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Influence of kenaf (*Hibiscus cannabinus* L.) leaves powder on the physico-chemical, antioxidant and sensorial properties of wheat bread

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Abstract

Hibiscus cannabinus L. or commonly known as kenaf is a multipurpose crop with various medicinal and nutritional properties. The aim of this study was to determine the antioxidant, nutritional, and functional properties of kenaf leaves powder. In addition, the effect of kenaf leaves powder fortification in bread properties and consumer acceptability were evaluated by production of three formulations of bread with 0% (control), 4% and 8% substitution of kenaf leaves powder per weight of flour used. From the sensory evaluation, 4% leaves powder bread was the most preferred by the panelists followed by 0% leaves powder bread then 8% leaves powder bread. Therefore, 4% leaves powder bread was chosen as the best formulation for this study and it showed significantly higher value of DPPH free radical scavenging capacity (8.05 mg TE/100 g), total phenolic content (12.9 mg GAE/100 g) and total flavonoid content (13.3 mg QE/100 g) compared to control bread (1.38 mg TE/100 g, 8.17 mg GAE/100 g and 8.77 mg QE/100 g, respectively). Besides, 4% leaves powder bread also showed improvement in calcium and total dietary fibre compared to control bread. Given these results, kenaf leaves powder can be used in baking formulation up to 4% with flour to enhance the functional properties of baking formulation without compromising eating quality.

Keywords *Hibiscus cannabinus* L. · Kenaf · Functional food · Antioxidant properties · Nutritional properties · Bread

Introduction

As technology and education advances, consumers nowadays are gaining more conscious on the fact that food directly contribute to their health and are shifting from choosing food that solely provide satiety to food that have additional nutritional values. Functional food is known as food with additional component, which are health improving, beyond just providing basic nutrition. Functional food began in Japan in the 1980s, when the government aim to improve overall population health and reduce cost on healthcare [1]. Recent years, functional food added with antioxidant properties are many research interests as the reactive oxygen species and free radicals are powerful oxidants that cause several health problems. Moreover, lipid oxidation during the manufacturing process and food storage leads to an increase in the number of reactive radicals, which cause the loss of nutritional and sensory quality of foods [2].

With the wide acceptance of bakery product as a part of the daily meal worldwide, bread was seen to be a great vehicle to deliver functionality. Bread among all the baked goods are one of the staples to many developing countries and regions making it potentially favourable for fortification to be a functional food. Many researches were conducted on nutrient fortification of bread through addition of various plant parts. In addition, the rising demand for novel and healthy foods together with the increasing lifestyle diseases has dramatically driven a new market for bread using alternative flours to wheat [3].

Hibiscus cannabinus or commonly known as kenaf is a tough herbaceous plant from the *Malvaceae* family, which is natively grown in Africa [4]. It is a multi-purpose crop as it has many medicinal and nutritional values to human, it also is a great feed for domesticated farm animals. Many researches had shown that kenaf leaves are high in

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antioxidant content and calcium [5, 6]. Despite many researches were done on kenaf plants especially its leaves and many positive results shown on the medicinal potential of the leaves in antioxidant properties, such as hepatoprotective effect and anti-hyperlipidemia effect, fortification of kenaf leaves into bread to improve nutritional level was seldom conducted and none investigate on the antioxidant properties of the fortified bread [7]. Thus, in this research, kenaf leaves powder was fortified into bread to determine its effect on the antioxidant, nutritional and physical properties of the fortified bread as well as the antioxidant, nutritional and functional properties of the kenaf leaves powder.

Materials and methods

Preparation of leaves powder

The fresh kenaf (*Hibiscus cannabinus* L.) leaves were obtained from Lembaga Kenaf dan Tembakau Negara (LKTN, Malaysia). The leaves were rinsed using water then oven dried at 100 °C for 1 h using convection oven (SM400, MEMMERT, Germany). Then, the leaves were ground using a grinder (MX-GM1011H, Panasonic, Japan) till fine particle size of 1 mm. The leaves powder was then vacuum packed and wrapped in aluminium foil to reduce possibility of oxidation by air and light. Then, it was stored at − 20 °C until use for a maximum of 12 weeks.

