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Preparation and characterisation of protein concentrates from defatted kenaf seed

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ABSTRACT

Two kenaf varieties QP3 and V36 were used to obtain protein concentrates. Proximate analysis, foaming, water and oil absorption properties were studied. Significant ($P < 0.05$) differences were observed among the two varieties only in their content in oil and carbohydrates. The protein concentrate yield was 13.04% and 10.56%, respectively. The two varieties showed significantly different ($P < 0.05$) water and oil absorption capacities. QP3 showed higher foaming capacity than did V38, and it was increased with increasing salt and sugar concentration. Albumin was the main fraction representing 59.6% and 66.1% in QP3 and V36 varieties, respectively, followed by globulin, which represented 22.6% and 19.1%, respectively. The ratios of albumin, globulin, glutelin and prolamin were significantly different. Based on the data obtained from sodium dodecyl sulphate polyacrylamide gel electrophoresis, the main kenaf seed proteins present in the concentrates were five proteins with molecular weights ranging from 10 to 66 kDa. From differential scanning calorimetry data, QP3 and V36 protein concentrates had similar denaturation temperatures (82.6 and 81.8 °C, respectively).

1. Introduction

Kenaf (*Hibiscus cannabinus* L.) is an annual herbaceous plant which has great potential for fibre, energy and feedstock. Kenaf has been planted in Africa for more than 4000 years and can also been found in many countries, such as China, India, USA and Thailand (Dempsey, 1975). Malaysia has taken the initiative to grow up the kenaf plant for crops and other uses. The government has taken an action to cultivate kenaf as the fourth industrial crop in the nation in order to replace tobacco plantation. The kenaf plant was traditionally used as fibre source for making sacs, canvases, ropes and also carpet (Li, 1980). An intensive review of the hibiscus family showed that the bark and core extract of kenaf had a strong activity against tumoural cell lines, and the leaves showed very interesting volatile constituents (Maganha et al., 2010). Kenaf seed has been reported as a suitable source for livestock and horse feed due to its high content of protein (Nyam, Tan, Lai, Long, & Che Man, 2009). Kenaf seeds are good source for edible oil and flour for cooking (LeMahieu, Oplinger, & Putnam, 2003). The kenaf seed has always been wasted by the kenaf industry as agricultural waste or rendered into animal feed. Thus, intensive research is highly required in order to maximise the usage of the kenaf plant, especially the kenaf seed. Different authors reported the composition of kenaf seedoil, kenaf seedcake, and their antioxidant activity (Chan & Maznah, 2009; Mohamed, Bhardwaj, Hamama, & Webber, 1995; Webber, Bhardwaj, & Bledsoe, 2002).

The demand of relatively inexpensive sources of proteins that can be incorporated to value-added food products is increasing. Worldwide, much of the research is going on various sources of plant proteins (Chandi & Sogi, 2007; Rangel, Domont, Pedrosa, & Ferriera, 2003; Sogi, Garg, & Bawa, 2002), which may help to increase the nutritional value of food products at low cost. The development of new food components from kenaf seed by-products calls for more precise information to be obtained on its desirable and undesirable components. As a potential food ingredient, kenaf seed protein concentrate (KSPC) will be subjected to various processing conditions during food manufacturing, leading to conformational and structural changes in the protein. These changes could be either beneficial or detrimental in terms of the nutritional or functional properties of the processed food system (Adebiyi, Adebiyi, Hasegawa, Ogawa, & Muramoto, 2009). Differential scanning calorimetry (DSC) was used as a tool in assessing the potential of KSPC as functional ingredients.

This paper suggested that kenaf defatted seed protein concentrates might be used as a potential source of protein and as high valuable food ingredient. However, studies on the kenaf protein,

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its composition and functions are limited. To the best of our knowledge no other study exists, investigating functional properties of kenaf protein concentrates, such as absorption capacity, foaming capacity, solubility, and stability. This study was focused on the functional behaviour of kenaf seed protein concentrates.

2. Materials and methods

2.1. Materials

The kenaf seeds Qui Ping 3 (QP3), and V36 (V36) were purchased from the National Tobacco Board, Pasir Putih, Kelantan, Malaysia.

2.2. Kenaf seed protein, moisture, ash, and lipid analysis

Protein, moisture, and ash were determined according to the standard methods of the Association of Official Analytical Chemists (AOAC, 1995). A forced draft oven BS (Gallenkamp, Model OV-160, England) was used for the determination of moisture and volatile matter. Under the condition of the test, the temperature was adjusted at 130 ± 2 °C. Proteins were calculated from the nitrogen content by the Kjeldahl method using the conversion factor 6.25. Ash was determined by incinerating at 500 °C in a muffle furnace for 6 h. Crude fibre was estimated by standard methods (AOAC, 1995). The total carbohydrate content (on dry weight basis) was calculated by the difference [100 − (protein + lipids + ash + crude fibre)]. The nitrogen and protein contents of the kenaf seed protein concentrates were also determined by Kjeldahl method (AOAC, 1995).

