefore, this study aimed to evaluate the nutritional contents of the whole, dehulled kenaf seeds and kenaf seed by-products (meals and dregs). Following this, the DF from the most potential source was fractionated to quantify its composition.

METHODS

Design, location, and time

The study consisted of two parts. The first part evaluated the nutritional composition and the DF contents of the whole, dehulled and by-products (meals and dregs) of kenaf seeds. Secondly, the DF from the most potential source was fractionated to quantify its composition. This project was carried out at the Laboratory of Functional Carbohydrate and Protein, Faculty of Food Science and Technology, Universiti Putra Malaysia, from November 2020–August 2021.

Materials and tools

Kenaf seeds variety V36 and defatted kenaf seeds resulting from cold-press kenaf seed oil were provided by the National Kenaf and Tobacco Board (LKTN) in Kangar, Perlis, Malaysia. All chemicals used in this study were of analytical grade.

Procedure

Sample preparation. The whole seed impurities, including immature seeds, dust, chaffs, and stones, were eliminated. The clean, whole seeds were rinsed three times under running tap water, oven-dried at 40°C for 5 h, and then kept in plastic bags at room temperature (25±2°C) until use.

Kenaf seed dregs were the residue left after the extraction of kenaf seed milk (Karim *et al.* 2020). Briefly, cleaned kenaf whole seeds were washed three times under running tap water, soaked at 60°C for 1 h, blended for 3 min (seed: water=1:8), and filtered. The remaining solids (kenaf seed dregs) were oven-dried at 40°C for 40 h, ground and sieved (<500µm/35 mesh).

***Dietary Fiber* (*DF) analysis*.** The total, soluble and insoluble DF (TDF, SDF, and IDF) of samples were determined using the total DF assay kits (K-TDFR-100A) from Megazyme

(Bray, Ireland). The procedure was carried out according to AOAC Method 991.43, "Total, Soluble, and Insoluble Dietary Fiber in Foods" (First Action 1991) and AACC Method 32-07.01, "Determination of Soluble, Insoluble, and Total DF in Foods and Food Products" (Final Approval 10-16-91). Specifically, 1 g of dried materials (in duplicate) were sequentially digested with heat-stable α-amylase, protease, and amyloglucosidase. The IDF was filtered, and the residue was rinsed with warm distilled water. For SDF determination, a solution containing filtrate and water washings was precipitated with 4 volumes of 95% ethanol. The precipitate was filtered and oven-dried at 103°C overnight. For the final calculation of SDF and IDF values, both SDF and IDF residues were corrected for protein, ash and blank. For TDF determination, after ethanol precipitation of the SDF, the precipitate was filtered, dried and weighed. The TDF value was corrected for protein and ash content.

Sequential fractionation of DF. The sequential fractionation method (Alba *et al.* 2018) was used to isolate and quantify DF fractions (Figure 1).

Physicochemical analysis. The moisture, ash, fat, protein and DF contents were determined using the AOAC procedures of 925.09, 923.03, 920.85, 920.87, and 991.43, respectively. The procedure proposed by Parrot and Thrall was used to determine the direct and bulk densities of samples (Parrot & Thrall 1978). By placing the powdered samples in a calibrated 10-ml cylinder, the direct density (in g/ml) was determined. Before emptying the cylinder, the samples settled at the bottom and were weighed. Bulk density (in g/ml) was calculated by loading 2 g of sample into a graduated syringe. The samples were packed in the syringe with sufficient pressure. Following that, the sample volume in the syringe was measured.

Calculations on powder cohesiveness (HR) and powder compressibility (CI) were performed using the equations below (Jinapong *et al.* 2008):

$$HR = DD/BD \quad (1)$$

$$CI(\%) = \left(\frac{DD - BD}{DD}\right) X100 \quad (2)$$

Where HR=Hausner Ratio, CI= Carr Index, DD=Direct Density, and BD=Bulk Density.