Proximate analysis

The moisture, ash, crude fat, crude protein, and crude fibre were determined using the AOCS Methods Ba 2a-38, Ba 5a-49, Ba 3-38, Ba 4a-38, Ba 6-84 respectively [8], while total dietary fibre was determined using AOAC method 985.29 [9]. Total protein was calculated using conversion

factor (6.25) and total carbohydrate was calculated based on difference by using the formula below:

Total carbohydrate in leaves powder(%)

$$= 100\% - (\% \text{ of moisture}$$
$$+ \% \text{ of ash} + \% \text{ of fat} + \% \text{ of protein}$$
$$+ \% \text{ of crude fibre})$$

Total carbohydrate in bread(%)

$$= 100\% - (\% \text{ of moisture} + \% \text{ of ash}$$
$$+ \% \text{ of fat} + \% \text{ of protein}$$
$$+ \% \text{ of total dietary fibre})$$

Calcium analysis

Sample was extracted according to the method of Uzockwe and Mohammed [10], with slight modifications. Five grams of sample and a tablet of selenium catalyst were added into 20 mL of nitric acid and then heated on a hotplate at 280 °C until the mixture was clear. The mixture was then filtered into a 50 mL volumetric flask and topped up to mark with distilled water. Then, 9.8 mL of sample extract was added to 0.2 mL of 5% lanthanum chloride solution. The calcium content was measured using flame atomic absorption spectrophotometer (FAAS) (AAnalyst 100, Perkin Elmer, USA).

Antioxidant properties

Sample extraction

The sample for antioxidant analysis was extracted base on Nyam et al. with slight modification [11]. First, 0.1 g of leaves powder were transferred into a 15 mL falcon tube and added with 10 mL of methanol then shaken using a mechanical shaker at 4×*g* (200 rpm in a 1633 motor, EBA280, Hettich, Germany) for 1 h. The mixture was then filtered, and the filtrate was obtained and stored at 4℃ until use (maximum 1 day). For bread sample, 2.0 g of the sample was used and the procedures were repeated.

DPPH radical scavenging activity

The DPPH radical scavenging activity was determined according to Jin et al. [12] method. One mL of the sample extract was added to 4 mL of 0.15 mM methanolic DPPH solution and vortex to mix well. The mixture was then kept in the dark at room temperature for 30 min before measured at 517 nm using a UV–Vis spectrophotometer (SECO-MAM UviLine 9400, Kisker Biotech, Germany). The DPPH

Table 1 Formulations of fortified bread

Ingredient	Control (0%) (g)	4% (g)	8% (g)
High protein flour	50	48	46
Kenaf leaves powder	0	2	4
Active dry yeast powder	2	2	2
Sugar	2	2	2
Salt	0.6	0.6	0.6
Butter	4	4	4
Water	40	40	40
Milk powder	1.4	1.4	1.4
Total weight	100	100	100

radical scavenging activity was expressed as mg Trolox equivalent (TE)/100 g sample and in % activity using the equation below:

DPPH radical scavenging activity$(\%) = (1 - A/B) \times 100$

where A = absorbance of sample, B = absorbance of control (without sample).

ABTS cation radical scavenging activity

The ABTS cation free radical scavenging activity was determined according to Cao et al. [13] method. ABTS cation stock solution was made by reacting the 7 mmol/L ABTS stock with 2.45 mmol/L potassium persulfate solution and kept in the dark at room temperature for 12–16 h before usage. For analysis, the ABTS cation stock solution was diluted with 80% methanol to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 4.85 mL of the diluted ABTS solution was added to 0.15 mL of sample extract and vortex to mix well. The absorbance reading at 734 nm were taken 6 min after initial mixing using a spectrophotometer (SECOMAM UviLine 9400). ABTS cation free radical scavenging activity was expressed as mg Trolox equivalent (TE)/100 g sample and in % activity using the formula below:

ABTS cation free radical scavenging activity$(\%) = (1 - A/B) \times 100$

where A = absorbance of sample, B = absorbance of control (without sample).

Total phenolic content (TPC) and total flavonoid content (TFC)

The total phenolic content was determined according to Liu et al. [14] method. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/sample. The total flavonoid content was determined according to Jin et al. [12] method. The total flavonoid content was expressed by mg quercetin equivalent (QE)/100 g sample.

