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Optimization of trypsin-assisted extraction, physico-chemical characterization, nutritional qualities and functionalities of palm kernel cake protein

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ABSTRACT

The extraction of palm kernel cake protein (PKCP) was enhanced by trypsin-assisted assay. From the Response Surface Methodology (RSM) generated model, the optimum conditions were using trypsin at concentration 1.36 g/100 g, reacted on palm kernel cake (PKC) slurry (1.1 g/100 mL) at 50 °C and pH 9.5. The trypsin extracted protein yield (61.99 ± 0.74 g/100 g) was significantly ($P < 0.05$) higher than the alkaline (pH 9.5) method (10.21 ± 0.24 g/100 g). Surface hydrophobicity of PKCP (159.36) was significantly ($P < 0.05$) higher than soy protein isolate (SPI) (51.51). PKCP had a lower denatured temperature (66.88 °C) than SPI (101.41 °C). The electrophoretograms of PKCP showed 3 bands at 2.20, 3.51 and 4.28 kDa, compared to SPI which had 22 bands with molecular weight ranging from 2.00 to 82.79 kDa. Except lysine, the essential amino acids of PKCP met the suggested requirements of FAO/WHO for 2–5 year old infants. The in-vitro digestibility, essential amino acids/total amino acids (E/T) value, biological value and computed-protein efficiency ratio (C-PER) value for PKCP were significantly ($P < 0.05$) higher than that of SPI. PKCP also showed better solubility (30.12–97.79 vs. 9.15–69.78 g/100 g) emulsifying activity (143.25 vs. 32.57 m^2/g); but lower emulsifying stability (37.83 vs. 43.08%), foaming capacity (22.5 vs. 100.0 ml/100 ml) and foam stability (3.70 vs. 19.20 ml) than the SPI.

1. Introduction

Palm kernel cake (PKC) is an underutilized co-product of palm oil milling industry. In Malaysia alone, approximately 2310 Gg of palm kernel cake was produced in the year of 2009 (MPOB, 2010). Unfortunately, it has limited applications as ruminant, poultry and swine feed ingredients (Alimon, 2005), and fertilizer (Kolade, Coker, Sridhar, & Adeoye, 2005). Palm kernel cake contains valuable dietary source of protein (14.5–19.5 g/100 g) (Alimon, 2005); but until today, PKC protein concentrate and isolate are not commercially produced due to the lack of a commercially feasible extraction method.

The alkaline method is the most common procedure for protein extraction from plants, including red pepper seed (Firatligil-Durmus & Evranuz, 2010), tea (Shen, Wang, Wang, Wu, & Chen, 2008), pulse (peas, chickpeas, lentils, and beans) (Boye et al., 2010) and coconut milk press cake (Chambal, Bergenståhl, & Dejmek, in press). By using sodium hydroxide solution, 24.48 g/100 g of nitrogen was also extracted from defatted palm kernel cake (Balogun, 1982). Our previous findings also showed that only 11.91 g/100 g PKC protein recovery with the alkaline method. A better protein yield from PKC is always obtained at more alkaline conditions, which may encourage the formation of lysinoalanine and reduce the nutritive value of protein. In addition, high alkaline conditions could darken the colour of protein due to enhancement of Maillard reaction; caused hydrolysis and denaturation of proteins and lowered the quality of protein isolated due to increased extraction of non-protein components that co-precipitated with protein (Wang, Hettiarachchy, Qi, Burks, & Siebenmorgen, 1998).

To avoid the denaturations of protein during alkaline extraction, enzyme-assisted extraction has been considered as a good option for protein extraction. Carbohydrases (cellulose, hemicellulase, pectinase and viscozyme L) can hydrolyze the α -1, 4 linkage of polysaccharide and indirectly increases the protein recovery by liberating polysaccharide-bound protein (Ansharullah, Hourigan, & Chesterman, 1997; Guan & Yao, 2008). Other than that, protease makes the protein more soluble for extraction by hydrolyzing protein into peptides (Shen et al., 2008; Tang, Hettiarachchy, & Shellhammer, 2002). Onuora and King (1985) also reported that

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the proteolytic enzymes used (proteases, pepsin, trypsin and bromelain) had increased the PKC protein (nitrogen) solubility by 34–62 g/100 g.

The efficiency of protein extraction may be affected by several factors (temperature, pH, enzyme concentration and the quantity (weight) of substrate, etc.) in the enzyme-assisted extraction process, and their effects may be either independent or interactive. Therefore, response surface methodology (RSM) becomes a useful tool to optimize the extraction parameters, evaluate the multiple parameters and identify their significant interactions with the reduced number of experimental trials (Wani, Kaur, Ahmed, & Sogi, 2008).

Following protein preparation, characterization of the protein is needed for better understanding of its physico-chemical characteristics (surface hydrophobicity, molecular size distribution, and thermal properties) and nutritive quality. These protein characterization studies are very important and relevant to the suitability of protein functionalities for food application. Basic functionalities of protein isolate including solubility, emulsification and foaming ability from various sources have been studied, such as soy protein isolate (Chovea, Grandison, & Lewis, 2007), alfalfa leaf (Lamsal, Koegel, & Gunasekaran, 2007) and guava seed (Bernardino-Nicanor, Scilingo, Anon, & Davila-Ortiz, 2006).