Data analysis

The physicochemical tests were conducted in triplicate. Minitab statistical software version 17.0 (Minitab Inc, State College) performed a one-way ANOVA on raw data. Research Part I consisted of one factor (kenaf seeds) and three levels (whole seeds, dehulled seeds, seed hulls), whereas Part II consisted of one factor (DF fractions) and four levels (ASP, CBP, HEM,

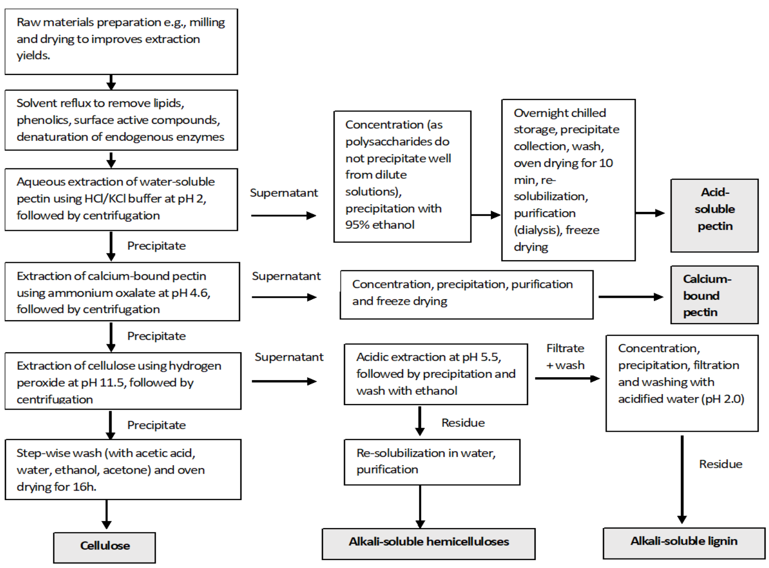

Figure 1. Sequential fractionation method (adopted from Alba *et al.* 2018)

CEL). Tukey's multiple comparison test at a 95% confidence level ($p<0.05$) was used to determine the significant difference, and the results were reported as means and Standard Deviation (SD).

RESULTS AND DISCUSSION

Composition of whole and dehulled kenaf seeds

The structure of kenaf seeds comprised of the seed and hull (Figure 2) with a ratio of 1.6:1. The composition of whole and dehulled kenaf seeds are presented in Table 1. Whole kenaf seeds contained 39.05% protein, 27.15% fat and 20.63% DF. After the dehulling process, the protein and fat contents were found to be accumulated in the seeds.

In terms of DF, the whole kenaf seeds had the highest value (20.63%) compared with the seed and hull, with the majority of insoluble fiber constituents. This value represented the total fiber content of the seed and its hull. However, the dehulled kenaf seed exhibited the highest soluble fiber content (11.80%), whereas its hulls contained a higher insoluble fiber fraction (13.16%). The concentration of cellulosic and non-cellulosic polysaccharides differs significantly between cotyledon (dehulled seed) fibers and hulls. Soluble DF, such as soluble hemicelluloses, pectins, and gums, are common components of cotyledon fibers, whereas the hulls are primarily composed of insoluble fiber, i.e., cellulose, hemicelluloses and lignin (Shen *et al.* 2020).

Kenaf seeds can be considered as one of the potential sources of functional food ingredients from non-conventional oilseeds due to their considerable amount of protein, fat and DF contents. Figure 3 displays the composition of kenaf seeds and several commercial oilseeds, i.e., soybean, almond and hemp seeds. The moisture content of kenaf seeds (7.34%) was lower than soybean (8.54%) but higher than almond (5.25%) and hemp seeds (5.90%). Moisture content is one of the most fundamental and essential analyses that can be performed on a food product to indicate its storage stability and also as one of the quality factors (Nielsen 2017). Interestingly, kenaf seeds showed the highest ash content (6.92%) among other oilseeds (3.11–4.87%). The ash content in foods represents their mineral content. As certain foods are rich in particular minerals, ash content can be significant from nutritional, toxicological, and food quality perspectives (Nielsen 2017).

