



The Effect of Soaking Treatment on Physicochemical Properties of Kenaf Seeds MH8234

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ABSTRACT

Kenaf seeds are considered to be one of the underutilised biomaterials generated from the kenaf industry. These kenaf seeds contain essential nutrients, including dietary fibre, protein, fat, carbohydrate and phytochemicals. Consequently, they have attracted the attention of many researchers. This study aims to determine the effect of soaking treatment on the physicochemical properties of kenaf seeds MH8234. The soaking treatment can give many benefits such as easing the blending process, maximising the yield production of kenaf extract (kenaf seeds milk), improving the kenaf extract's nutritional values and decreasing the presence of anti-nutritional compounds in the seeds. Twenty grams of kenaf seeds were manually cleaned to eliminate foreign debris and soaked in excessive distilled water. Soaking was carried out for 3, 6 and 12 hours in ambient conditions. The kenaf seeds were soaked before being analysed for size distribution, proximate analysis, water absorption, colour difference and microscopic appearance. Results showed that 12-hour soaking significantly increased seed size, moisture content (16.09%) and crude protein (20.14%), while slightly decreasing crude fat, ash content and total carbohydrates. Water absorption capacity ranged from 46.57% to 81.79%. The colour difference between soaked and unsoaked seeds was notable, shifting from dark brown to light brown. Overall findings suggested that a 12-hour soaking treatment demonstrates the highest water absorption capacity that enhanced seed size, protein content, as well as pleasant colour development, making it suitable for domestic and commercial practice.

1. Introduction

Kenaf seeds or *Hibiscus cannabinus L.*, belongs to the Malvaceae family, classified under the genus *Hibiscus* and are widely grown in Sudan, Africa and Asia [1]. The kenaf seeds have irregular shapes

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and sizes, resembling shark teeth, often triangular and occasionally kidney-shaped with pointy corners. The colour of fresh and dried kenaf seeds typically appears as mixed with ash grey and pale brown. However, upon wetting, this colour transforms to a dark brown hue.

Kenaf seeds exhibit a firm texture, which can be tenderised through soaking. Soaking is widely employed as a traditional technique that is considered the easiest and most commonly used method to improve the nutritional value and decrease the presence of anti-nutritional compounds in seeds. Soaking is used in domestic technology to hydrate seeds by immersing them in water for a few hours, allowing them to absorb water [2]. Extended immersion resulted in the softening of the seeds as water can permeate the seed coat and reach the inner part of the seeds. The seed's structure become less stiff as it absorbs water. In turn, it reduces and eradicates the anti-nutritional compound found in seeds [3].

Soaking is done before various treatments, such as germination, cooking and fermentation [4]. Furthermore, the nutritional quality of the legume seeds can be enhanced by eliminating or reducing the presence of anti-nutritional compounds through soaking, germination and cooking. Soaking has been scientifically proven to remove anti-nutritional compounds effectively by discarding the soaking solution [3,4]. In addition, some metabolic reactions may occur during soaking, increasing protein solubility and digestibility. The specific effect of soaking differed depending on the cultivars of seeds and the parameters of the soaking process, including the type of soaking solution, duration of soaking and temperature. Several studies have indicated that soaking seeds for 12 to 18 hours was optimal for reducing the presence of enzyme inhibitors. These substances are wholly or partially dissolved in water used for soaking [2,3].

Over the past few years, numerous researchers have worked on researching the physical and mechanical characteristics of nuts, grains and seeds, such as chia [5], jatropha [6], cucurbit [7] and cotton [8]. Several researchers reviewed raw and soaked legume seeds' proximate composition, mineral contents and antinutritional aspects [9,10]. However, from an extensive review of the scientific literature, very little information can be found on the physicochemical properties of the novel kenaf seeds MH8234. Therefore, this research aims to study the effect of soaking treatment on the physicochemical properties of kenaf seeds MH8234, especially in terms of size distribution and expansion, proximate contents, colour changes, water absorptivity and microscopic appearance.

2. Methodology

2.1 Kenaf Seeds

The kenaf seeds of MH8234 were purchased from Zhanphu Zholong Kenaf Seed Co. Ltd, Fujian, China and stored at a chilled temperature of 0 to -5°C with relative humidity ranging from 85% to 95%. The physicochemical properties of raw and soaked kenaf seeds were evaluated at the Food Technology Laboratories, University of Technology Sarawak.

2.2 Preparation of Soaking Treatment

The soaking treatment was performed according to the methodology employed [11]. A total of 20g of kenaf seeds was manually rinsed with distilled water to wash away and eliminate any impurities and soaked in excessive distilled water. Soaking was carried out for 3, 6 and 12 hours at the ambient conditions (24 - 26°C, 65 - 75% RH).