Functional properties

Swelling capacity

The swelling capacity was determined according to the method of Rosell et al. [15], with modifications. One gram of sample was weighed into a 50 mL falcon tube and added 20 mL of distilled water then allowed to hydrate for 24 h at room temperature $(25 \pm 1\ °C)$. The volume of distilled water in the tube was measured after 24 h. Swelling capacity was expressed as mL/g of sample.

Water holding capacity

The water holding capacity was determined according to the method of Sangnark and Noomhorm [16], with modifications. One gram of sample was weighed into a 50 mL falcon tube and added with 20 mL of distilled water then allowed to hydrate for 24 h at room temperature $(25 \pm 1\ °C)$. After 24 h, the excess water was filtered off and the weight of hydrated sample was measured. Water holding capacity was expressed as g of water/g of sample.

Water retention capacity

The water retention capacity was determined according to the method of Nyam et al. [11]. One gram of sample was weighed into a 50 mL falcon tube and added 30 mL of distilled water then allowed to hydrate for 24 h at room temperature $(25 \pm 1\ °C)$. After 24 h, the falcon tube was centrifuged at $1008 \times g$ (3000 rpm) for 20 min. The supernatant was weighed and recorded. Water retention capacity was expressed as g of water/g of sample.

Oil holding capacity

The oil holding capacity was determined according to Alfredo et al. [17] method. One gram of sample was weighed into a 50 mL falcon tube and added 20 mL of corn oil and stored in a cabinet for 24 h at room temperature $(25 \pm 1\ °C)$. After 24 h, the falcon tube was centrifuged at $542 \times g$ (2200 rpm) for 30 min. The supernatant was weighed and recorded. Oil holding capacity was expressed as g of oil held/g of sample.

Organic molecule adsorption capacity

The organic molecule adsorption capacity was determined according to Alfredo et al. [17]. Three grams of sample was transferred into a 50 mL falcon tube and added 10 mL corn oil and stored in a cabinet for 24 h at room temperature $(25 \pm 1\ °C)$. After 24 h, the tube was centrifuged at $448 \times g$ (2000 rpm) for 15 min. The supernatant was weighed and recorded. Organic molecule adsorption capacity was expressed as g of oil held/g of sample.

Emulsifying ability

The emulsifying ability was determined according to Alfredo et al. [17] method. Two grams of sample was transferred into a beaker and added 100 mL of distilled water. The mixture was homogenized for 2 min and then added 100 mL of corn oil, followed by homogenization for 1 min. The emulsion was then transferred to a 50 mL falcon tube and centrifuged at 1200 rpm for 5 min. The emulsion volume

was recorded. The emulsifying activity was expressed as mL of emulsion/100 mL emulsion volume.

Emulsifying stability

The emulsifying stability was determined according to Alfredo et al. [17] method. The emulsion from emulsifying ability analysis was heated at 80 °C for 30 min and then allowed to cool to room temperature. The emulsion was then homogenized for 1 min and transferred to a 50 mL falcon tube. The tube was centrifuged at $161 \times g$ (1200 rpm) for 5 min. The emulsion volume was recorded. The emulsifying stability was expressed as mL of emulsion/100 mL emulsion volume.

Preparation of bread

Three formulations of bread were prepared for this study based on 0%, 4% and 8% substitution of high protein flour with the kenaf leaves powder, respectively (Table 1). Control (0% substitution) was prepared base on 50 g of high protein flour and used as a comparison to the other formulations. The breads were baked at 150 °C for 20 min in a convection oven (MEMMERT).

Physical properties

The loaf volume was determined according to the method of Abdul-hamid and Luan [18]. While, the dough expansion was determined according to the method of Sangnark and Noomhorm [16]. The bread texture was determined according to AACC method 74-10.02 [19]. A slice of bread sample with thickness of 2.5 cm was prepared. The bread slice was placed on the texture analyser platform (CT3 Texture Analyzer, Brookfield, USA) and compressed using the following settings (speed: 2 mm/s, distance: 10 mm, trigger: Auto 5 g trigger, probe: 38.1 mm Perspex cylinder). The hardness, springiness, cohesiveness, chewiness and adhesiveness were recorded. The crumb colour was determined according to Nyam et al. [20] method. A slice of bread sample was placed in the sample cup and measured in a colorimeter (Colour-Flex EZ, Hunterlab, USA) for surface colour L—brightness, a—redness and b—yellowness.