In terms of protein content, kenaf seeds showed a higher value (39.05%) than other

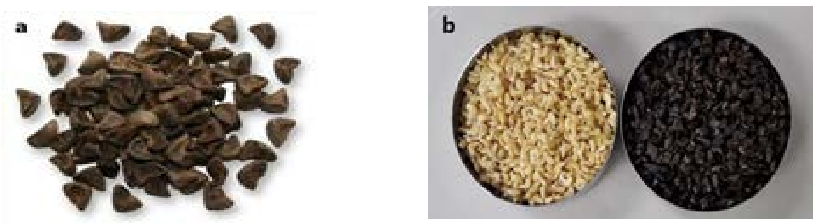

Figure 2. (a) Whole kenaf seeds (Mariod *et al.* 2017); (b) dehulled seeds (white) and seed hulls (black)

Table 1. Composition of whole and dehulled kenaf seeds

Parameters	Whole kenaf seeds	Dehulled kenaf seeds	
		Seeds	Hull
Moisture content (% db)	7.34±0.21[a]	4.03±0.12[b]	7.24±0.26[a]
Ash content (% db)	6.92±0.02[a]	5.43±0.07[b]	2.41±0.02[c]
Crude protein content (% db)	39.05±0.50[b]	42.12±0.87[a]	11.05±0.30[c]
Crude fat content (% db)	27.15±0.04[b]	30.70±0.12[a]	1.77±0.05[c]
DF content (% db)	20.63±0.92[a]	13.52±0.66[b]	16.65±0.71[ab]
Soluble (% db)	2.77±0.14[b]	11.80±0.49[a]	3.49±0.15[b]
Insoluble (% db)	17.86±0.78[a]	1.72±0.07[c]	13.16±0.65[b]

The data are expressed as mean±standard deviation

Different letters in the same row denoted statistically significant differences ($p\leq0.05$)

DB:Dry Basis

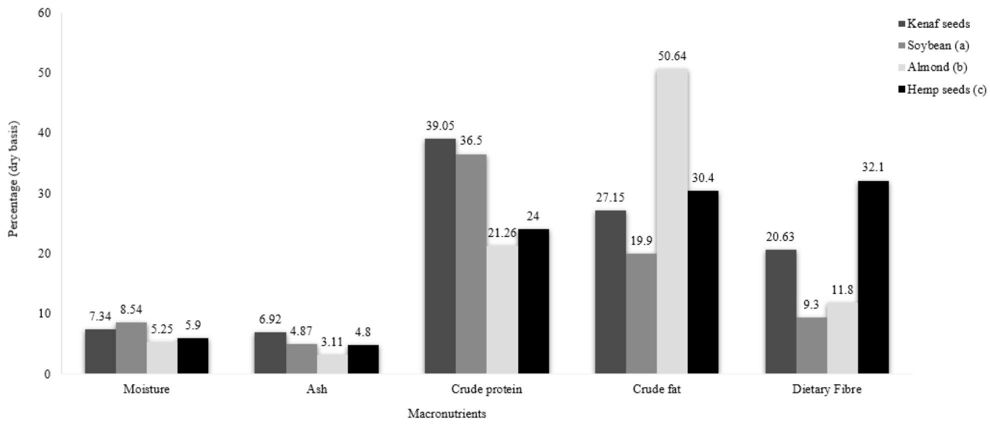

Figure 3. Macronutrients contents of kenaf seeds and several commercial oilseeds a (Colletti *et al*. 2020); b (Yada *et al.* 2013); c (Leonard *et al.* 2020).