2.3 Size Distribution

The size of kenaf seeds was evaluated by measuring their length, width and height before and after soaking. Vernier callipers were utilised for precise measurements as a similar method was employed [12].

2.4 Proximate Analysis

The proximate composition of the soaked and unsoaked kenaf seeds was analysed using the standard procedures of the Association of Official Analytical Chemistry [13], which encompassed the determination of the moisture content, crude protein, crude fat, ash and carbohydrate.

2.5 Water Absorption Capacity

The water absorption capacity was measured using the methodology developed [14]. Twenty grams of kenaf seeds was initially weighed and soaked accordingly. The soaked kenaf seeds were strained using a muslin cloth and the weight of the soaked sample was measured. The water absorption capacity was calculated using the Eq. (1).

$$\text{Water Absorption Capacity: } \frac{W_2 - W_1}{W_1} \times 100\% \quad (1)$$

Where, W_1 is the weight of kenaf seeds before soaking, (g) and W_2 is the weight of kenaf seeds after soaking, (g).

2.6 Colour Difference

The colour differences were measured using a chromameter (Konica Minolta, Japan), which measures the parameters of L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness). The calculation of the colour difference was based on Eq. (2), a similar method applied [15].

$$\Delta E = \sqrt{(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2} \quad (2)$$

2.7 Microscopic Observation

The microstructure or microscopic morphology of the kenaf seeds was examined using a digital microscope (HIROX, USA). Each sample was placed on the microscope stage and illuminated at 35 times magnification with the appropriate light intensity and illumination angle.

3. Results and Discussion

3.1 Size Distribution and Proximate Analysis

Table 1 shows a significant increase in the size of kenaf seeds following the soaking treatment, with noticeable expansions observed in all directions, including length, width and height due to imbibition [16]. This expansion in size is likely attributed to the moisture absorption during soaking treatment. This finding aligns with previous studies documenting the effects of soaking treatments

on the expansion of seed size and morphology for maize [17], chickpeas [18] and lotus [19]. The size and morphology of seeds play pivotal roles in the water absorption and germination process. Larger bean seeds have been observed to undergo expansion and germination at a swifter pace compared to smaller and medium-sized seeds [20].

Table 1
The average distribution of kenaf seed size as affected by the soaking treatment

Samples	Soaking Time (h)	Average size distribution (mm)		
		Length	Width	Height
Unsoaked	0	5.35±0.05	3.83±0.06	3.29±0.09
Soaked	3	6.11±0.14	4.27±0.03	4.02±0.13
	6	6.79±0.07	5.10±0.13	4.77±0.07
	12	7.02±0.13	6.12±0.08	5.13±0.12

The raw kenaf seeds had a moisture content of 10.14%, as depicted in Table 2. Dry seeds generally have less than 15% moisture, significantly reducing their seed activity and preventing microbiological damage. The embryo's germination occurs when dry seeds' moisture content reaches up to 24%. After soaking for 12 hours, the moisture contents of kenaf seeds MH8234 increased to 16.09%, still below the threshold for embryo germination. The moisture content of the kenaf seeds has elevated due to moisture absorption as the result of soaking treatment. The disintegration of legume cell walls, which leads to rapid water uptake, causes the change in the moisture content observed for soaked kenaf seeds. Similar moisture contents increases have been reported for maize, chickpea and lotus seeds [17-19]. The longer the soaking duration, the higher the moisture content until it reaches its peaks at a specific point [20].

Table 2
The effect of soaking treatment on the proximate contents of Kenaf seeds MH8234

Samples	Moisture content (%)	Crude protein (%)	Crude fat (%)	Ash content (%)	Carbohydrate content (%)
Unsoaked	10.14±1.05	19.23±0.12	27.31±1.16	5.17±1.10	34.15±1.03
Soaked (12 hours)	16.09±1.63	20.4±0.22	25.12±0.94	5.09±0.05	33.56±1.16

The soaking treatment slightly increases the protein content of kenaf seeds MH8234, from 19.23 to 20.14% after 12 hours of soaking treatment. Soaking is the earliest phase of water penetration, where the dormant tissues are transformed into active tissues. At this stage, the metabolic mechanism is activated to be ready for germination [21]. Moreover, it has been demonstrated that immersing and sprouting cereal and legumes can reduce the presence of trypsin inhibitor activity and oligosaccharides such as stachyose and raffinose, which are responsible for causing flatulence. Consequently, this soaking treatment increases the ability to break down proteins and enhances the sensory qualities. Soaking leads to the biological breakdown of complex molecules into simpler ones and extending the soaking duration to 12 hours significantly increases the total protein content [22]. In this study, the kenaf seeds had a crude protein level of 19.24% of the dry weight. This value is slightly lower than the findings reported by Abd El-Hady *et al.*, [22] and Ab Razak *et al.*, [23]. The difference in values could be due to the type of kenaf seeds, cultivation conditions and soil composition of the kenaf plant.