Sensory analysis

Twenty trained panelists were involved in the hedonic test. Each panelist was given a set of sensory evaluation questionnaire and required to taste the bread samples (control, 4% fortified bread and 8% fortified bread), which were randomly

labelled with three-digit numbers to avoid bias. The panelists were required to rate according to their preferences each of the sample based appearance, aroma, flavour, texture and overall acceptability. The sensory attributes were rated based on a nine-point hedonic scale in which "1" was dislike extremely, "5" was neither like nor dislike and "9" was like extremely.

Statistical analysis

For each analysis the means and standard deviation were determined and analysed using One-way ANOVA Minitab (Version 18, Minitab Pty Ltd., Sydney, Australia). Significant difference was determined when $p < 0.05$.

Results and discussion

Proximate, antioxidant and functional analyses

Table 2 shows the result for proximate, antioxidant and functional analysis of kenaf leaves. The kenaf leaves powder contained 2.84% of moisture. The moisture content was lower compare to previous study by Nasir et al. [21], which stated that 9% moisture in flour was suitable for storage, therefore indicating the longer shelf life of kenaf leaves powder. The

Table 2 Proximate, antioxidant and functional analyses of kenaf leaves powder

Characteristic	Leaves powder
Moisture (%)	2.84 ± 0.17
Crude fat (%)	5.70 ± 0.38
Crude protein (%)	25.0 ± 0.00
Ash (%)	3.49 ± 1.77
Crude fibre (%)	8.57 ± 1.96
Total carbohydrate (%)	64.4
Calcium (mg/100 g)	163.0 ± 1.71
Total phenolic compound (g GAE/100 g)	10.3 ± 0.58
Total flavonoid compound (g QE/100 g)	14.6 ± 0.04
DPPH radical scavenging activity (g TE/100 g)	6.97 ± 0.28
DPPH radical scavenging activity (%)	84.2 ± 3.53
ABTS cation radical scavenging activity (g TE/100 g)	14.6 ± 0.21
ABTS cation radical scavenging activity (%)	94.2 ± 0.21
Swelling capacity (mL/g)	12.9 ± 3.01
Water holding capacity (g/g)	3.94 ± 0.74
Water retention capacity (g/g)	4.16 ± 1.20
Oil holding capacity (g/g)	1.34 ± 0.37
Oil molecule adsorption capacity (g/g)	0.98 ± 0.18
Emulsifying ability (ml/100 mL)	85.4 ± 5.37
Emulsifying stability (mL/100 mL)	49.5 ± 9.85

ash content of kenaf leaves powder was 3.49%. Kenaf leaves contained many minerals, such as iron and calcium, it contained 1.4 times more of iron compared to roselle leaves [5]. Based on the results, kenaf leaves powder had 163 mg/100 g calcium. This was especially important in Malaysia, which the calcium intake was low in children, adolescence and women [22].

The kenaf leaves powder also showed low crude fat content (5.70%). Kenaf leaves powder had a high crude protein content of 25.0%, allowing it to be ranked in the higher end of dried leafy vegetables, which typically range from 15 to 30% [23]. The crude fibre content of kenaf leaves powder was 8.57%. These results were slightly lower compared to previous finding on the nutritional content of kenaf as feed crops, which found that dried kenaf leaves had 11.6% crude fibre due to the method used, which some true fibre might be solubilised particularly hemicellulose, pectin and lignin [24].

The DPPH radical scavenging activity of kenaf leaves powder was high (6.97 g TE/100 g). This was supported by the TPC and TFC results of kenaf leaves powder and consistent with the result of previous studies [25]. The ABTS cation radical scavenging activity for kenaf leaves powder was 14.6 g TE/100 g. This was higher than DPPH radical scavenging activity as ABTS assay is more suitable for hydrophilic antioxidant system; highly pigmented plants, fruits and vegetables also shown better result when analysed with ABTS assay. As kenaf has higher hydrophilic antioxidants, such as catechins and phytol, thus, ABTS assay was better at extracting the antioxidant presents within the matrix.

