Food Research International 62 (2014) 726–734

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

# Identification and characterization of papain-generated antioxidant peptides from palm kernel cake proteins

Mohammad Zarei [a], Afshin Ebrahimpour [a], Azizah Abdul-Hamid [a], Farooq Anwar [b], Fatimah Abu Bakar [a], Robin Philip [c], Nazamid Saari [a,*]

[a] Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[b] Department of Chemistry, University of Sargodha, Sargodha 40100, Pakistan
[c] Agilent Technologies, 1 Yishun Avenue 7, Singapore 768923

ARTICLE INFO

*Article history:*
Received 25 January 2014
Accepted 19 April 2014
Available online 26 April 2014

*Keywords:*
Antioxidant
Palm kernel cake
Protelysates
Bioactive peptide
Metal chelating activity
Radical scavenging activity

ABSTRACT

Novel peptides with antioxidant activity were isolated and identified from papain generated palm kernel cake (PKC) proteolysate. The proteolysate was fractionated into individual peptides based on hydrophobicity and isoelectric point using reversed-phase high-performance liquid chromatography and isoelectric focusing techniques. The individual peptides were identified by tandem mass spectrometry and their respective antioxidant activities were evaluated using 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and metal chelating activity assays. Peptide sequences, AWFS, WAF, and LPWRPATNVF showed the highest radical scavenging activities of 71%, 56%, and 50%, respectively, while peptide sequences GGIF, YGIKVGYAIP and YLLLK showed the highest metal chelating activities of 56%, 53%, and 50%, respectively. However, the best $IC_{50}$ values of peptides measured by DPPH• assay were displayed by GIFE, GVQEGAGHYALL and GGIF at 0.02 μM, 0.09 μM and 0.35 μM, respectively, while the best half maximal inhibitory concentration values measured using metal chelating activity were shown by LPWRPATNVF, AWFS and YGIKVGYAIP at 0.001 μM, 0.002 μM and 0.087 μM, respectively. It can be concluded that the peptides derived from PKC proteolysate were more potent and distinctive compared to those previously reported from other plant protein sources.

© 2014 Published by Elsevier Ltd.

## 1. Introduction

Palm kernel cake (PKC) is a by-product of palm oil industry with an estimated total production of 2.4 million tons per year in Malaysia. It has been solely used for animal feeds of swine, aquaculture and ruminant due to its high protein content ranging from 15 to 21%. Due to the amount of generated PKC and its derived protein that is significant enough to be considered as a useful raw material for many industries, it can be a potential bioresource for production of functional components such as bioactive peptides.

Enzymatic proteolysis is one of the fastest, safest and most controllable ways to produce the bioactive peptides and enhance the added value of PKC in diversifying its functional bioactivities such as antioxidative, anti-hypertension and antimicrobial functions. It is estimated that 202,000 tons of peptides can be generated from PKC yearly in Malaysia. Recently, we have reported the use of PKC for the production of an antioxidative proteolysate (Zarei, Ebrahimpour, Abdul-Hamid, Anwar, & Saari, 2012). In another study, Ng, Ayob, Said, Osman, and Ismail (2013) optimized a method to produce the antiradical PKC proteolysate by alcalase. Tan, Ayob, and Wan Yaacob (2013) reported the antibacterial peptide-containing compounds that are generated from PKC using alcalase. These authors also reported antibacterial activity of different hydrolysates of PKC protein (Tan, Ayob, Osman, & Matthews, 2011). Hence, retarding the progress of oxidative stress using some antioxidant additives can be one of the most important and critical steps towards alleviating stress-related diseases (Butterfield et al., 2002; Sarmadi & Ismail, 2010).

Several synthetic antioxidants have been widely used in the food, pharmaceutical and cosmetic industries (Di Bernardini et al., 2011). However, the use of these synthetic antioxidants, as radical scavengers possesses a potential health risk to the human body. Their applications in the food and pharmaceutical products must be strictly controlled (Pokorný, 2007). Therefore, natural antioxidants are in high demand as dietary supplements to reduce the risk of aging, inflammation and cardiovascular diseases consequently improve the human health (Di Bernardini et al., 2011).