The fat content of raw kenaf seeds reduced from 27.31 to 25.12% after a 12-hour soaking period. Immersing the kenaf seeds resulted in a notable reduction in their lipid content. The crude fat content

of kenaf seeds decreases by 2.19%. The reduction in fat content may be related to the increase of lipolytic enzymes during soaking and germination or the breakdown of fat through β -oxidation for energy purposes in embryo development [24]. The results align with the study conducted by Inyang *et al.*, [27], which observed a reduction in the fat content of malted millet during the soaking process. A similar decreasing pattern was also observed in raw soybeans, with the initial fat content at 10.6%. Following the soaking process, the fat percentage decreased to 4.48% [25-28].

Table 2 also demonstrated that the ash content of kenaf seeds slightly decreased as the treatment time was prolonged to 12 hours. The immersion of the material in water resulted in a reduction of 0.08% in ash content, which might potentially be leached off throughout the process. The reduction in ash content was noticed after a specific length of soaking time for soybeans, koro beans and sorghum seeds [29-31]. This reduction is produced by mineral leaching and a decrease in antinutrients that can bind to minerals during soaking.

The carbohydrate content of unsoaked and soaked kenaf seeds slightly decrease from 34.15 to 33.56%. Soaking alters the chemical composition of kenaf seeds. This disparity could be ascribed to the loss of the constituent with the bran and outer layers of the endosperm [32]. The soaking process resulted in a reduction of the solubilised carbohydrate contents of kenaf seeds. El-Safy *et al.*, [33] documented decreased carbohydrate contents linked to amylose leaching during soaking of some legumes and cereals grain seeds.

3.2 Influence of Soaking Treatment on the Water Absorption Capacity

During soaking treatment, water permeates into the kernel, resulting in an enlargement of the seeds. Table 3 provides the absorption measurement findings at 3, 6 and 12 hours. The soaking duration has a substantial effect on water absorption. Water absorption is directly proportional to the soaking duration, as evidenced by the increase in soaking duration from 3 to 12 hours, which resulted in a rise in water absorption from 14 to 18%. A study by Vasudeva *et al.*, [34] found that the high hydration in the seeds could be attributed to the presence of hemicellulose and pentosans in the seed coat, facilitating water absorption. Legumes use soaking to absorb water from their environment to trigger metabolic activities [33,34]. Dry legumes exhibit quick water absorption, regulated by their structural composition. The gradual increase in water absorption over time can be attributed to the growing number of cells within the seed undergoing hydration [35]. Similar findings have been documented for various agricultural materials, including sorghum, milled rice, soybean and barley seeds [36].

Table 3
The effect of soaking treatment on the water absorption of kenaf seeds MH8234

Soaking duration	Water absorption (%)
0	-
3	46.57±3.46
6	60.30±4.28
12	81.79±2.73

3.3 The Effect of Soaking Treatment on the Colour of Kenaf Seeds

Figure 1 depicts images of kenaf seeds before and after soaking for 3, 6 and 12 hours. The colour changed from ash grey or pale brown for dried fresh seeds to dark brown upon contact with water. After soaking for 6 to 12 hours, the colour of the kenaf seeds shifted to a light brown hue.



Fig. 1. Images of unsoaked and soaked kenaf seeds MH8234 (a) Unsoaked kenaf seeds (b) 3 hours of soaking (c) 6 hours of soaking (d) 12 hours of soaking

The dried fresh seeds exhibit L^* , a^* and b^* values of 22.13, 2.35 and 7.62, respectively (Table 4). Upon contact with water, the lightness value drops drastically from 22.14 to 21.77 and a dark brown colour immediately appears. These rapid colour changes could be attributed to water absorption by the dried surface of the seeds, reducing the lightness intensity. Extending the soaking duration from 3 to 12 hours demonstrates an increasing trend in the redness or a^* values, moving towards positive values and a more pleasant colour. Similar trends are observed in the yellowness or b^* values.