Based on Table 2, kenaf leaves powder had 10.3 g GAE/100 g total phenolic content (TPC). This high TPC content was due to the abundance of phenolic compound, in which kaempferitrin was the most abundance in methanolic extract of kenaf leaves based on a UPLC analysis [25]. The

kenaf leaves powder also showed higher total flavonoid content (TFC) (14.6 g QE/100 g) compared to plants of the same species, such as roselle. According to Hosomi et al. [5], kenaf contained as least 3 time more of epicatechin and 1.7 times more of epigallocatechin than roselle leaves powder.

Kenaf leaves powder showed high swelling capacity (12.9 mL/g) and this may be due to low fat content (5.70%) of the kenaf leaves as hydration properties improve with the decrease in lipid as residual oil trapped within the fibre matrix may restrict the water entry [26]. Kenaf leaves powder also had higher water holding capacity (3.94 g/g) and water retention capacity (4.16 g/g) than its oil holding capacity (1.34 g/g). This phenomenon was due to the presence of more starch and soluble fibres allowed more polysaccharides, which are hydrophilic with hydroxyl groups to bind more water molecules. The low oil holding capacity beneficial for production of low-fat bakery product. The organic molecule adsorption capacity of kenaf leaves powder was 0.98 g/g. This is relatively low compare to chia seed fibre (1.09 g/g) and wheat hull (1.98 g/g) but higher than soybean fibre and passion fruit fibre with 0.65 g/g and 0.28 g/g respectively [17]. Kenaf leaves powder had a high emulsifying ability of 85.4 mL/100 mL and emulsifying stability of 49.5 mL/100 mL. This indicated kenaf emulsion did not break down easily upon thermal treatment.

Physical analysis

The loaf volume and dough expansion showed a decrease in value when the fortification of kenaf leaves powder increased as shown in Table 3. Significant difference was observed between the loaf volume of 8% and 4% kenaf leaves powder. The decrease in dough expansion upon fortification of kenaf leaves powder are due to particle size of kenaf leaves powder and the presence of phenolic compounds. Coarser particle

Table 3 Effect of kenaf leaves powder flour substitution on physical characteristics of the bread

Characteristics	Control	4% leaves powder	8% leaves powder
Loaf weight (g)	93.8 ± 1.79^a	96.2 ± 3.69^a	96.17 ± 0.88^a
Loaf volume (cm^3)	348 ± 2.89^a	370 ± 23.1^a	242 ± 9.24^b
Specific volume (cm^3/g)	3.70 ± 0.04^a	3.84 ± 0.09^a	2.51 ± 0.07^b
Colour			
L*	76.9 ± 0.09^a	54.6 ± 1.24^b	42.5 ± 1.25^c
a*	1.53 ± 0.16^b	2.33 ± 0.60^b	3.97 ± 0.35^a
b*	21.7 ± 0.16^b	34.0 ± 2.78^a	31.8 ± 0.49^a
Hardness	310 ± 82.9^c	466 ± 72.1^b	726 ± 34.1^a
Cohesiveness	0.88 ± 0.04^a	0.84 ± 0.04^a	0.89 ± 0.07^a
Springiness	9.45 ± 0.13^a	9.25 ± 0.33^a	9.60 ± 0.18^a
Adhesion	0.60 ± 0.00^a	0.55 ± 0.58^a	0.63 ± 0.13^a
Chewiness	2556 ± 651^b	3658 ± 799^b	6236 ± 764^a

Mean and standard deviation (n = 4) values in the same row with different superscripts differ significantly (p < 0.05)

Fig. 1 Sliced 0% leaves powder bread (left), 4% leaves powder bread (middle), 8% leaves powder bread (right)

Table 4 Sensory attributes of three different formulations of bread in hedonic test

Sensory attributes	Control	4% leaves powder	8% leaves powder
Appearance	6.25 ± 1.52^a	6.65 ± 1.42^a	6.45 ± 1.64^a
Aroma	6.37 ± 1.21^a	6.65 ± 1.23^a	6.60 ± 1.64^a
Flavour	6.35 ± 1.27^{ab}	6.60 ± 1.47^a	5.40 ± 1.73^b
Texture	6.45 ± 1.43^b	7.53 ± 0.77^a	6.15 ± 1.57^b
Overall acceptability	6.20 ± 1.06^{ab}	6.58 ± 1.14^a	5.85 ± 1.46^b

Mean and standard deviation (n = 20) values in the same row with different superscripts differ significantly (p < 0.05)

of leaves powder tend to absorb more water and dilute the gluten structure thus impaired the carbon dioxide retention, leading to low dough expansion [16]. Phenolic compounds were able to form reversible complexes with the protein and polysaccharides in the dough. These interactions increased the molecular weight of the gluten-phenolic network and thus reducing the max resistance of extensibility [27].