2.3. Oil extraction

Seeds (50 g) were ground using a blender (Waring, Torrington, CT, USA) at a high speed for approximately 2 min. The oil was extracted from the ground seeds by extraction with petroleum ether (60–80 °C) in a Soxhlet apparatus for 6 h following the AOCS (1998) Method No. Am 2-93. The ratio of solids to solvent used was 1:10. The oil was then recovered by evaporating off the solvent using a rotary evaporator Model N-1 (Eyela, Tokyo Rikakikal Co., Ltd., Japan). The residual solvent was removed by flushing with 99.9% nitrogen. The residue was dried at 50 °C in an oven and used further for protein concentrates extraction.

2.4. Preparation of kenaf seed protein concentrates (KSPC)

The dried defatted seed was weighed and suspended in distilled water at a 1:100 ratio. With the use of a magnetic stirrer, the mixture was stirred for 1 h while adjusting the pH at 9.0 with NaOH solution (4 M). Then, the mixture was centrifuged at 3500 rpm for 15 min at room temperature. The supernatant was transferred into a beaker, stirred for another 30 min, and the pH adjusted to 4.5. The supernatant was left undisturbed for cold precipitation overnight at 4 °C in a freezer. After that, the supernatant was carefully siphoned off and the protein slurry was washed three times with distilled water by centrifuging at 3500 rpm for 10 min, at 4 °C. The pellet was then resuspended in distilled water, and the pH was adjusted at 7.0. The slurry was kept overnight at −80 °C and then it was freeze dried (Chandi & Sogi, 2007). The sample inside the freeze-dryer took 2–3 days before it was completely dried. The protein concentrates obtained were weighed using analytical balances.

2.5. Water absorption capacity (WAC)

The method of Jyothirmayi, Prabhakara Rao, and Walde (2006) was followed with some modifications; 0.1 g of sample was taken and mixed with 1 ml of distilled water. The slurry was centrifuged at 3000*g* for 15 min. The supernatant was removed. The pellet was drained for 30 min and the gain in weight per unit weight was reported as water absorption capacity (g/g).

2.6. Oil absorption capacity (OAC)

Sample (1.0 g) was taken and mixed with 10 ml of refined sunflower oil, vortexed thoroughly, and centrifuged at 3000*g* for 15 min. The oil absorbed by the samples was noted and expressed as oil absorption capacity (g/g) (Beuchat, 1977).

2.7. Foaming capacity and foam stability

The foaming capacity (FC) of proteins was determined by measuring the volume of foams immediately after the introduction of air (90 cm^3/min) for 15 s into 5 ml of 0.2% protein solution, in a 0.05 M phosphate buffer (pH 7.4), in a glass tube (2.4 × 30 cm). Foaming stability (FS) was calculated from the following equation:

$$FS = V_0(\Delta t/\Delta V)$$

Here ΔV is the change in the volume of foam (V) occurring during the time interval, Δt (30 min), and V_0 is the volume of foam at 0 time (Kato, Lee, & Kobayashi, 1989).

2.7.1. pH system (5, 7, and 9)

Protein (0.25 g) concentrates was dispersed in 25 ml of citrate buffer set at pH 5, 7, and 9. The dispersion was whipped in a blender for 2 min and immediately transferred to a measuring cylinder. The foam height was noted with time till it collapsed half life.

2.7.2. Salt system (0.5%, 1.0%, and 1.5%)

Protein (0.25 g) concentrates was dispersed in 25 ml of citrate buffer pH 7 with sodium chloride (0.5%, 1.0%, and 1.5% w/w). The dispersion was whipped in a blender for 2 min and immediately transferred to a measuring cylinder. The foam height was noted with time until it collapsed.

2.7.3. Sugar system (5%, 10%, and 15%)

Protein (0.25 g) concentrate was dispersed in 25 ml of citrate buffer pH 7 with sucrose (0.5%, 1.0%, and 1.5% w/w). The dispersion was whipped in a blender for 2 min and immediately transferred to a measuring cylinder. The foam height was noted with time until it collapsed.

2.8. Isolation of protein fractions

Isolation of protein fractions was carried out by adapting the method of Ju, Hettiarachchy, and Rath (2001). The albumin, globulin, and glutelin were precipitated from their supernatants by adjusting the pH to their isoelectric points (IPs). The IPs were determined by subjecting each supernatant to a pH ranging from 3.0 to 10.0 and determining the turbidity (optical density at 320 nm) with a spectrophotometer (Shimazu Co., Ltd., Kyoto, Japan). The pH that gave the maximum turbidity was taken as the IP. The prolamin was precipitated by adding acetone to the supernatant, according to Tecson, Esmama, Lontok, and Juliano (1971). The precipitated proteins (albumin, globulin, glutelin, and prolamin) were washed twice with distilled water, adjusted to pH 7.0, freeze-dried, and then stored at 4 °C.