The increase in redness and yellowness could be attributed to the native phenolic compounds in the kenaf seeds. The non-degradative soaking treatment at ambient temperature causes the seeds to absorb water, expanding and exposing undamaged carotenoids and flavonoids compounds. The rise in redness (a^*) and yellowness (b^*) values observed throughout the soaking duration shows an inverse correlation with the L^* value. Similar findings have been reported by Lopez *et al.*, [37] and Bayram *et al.*, [38].

Table 4
 Colour properties of soaked and unsoaked Kenaf seeds MH8234

Samples	Soaking times (hours)	Colour			
		L^*	a^*	b^*	ΔE
Unsoaked	0	22.14±0.53	2.35±0.41	7.62±0.13	-
Soaked	3	21.77±0.03	3.34±0.22	7.73±0.42	1.06±0.15
	6	21.06±0.45	4.92±0.58	8.52±0.64	2.93±0.25
	12	20.89±0.74	6.45±0.34	9.67±0.18	4.72±0.37

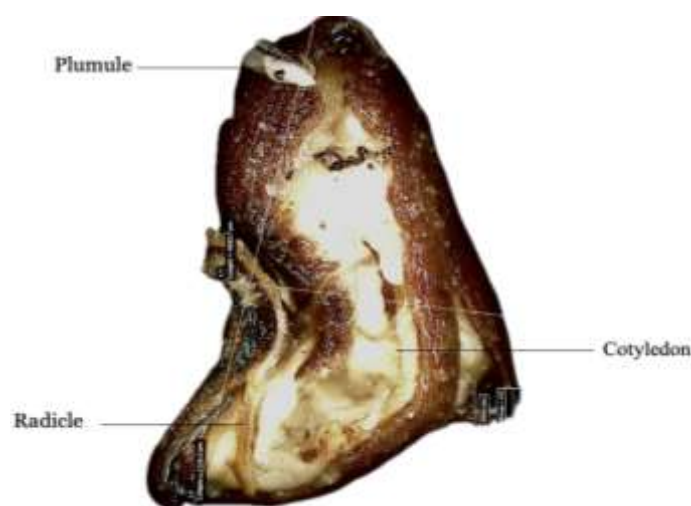
3.4 The Effect of Soaking Treatment on Microscopic Observation

The image from a digital microscope enables clear viewing of the seeds' colour, surface structure and seed components such as seed coat, embryo and endosperm. Figure 2 shows a typical kenaf seed's external structure and Figure 3 shows the internal structures after being soaked and cut open.



External appearance

Fig. 2. The external appearance of kenaf seeds MH8234



Embryo cut opened

Fig. 3. The internal appearance of kenaf seeds MH8234

The structure of kenaf seeds closely resembles that of legume seeds, comprising a micropyle, seed coat, dicotyledon and embryonic axis (radicle and plumule). The cotyledon, rich in protein and starch, is the primary nutritional component of legume seeds [39]. During soaking, kenaf seeds undergo a physiological process known as imbibition, wherein water permeates structures such as the micropyle and seed coat, leading to seed swelling [40]. Initially, dry kenaf seeds show no signs of water uptake, as depicted in Figure 2 and Figure 4.



Fig. 4. Digital microscope images of kenaf seeds MH8234, (a) Fresh, unsoaked seed (b) Cross-sectional of unsoaked seed

However, prolonged soaking results in a discernible increase in seed size due to water absorption, as shown in Figure 5. This swelling is a direct consequence of imbibition, during which water molecules penetrate the seed's outer layers, causing internal tissues to swell. Imbibition marks a crucial stage in seed germination, initiating metabolic processes within the seed. This water uptake rehydrates the seed and facilitates essential enzymatic reactions to break dormancy and initiate cellular respiration [40,41].



Fig. 5. Digital microscope images of kenaf seeds, (a) Soaked Seed, (b) Cross-sectional soaked seed

4. Conclusions

Overall, the effect of the soaking treatment on the physicochemical properties of kenaf seeds suggests that after 12 hours of soaking treatment, the size distribution of dried kenaf seeds increased from 3-5mm to 5-7mm, which is supported by the water absorption value of 82%. The soaking technique demonstrated significant efficacy in enhancing the availability of certain nutritional

constituents, particularly crude proteins, which increased to 20%. The colour transitioned from ash grey or pale brown for dried fresh seeds to dark brown upon contact with water and further changed to light brown. This soaking treatment caused the kenaf seeds to swell, resulting in a better appearance and a more pleasant colour. Therefore, soaking for 12 hours is deemed suitable for both domestic and commercial practices of kenaf seeds MH8234. Extending the soaking duration to more than 12 hours could be suggested for future study.

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