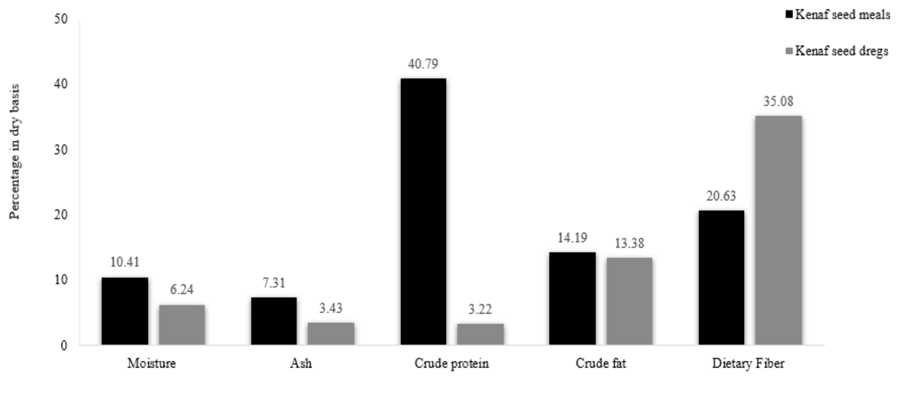

Figure 4. Compositions of kenaf seed by-products

commercial oilseeds. Ibadullah *et al.* (2021) extracted the protein concentrate from defatted kenaf seeds and found that it was high in essential and non-essential amino acids with several functionalities. They proposed that kenaf seed protein concentrate can be incorporated as an ingredient in tofu, butter, mayonnaise, and a meat extender. Nowadays, plant proteins are regarded as biologically active components rather than essential nutrients (Oomah *et al.* 2011). Hence, kenaf seeds may be a potential source of inexpensive plant protein to tackle severe problems in developing countries, such as protein deficiency.

Furthermore, the fat content of kenaf seeds (27.15%) was lower than almond (50.64%) and hemp seeds (30.40%) but higher than that of soybean (19.90%). The bioactive compounds such as tocopherols, tocotrienols, phytosterols, and phenolics in kenaf seed oil provide significant health benefits, i.e., antioxidant activity, anti-hypercholesterolemic activity, anti-cancer, anti-inflammatory, anti-ulcer, and anti-thrombotic activity) (Chew & Nyam 2019). The DF of kenaf seeds (20.63%) were higher than soybean (9.30%) and almond (7.9-12.24%) but lower than hemp seeds (32.10%). These findings emphasized that kenaf seeds are potential ingredients for producing high-protein and high-fiber food products. A recent study has shown that pasta enriched with 30–40% of hemp flour showed acceptable sensory properties by consumers (Teterycz *et al.* 2021). In another study, Martínez *et al.* (2021) substituted wheat flour with a variety of oilseed meals, including poppy, flax, chia, and sesame, to enhance the nutritional content of commercial cookies. Based on consumer tests, they discovered that sesame and flax cookies were equally well-received as commercial wheat cookies in all evaluated parameters. Several works have indicated that ingredients derived from plants, such as oilseed protein or fiber, can be substituted into healthier meat-based processed foods (Nevara *et al.* 2022), such as burgers enriched with chia and poppy seed meals (Souza *et al.* 2015).

The most notable attributes of oilseeds are the fat and protein contents; however, less focus is on the DF of oilseeds. The abundance of commercial seed oils and plant-based milk available in the market, such as soybean oil, almond oil, sunflower oil, soybean milk, almond milk, etc. Interestingly, more work is needed to valorise the by-products generated after the oil and milky extracts are removed from the seeds. In light of their DF content, these by-products are the potential ingredients for enriching food products such as bakery and pasta (Nevara *et al.* 2022). Incorporating DF into the formulations could enhance the nutritional values of the final food products (Alba *et al.* 2018). Providing consumers with a variety of high-fiber meals will eventually assist in resolving the deficiency problems prevalent in developing countries.

Composition of kenaf seed by-products

With an increasing world population and rising demand for food, it is critical for sensible and resource-saving use of raw materials, which include the use of underutilized oilseed by-products. Figure 4 presents the proximate composition of kenaf seed meals and dregs. Generally, protein and DF are desirable compounds after the oil and milk components are extracted from the seeds.