Palm kernel cake, which is relatively rich in protein, may serve as a potential edible protein source for human consumption. There are very limited information on PKC protein production and no scientific data is available on its physico-chemical and technological functional properties; and nutritional value for human. Thus, the objectives of the present work are (1) to investigate the effects of extraction parameters including incubation temperature, pH, amount of PKC substrate and trypsin concentration on the optimization of trypsin-assisted PKC protein extraction; (2) to evaluate its physico-chemical characteristics, nutritional qualities and functionalities.

2. Materials and methods

2.1. Materials

The palm kernel cake, PKC (supplied by FELDA Kernel Products Sdn. Bhd., Malaysia), which was defatted and in powder form, was ground into fine powder of particle size $\leq$ 0.1 mm. Its proximate content is (on a dry matter basis) 14.86 g/100 g protein (N x 5.50), 10.02 g/100 g crude fibre, 5.70 g/100 g fat and 3.55 g/100 g ash (Unpublished data). Trypsin, from porcine pancreas (2 BTEE units/mg solid) was obtained from Sigma Co., Malaysia. Soy protein isolate (Soypro900) was purchased from Crown Soya Protein Group, Qingdao, China. All other chemical reagents were of analytical grade and purchased from Sigma Co., Malaysia.

2.2. Optimal extraction parameter

2.2.1. Enzymatic pretreatment and protein extraction

The finely ground PKC powder was dispersed in 100 ml phosphate buffer (0.01 mol/L) solution followed different levels of independent variables, such as temperature, x_1 (30–50 °C); pH, x_2 (6.5–9.5); amount of PKC, x_3 (0.5–1.5 g/100 mL) and trypsin concentration, x_4 (0.5–1.5 g/100 g). The slurries containing enzyme were incubated in a water bath shaker (HOTECH INSTRUMENTS CORP., Taiwan) and continuously shaken at 180 rpm for 6 h. The trypsin assay variables were shown in Table 1. Then the solution was heated to 90 °C for 10 min and centrifuged at 4830$\times$ g (Jouan KR4i, ThermoFisher Scientific) for 10 min. The supernatants were collected and analysed for their protein content.

Table 1
Experimental values and coded levels of the independent variables for central composite rotatable design (CCRD).

Independent variable	Symbols		Levels				
	Coded	Uncoded	−2	−1	0	+1	+2
Temperature (°C)	x_1	X_1	20	30	40	50	60
pH	x_2	X_2	5.0	6.5	8.0	9.5	11.0
PKC weight (g/100 ml)	x_3	X_3	0.00	0.50	1.00	1.50	2.00
Trypsin concentration (g/100 g)	x_4	X_4	0.00	0.50	1.00	1.50	2.00

2.2.2. Protein determination

The protein content of PKC powder, enzyme (commercial trypsin), supernatants and protein powder (PKCP and SPI) was determined using the Kjeldahl method (AOAC, 2005) and multiplying the nitrogen content with protein conversion factor of 5.50 (Ezeagu, Petzke, Metges, Akinsoyinu, & Ologhobo, 2002). The extracted PKC protein was expressed as:

$$\text{Extracted protein(g/100 g)} = [(\text{total protein in supernatant} - \text{protein in enzyme})/\text{total protein in PKC}] \times 100 \quad (1)$$

2.2.3. Experimental design

A central composite rotatable design (CCRD), with four variables and utilizing five levels, was applied to obtain a quadratic model. The quadratic effects and central points were estimated with the protein recovery as response. The response pattern and the optimum combination of variables of trypsin-assisted protein extraction assay were determined. The independent variables X_i were coded as x_i, which are defined as dimensionless, according to the Equation (2):

$$x_i = (X_i - X_0)/\Delta X_i \quad (2)$$

where, x_i is the coded value of an independent variable, X_i is the real value of an independent variable, X_0 is the real value of an independent variable at the centre point, and X_i is the step change value. The independent variables and their levels are presented in Table 1. Six replicates at the centre of design were used to estimate a pure error sum of squares.

2.2.4. Statistical analyses

The responses obtained were subjected to multiple non-linear regression analysis to obtain the coefficients. Estimates of coefficients with levels higher than 95% ($P < 0.05$) were included in the CCRD models. The protein extractability can thus be expressed as function of independent variables by the second-order polynomial, i.e. (Equation (3))

$$Y = \beta_0 + \sum \beta_j x_j + \sum \beta_{jj} x_j^2 + \sum \beta_{jk} x_j x_k \quad (3)$$

where, Y is the response here in terms of protein recovery (g/100 g), β_0 is the intercept, β_j, β_{jj}, β_{jk} are linear, quadratic and interactive coefficients, respectively. The responses obtained were statistically evaluated and the model was built based on the variables with confidence levels more than 95%. All analyses were done by Design-Expert® 6.0 (Stat-Ease Inc., Minneapolis, USA). Fifty-four experiments were run and the experimental errors for protein extraction were calculated based on the standard deviation of the centre point with six runs.

2.2.5. Verification of the model

From the RSM generated model, the optimum conditions of trypsin-assisted protein extraction depended on temperature, pH,