Thus, identifying the safe and natural antioxidants sources would be beneficial for use in the food industry and to prevent oxidative stress related human health disorders.

* Corresponding author. Tel.: +60 3 89468385; fax: +60 3 89423552.
*E-mail addresses:* mzarei.mail@gmail.com (M. Zarei), a_ebrahimpour@yahoo.com (A. Ebrahimpour), azizah@food.upm.edu.my (A. Abdul-Hamid), fqanwar@yahoo.com (F. Anwar), fatim@upm.edu.my (F.A. Bakar), robin_philp@agilent.com (R. Philip), nazamid@upm.edu.my (N. Saari).

http://dx.doi.org/10.1016/j.foodres.2014.04.041
0963-9969/© 2014 Published by Elsevier Ltd.

Recently, interest has been emerging to produce and characterize bioactive peptides from plant and animal sources due to their availability, safety and nutritional aspects. Bioactive peptides are short protein sequences that are inactive within the parent protein but may reveal some biofunctional and physiological activities after releasing from their protein sources. Depending on their amino acid contents, sequences and structures, peptides generated can play different functions, such as immunomodulatory, antimicrobial, antioxidative, antithrombotic (Rojas-Ronquillo et al., 2012) hypocholesterolemic and angiotensin converting enzyme (ACE) inhibitory. Hence, this study was aimed to isolate and characterize the antioxidative peptides generated from palm kernel cake protein to be used as functional ingredients in food systems.

## 2. Methods

### 2.1. Materials

Palm kernel cake (PKC) used in this study was obtained from My-4-Season's Company, Serdang, Selangor, Malaysia. Alcalase was obtained from Novoenzyme (Nottingham, UK). Pepsin, flavourzyme, O-phthaldialdehyde (OPA) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich (Munich, Germany). Papain, bromelain and glutathione were from Acros Organics (St. Louis, MO, USA). Trypsin was obtained from Fisher Scientific (Georgia, USA). Chymotrypsin was purchased from Calbiochem (South Africa). Ethanol, acetic acid, sodium acetate, potassium phosphate, Tris-base and potassium chloride were obtained from Merck (Darmstadt, Germany). Ferrozine and $FeCl_2$ were purchased from Merck KGaA (Darmstadt, Germany).

### 2.2. Isolation of palm kernel cake protein

PKC protein was isolated according to the method described by Arifin et al. (2009) with minor modification. Briefly, PKC was defatted with petroleum ether and the solvent was removed by rotary evaporation. The defatted PKC was dried in a ventilator at 20 °C for overnight. PKC protein isolate was obtained by dispersing the defatted PKC in NaOH solution (0.03 *N*), at a ratio of 1:30 (w/v), and extracted by shaking at 150 rpm for 2 h using a water bath shaker. After filtration, pH of the supernatant was adjusted to 3.5 using HCl and the precipitate was collected by centrifugation at 10,000 ×*g* for 10 min and stored at −80 °C for further analysis.

### 2.3. PKC protein hydrolysis

PKC protein was hydrolyzed by mixing 0.65 g of PKC protein with papain (phosphate buffer, pH 6.5, 65 °C), alcalase (phosphate buffer, pH 7.5, 55 °C), pepsin (KCl–HCl buffer, pH 1.5, 37 °C), trypsin (tris–HCl buffer, pH 8, 37 °C), flavourzyme (tris–HCl buffer, pH 8, 55 °C), bromelain (acetate buffer, pH 5, 55 °C), or chymotrypsin (phosphate buffer, pH 6.8, 50 °C) at a ratio of 50:1 (w/w) in 35 ml of buffer solution and incubated in a water bath shaker at an agitation rate of 150 rpm under the optimal hydrolysis condition for each enzyme. Total hydrolysis time was 6 h. The enzyme was added to hydrolysis mixture every 6 h at 1:50 ratio. The hydrolysis process was stopped by heating the mixture in boiling water (100 °C) for 10 min. After centrifugation (10,000 ×*g* for 10 min), supernatant was collected and used for antioxidant activity assays.