In terms of protein content, the kenaf seed meals (40.79%) had significantly ($p<0.05$) higher values than kenaf seed dregs (3.22%) (Figure 4). The increasing trend is similar to that of soybean by-products, where the protein content of soybean meals (33.04–56.27%) is greater than that of soybean okara (28.52%) (Redondo-Cuenca *et al.* 2008; Rosset *et al.* 2014).

In contrast, the DF value of kenaf seed meals (20.63%) was considerably ($p<0.05$) lower than that of kenaf seed dregs (35.08%). This finding is comparable to soybean by-products, whereby the soybean meals contain lower DF content ranging from 17–20.70%, compared with okara (50–55.48%) (Grieshop *et al.* 2003; Redondo-Cuenca *et al.* 2008; Li *et al.* 2012). Furthermore, Li *et al.* (2012) suggested that okara was a good source of DF because DF made up about 50% of its composition. In addition, Redondo-Cuenca *et al.* (2008) found that the beneficial properties of soybean seeds fiber are attributed to its okara due to their similar monomers composition comprising of glucose, galactose, arabinose, xylose and uronic acids.

Based on these results, kenaf seed dregs could be a potential source of DF compared to kenaf seed meals. The low protein content of the dregs can serve as a suitable raw material for isolating the soluble and insoluble DF fractions, as described in the following section.

Fractionation and physical characteristics of DF fractions

In this study, kenaf seed dregs were used as the raw material for the DF source. Since DF refers to the complex mixture of undigested polysaccharides, fractionation was conducted to quantify the DF compositions. Following the sequential fractionation protocol by Alba *et al.* (2018), four DF fractions were isolated from defatted kenaf seed dregs, i.e., acid-soluble and calcium-bound pectins, alkali-soluble hemicellulose, and cellulose (Figure 5) with the yield of 1.86%, 1.01%, 6.33%, and 66.33%, respectively.

The effectiveness of DF in promoting health benefits is determined by the source

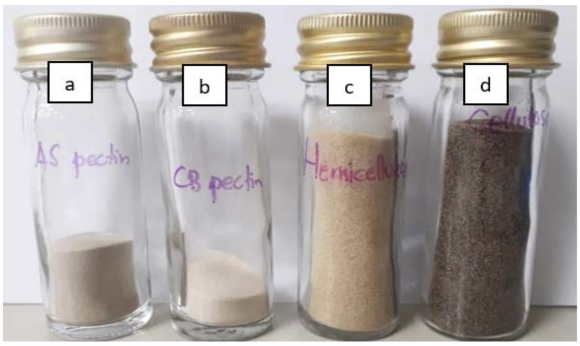

Figure 5. DF fractions from kenaf seed dregs (a) acid-soluble pectin; (b) calcium-bound pectin; (c) alkali-soluble hemicellulose; (d) cellulose

and composition of the DF (Alba *et al.* 2018). Fractionating the DF into its constituents is essential for isolating and quantifying fractions and eliminating undesirable compounds. Moreover, the properties of DF fractions could explain their technological and physiological functions in food products and their potential for other industrial applications, such as pharmaceutical sectors.

Several health-beneficial properties of soluble DF fractions, such as pectin and hemicellulose, are related to the modulation of gut microbiota. Blanco-Pérez *et al.* (2021) emphasized the potential of pectins for allergy treatment. Likewise, oligosaccharides derived from hemicelluloses play a role in alleviating autoimmune diseases, urinary tract infections, diabetes, Antimicrobial Resistance (AMR), and cardiovascular diseases. Thus, these DF fractions have similar potential to conventional prebiotics and can be supplemented into functional foods (Jana *et al.* 2021). On the other hand, cellulose, as the insoluble DF fraction, has an excellent role in bodyweight management due to its water-holding capacity, where the swelled cellulose can improve satiety (Phirom-on & Apiraksakorn 2021).

Furthermore, a comprehensive understanding of DF characteristics is required for its incorporation into food. The interactions between DF and other ingredients can significantly influence the acceptability and microstructure of the final product (Alba *et al.* 2018).