Fortification of kenaf leaves powder contributed a green colour to the bread and the presence of fibre in the leaves increased the darkness of crumb colour as shown in Fig. 1 [15]. L* value decreased with the increased in kenaf leaves fortification indicating decrease in brightness. 4% kenaf leaves powder (54.6) and 8% kenaf leaves powder (42.5) were darker than control bread (76.9). For a* value, 4% leaves powder (2.33) and 8% leaves powder (3.97) were redder than control bread. For b* value, 4% leaves powder (34) was the most the yellowish followed by 8% leaves powder (31.8) and the least being control (21.7).

Based on Table 3, the hardness of bread increased with the substitution of kenaf leaves powder into the flour with 8% leaves powder (726) being the hardest followed by 4% leaves powder (466) and control bread (310) being the softest. The incorporation of fibre through addition of kenaf leaves was directly linked with the increased in bread hardness. This phenomenon was also observed when quinoa leaves (1–5%) was incorporated in the bread [28]. No significant difference was observed for cohesiveness, springiness and adhesion between different level kenaf leaves powder fortification.

In terms of chewiness, 8% leaves powder was significantly chewier compared to the 4% leaves powder and control bread. This phenomenon was due to the high content of phenolic compound that interfered with the gluten network and changed the nature of starch [27]. This was also observed in green tea extract fortified bread and Shatavari (*Asparagus racemosu*s) fortified [29].

Sensory evaluation

In terms of overall acceptability, 4% leaves powder had the highest mean score of 6.58 followed by control bread at 6.20 and the least preferred being 8% leaves powder with a mean score of 5.85. Thus, 4% leaves powder bread was chosen as the best formulation. Based on Table 4, the appearance and aroma of 4% leaves powder was more preferred by panelist followed by 8% leaves powder and control. The score around 6 to 7 indicate that the panelists were not intimidated by the green colour and liked the tea fragrance of the fortified bread. The tea fragrance was due to presence of catechin a flavonoid compound found often in tea leaves.

The fortification of kenaf leaves powder provided the bread with a distinct sour taste and slight bitter aftertaste. The sour taste is due to the high level of malic and polylactic acids in kenaf leaves [30]. The bitter aftertaste is due to the presence of high amount of catechin in the leaves [5]. In terms of flavor, 4% leaves powder was the most preferred as it balanced the sour and bitter taste. The fortification of kenaf leaves powder increased the hardness and adhesion of the bread as shown in Table 3. The 4% leaves powder was the best formulation as it had a great balance between the hardness and adhesion, which provided a great mouthfeel. The control bread was too soft while 8% leaves powder was too firm and adhesive when chewed thus was less preferred.

Proximate and antioxidant analyses of control and 4% leaves powder bread

Based on Table 5, 4% leaves powder had significantly higher ash content compared to control bread. The higher content of ash was directly related to the higher mineral content of kenaf leaves powder. This was supported by the calcium analysis, which the 4% leaves powder bread (3.31 mg/100 g) had significantly higher amount (p < 0.05) of calcium compared to the control bread (2.20 mg/100 g). In terms of crude fat content, 4% leaves powder bread (0.50%) was significantly lower compared to control bread

Table 5 Proximate and antioxidant analyses of control and 4% leaves powder bread

Characteristic	Control	4% leaves powder
Moisture (%)	30.2 ± 4.84^a	30.7 ± 1.25^a
Crude fat (%)	1.00 ± 0.00^a	0.50 ± 0.26^b
Crude protein (%)	9.58 ± 0.08^b	10.4 ± 0.21^a
Ash (%)	2.05 ± 0.06^b	3.75 ± 0.62^a
Total dietary fibre (%)	2.76	4.00
Total carbohydrate (%)	53.3	51.4
Calcium (mg/100 g)	2.20 ± 0.02^b	3.31 ± 0.04^a
Total phenolic compound (mg GAE/100 g)	8.17 ± 0.77^b	12.9 ± 0.77^a
Total flavonoid compound (mg QE/100 g)	8.77 ± 1.09^b	13.3 ± 0.18^a
DPPH radical scavenging activity (mg TE/100 g)	1.38 ± 0.01^b	8.05 ± 1.18^a
DPPH radical scavenging activity (%)	29.5 ± 0.06^b	53.9 ± 4.34^a
ABTS cation radical scavenging activity (mg TE/100 g)	5.85 ± 0.68^b	15.9 ± 0.17^a
ABTS cation radical scavenging activity (%)	32.3 ± 1.31^b	51.9 ± 0.31^a