### 2.4. Free radical scavenging activity assay

1,1-Diphenyl-2-picrylhydrazyl free radical (DPPH•) scavenging activity of peptides was measured according to the method described by Hwang et al. (2010) with minor modification. Peptide solution (50 μL, 0.005–10 mg/ml) was mixed with 50 μL deionized water and 100 μL 0.15 mM DPPH• (in 80% ethanol). The mixture was kept in dark condition at room temperature (25 °C) for 45 min and absorbance at 517 nm was measured by using a 96 well microplate reader (Labomed, model UVD-2950, USA). The scavenging ability of each peptide was calculated by the following equation:

$$\text{DPPH•radical scavenging activity}(\%) = \left[\left(A_{Control} - A_{Sample}\right)/A_{Control}\right] \times 100 \quad (1)$$

where, $A_{Control}$ is the absorbance of control and $A_{Sample}$ is the absorbance of samples at 517 nm.

### 2.5. Metal chelating activity assay

Metal ion chelating activity was measured according to the method described by Decker and Welch (1990), with minor modification. Sample solution (100 μL) at concentrations of 0.005 mg/mL to 10 mg/mL was premixed with 5 μL of 2 mmol/L iron dichloride solution and 185 μL of double distilled water. Afterwards, 10 μL of 5 mmol/L ferrozine solution was added and mixed vigorously. The mixture was incubated at room temperature for 10 min and the absorbance was determined at 562 nm by a microplate reader (Power Wave X 340, Biotek Instruments Inc.). All analyses were run in triplicate, the results were averaged, and the chelating effect was calculated using the following equation:

$$\text{Metal ion chelating activity } (\%) = \left[\left(A_{Control} - A_{Sample}\right)/A_{Control}\right] \times 100 \quad (2)$$

where, $A_{Control}$ is the absorbance of control and $A_{Sample}$ is the absorbance of samples at 562 nm.

### 2.6. O-Phthaldialdehyde spectrophotometric assay

A rapid, sensitive and convenient o-phthaldialdehyde (OPA) based spectroscopic assay was performed to measure the degree of hydrolysis of PKC proteins in buffer solutions according to the method described by Church, Swaisgood, Porter, and Catignani (1983) with minor modification. The fresh OPA solution was prepared by mixing solutions A, B and C as follows: solution A: 7.62 g sodium tetrahydroborate and 200 mg of sodium dodecyl sulfate (SDS) dissolved in 150 ml deionized water; solution B: 160 mg of OPA dissolved in 4 ml of ethanol 96% and solution C: 400 μL of β-mercaptoethanol, adjusted to a final volume of 50 ml with deionized water. To study the degree of proteolysis, 36 μL of the sample was mixed with 270 μL of OPA reagent in a 96-well plate. The mixture was incubated at room temperature for 2 min, and then the absorbance was measured at 340 nm using a microplate reader system (Power Wave X 340, Biotek Instruments Inc.). All analyses were run in triplicate, the results were averaged, and the degree of hydrolysis was calculated using the following equation:

$$\text{Degree of hydrolysis}(\%) = \left[\left(A_{Sample} - A_{Control} - A_{Protein}\right)/A_{Sample}\right] \times 100 \quad (3)$$

where, $A_{Sample}$ is the absorbance of sample after proteolysis, $A_{Protein}$ is the absorbance of sample before proteolysis (negative control) and $A_{Control}$ is the absorbance of control.

### 2.7. Purification and characterization of PKC protein hydrolysates

#### 2.7.1. Fractionation using reverse phase high performance liquid chromatography

The PKC protein hydrolysate produced by papain was loaded onto a semi-preparative C18 reversed phase high performance liquid chromatography (RP-HPLC) column (9.40 mm × 250 mm, 5-μm particles, Agilent Technologies, USA). The sample injection volume and concentration were 250 μL and 0.2 mg of peptide per ml, respectively. The column was eluted by a linear gradient of acetonitrile (0–100%, v/v) containing 0.1% trifluoroacetic acid (TFA) at a flow rate of 4.0 ml/min. The elution

Download English Version:

https://daneshyari.com/en/article/6395922

Download Persian Version:

https://daneshyari.com/article/6395922

Daneshyari.com