The physical properties of kenaf seed fiber fractions are presented in Table 2. The direct and bulk densities are critical factors that affect powder functionality by providing information on the order and packing of powder particles (Niknam *et al.* 2020). The direct density of both Acid Soluble-Pectin (ASP) and Calcium-Bound Pectin (CBP) were higher than Alkali-Soluble Hemicellulose (ASH) and lower than Cellulose (CEL). The same trend is shown in the bulk density values. The flow properties of these fiber fractions are calculated based on the ratio of bulk and direct densities.

In terms of powder cohesiveness, ASH demonstrated the highest value (8.71), CEL showed the lowest (1.32), whereas the ASP and CBP were in the middle (3.64–4.01). According to Jinapong *et al.* (2008), the Hausner ratio (HR) can be categorized into three, i.e., high (>1.4), intermediate (1.2–1.4), and low (<1.2). It was observed that the ASH and CEL showed the highest and lowest fiber cohesiveness, respectively. Cohesiveness is the internal bond strength of a food structure determined by the structure's deformation resistance (Noorlaila *et al.* 2017). The cohesiveness of the powder could be helpful in the development of food formulation.

The trend for powder compressibility was the same as the powder cohesiveness property. The highest to the lowest Carr Index (CI) were ASH, CBP, ASP, and CEL, with values of 88.93, 75.02, 72.52, and 22.66, respectively. The powder compressibility can be divided into several categories based on very poor CI values (>45), poor (35–45), fair (20–35), good (15–20), and very good (<15%). All the soluble fractions (ASH, CBP, ASP) and insoluble fiber (CEL) had very poor and fair compressibility, respectively. A higher HR value of soluble fractions than insoluble fiber indicates that soluble fractions are very cohesive powders but have poor flowability compared to insoluble fiber. Overall, the soluble fractions of DF demonstrated lower flowability

Table 2. Physical properties of kenaf seed fiber fractions

Parameters	Kenaf seed fiber fractions			
	ASP	CBP	ASH	CEL
Bulk density (g/ml)	0.23±0.01[b]	0.21±0.01[b]	0.07±0.00[c]	0.57±0.02[a]
Direct density (g/ml)	0.45±0.01[b]	0.42±0.00[c]	0.30±0.00[d]	0.72±0.00[a]
Powder cohesiveness (HR)	3.64±0.18[b]	4.01±0.14[b]	8.71±0.01[a]	1.32±0.05[c]
Powder compressibility (CI) (%)	72.52±1.33[b]	75.02±0.87[b]	88.93±0.72[a]	22.66±1.01[c]

The data are expressed as mean±standard deviation

Different letters in the same row denote statistically significant differences (p≤0.05)

ASP: Acid-Soluble Pectin; CBP: Calcium-Bound Pectin; ASH: Alkali-Soluble Hemicellulose

CEL: Cellulose; HR: Hausner ratio; CI: Carr Index and compressibility than the insoluble fractions. Powder flowability and compressibility are essential properties in selecting a suitable processing methods, packaging, storing, and shipping method of the powder; hence it is necessary to examine these data for future application of the samples (Niknam *et al.* 2020).

CONCLUSION

The role of kenaf seed and its by-products as potential DF sources was analysed. The kenaf seed dregs showed higher (35.08%) DF content than kenaf seed meals (20.63%) which is comparable to soybean by-products. Four different fiber fractions were identified and isolated from kenaf seed dregs, which were acid-soluble pectin (1.86%), calcium-bound pectin (1.01%), alkali-soluble hemicellulose (6.33%), and cellulose (66.33%). Results for physical properties determination demonstrated that all the soluble DF fractions had a high Hausner Ratio (HR) and Carr Index (CI), implying that they are very cohesive powders but have poor flowability compared to the insoluble fiber fraction. This study provides fundamental data on kenaf seeds as Dietary Fiber (DF) sources. However, further investigations on the techno-functional and chemical properties of kenaf seed fiber fractions are required to elicit their functions in food products and provide clues about their physiological potential for industrial use.

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DECLARATION OF INTERESTS

The authors have no conflict of interest.

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