Mean and standard deviation (n=4) values in the same row with different superscripts differ significantly ($p < 0.05$)

Mean value for total carbohydrate and total dietary fibre (n=2), without standard deviation

(1.00%). The lower fat content was due to the low oil holding capacity of the kenaf leaves powder. An increase in protein content was observed in 4% leaves powder bread compared to the control bread. This was due to the high protein content of kenaf leaves powder as analyzed previously. This phenomenon showed that kenaf leaves powder is suitable to be substituted into flour to increase the protein content in bread products. In terms of total dietary fibre, 4% leaves powder bread (4.00%) also showed higher amount compared to control bread (2.76%). This was due to the high fibre content of kenaf leaves powder [5]. The total dietary fibre content of 4% kenaf leaves bread was higher compared to whole meal bread (0.4%) according to the Food Composition Database by Nutrition Society of Malaysia, this is beneficial in improving the digestive system for consumer suffering from indigestibility or constipation [31]

In terms of Trolox equivalent, the DPPH radical scavenging activity of 4% leaves powder bread (8.05 mg TE/100 g) significantly increased compared to the control bread (1.38 mg TE/100 g). The 4% leaves powder bread showed 82.8% increase in radical scavenging activity compared to control bread. Similar pattern was also observed for the ABTS cation radical scavenging activity of 4% leaves powder bread (15.9 mg TE/100 g), which had significantly increased compared to control bread (5.85 mg TE/100 g). The 4% leaves powder bread showed 60.9% increase in radical scavenging activity compared to control bread. The results from these radical scavenging activities both showed that phenolic compound and antioxidant properties of kenaf leaves were not destroyed at the high temperature of 150 °C during baking.

Even though higher fortification of kenaf may have better antioxidant properties but it may also lead to decrease in antioxidant if exceeded the optimum level. This was shown by fennel seed fortification in bread, which a drop in DPPH radical scavenging activity when it exceeded 5% fortification [32]. This phenomenon was due to the phenolic compound might react with the starch in wheat flour and form large complexes, which reduce the antioxidant properties as well as sensory properties [27].

Based on the results, 4% leaves powder bread (12.9 mg GAE/100 g) had higher total phenolic content than control bread (8.17 mg GAE/100 g). 4% leaves powder bread exhibited 57.7% increase in phenolic content compared to the control bread. The increase in total phenolic content of 4% leaves powder carried upon from the phenolic compound found naturally in kenaf leaves, such as kaempferitrin and caffeic acid mentioned previously. As shown in Table 5, 4% leaves powder bread (13.3 mg QE/100 g) had higher total flavonoid content compared to the control bread (8.77 mg QE/100 g). 4% leaves powder bread exhibited 51.2% increase in flavonoid content compared to the control bread. Catechin was able to be observed physically from the distinct tea aroma and bitter flavor of the 4% leaves powder bread. In a recent study, the substitution of catechin, such as epigallocatechin in flour for bread was able to reduce the formation of acrylamide a carcinogen during high temperature baking up to 37% compared to the control bread [33]. Thus, reducing the risk of consuming carcinogenic compounds.

Conclusion

The high antioxidant content, low fat content of the kenaf leaves powder is essential in the current functional food market targeting prevention of chronic disease caused by unhealthy lifestyle and diet. The fortification of kenaf leaves into bread also improved total dietary fibre compared to commercial white bread. This can help to reduce cholesterol and constipation. Kenaf leaves powder addition at 4% had the highest overall sensory acceptance. This research may stimulate further interest in developing healthy bakery products fortified with kenaf leaves powder to address the ever-rising occurrence of medical conditions among Asian and Chinese populations.

Compliance with ethical standards

Conflict of interest The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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