Journal of Oil Palm Research
DOI: https://doi.org/10.21894/jopr.2019.0002

ANTIOXIDANT PROPERTIES OF CRUDE AND COLD ETHANOL PRECIPITATED PROTEIN FROM PALM KERNEL CAKE (PKC) AS POTENTIAL COSMECEUTICAL AGENT

CHOI, W C*; HON, W M; MOHAMAD, M‡; KOK, A K D X‡; LAI, K S‡‡ and YAP, W S***

ABSTRACT

Palm kernel cake (PKC) is a by-product obtained from the production of edible oils using oil palm (Elaeis guineensis). *PKC is well-known for its high protein content, therefore, it was chosen as the target of this research to study its antioxidant properties, which is an important criteria in cosmeceutical industry. Our studies showed that, the extraction of crude protein at 80°C resulted in the highest total phenolic content (TPC) and protein yield. It was further seen that precipitation using 80% cold ethanol following protein extraction at 80°C gave the best protein yield of 56.6%. The antioxidative activity of this precipitated protein was expressed as IC_{50} for 1-diphenyl 1-2-picrylhydrazyl (DPPH) radical-scavenging capacity (517±0.016 mg ml^{-1}), 2, 2′-azino-bisC3-ethylbenzothia zoline-6-sulphonic acid (ABTS) radical-scavenging capacity (0.047±0.009 mg ml^{-1}) and ferrous ion-chelating (FIC) ability (21±0.13 mg ml^{-1}). Meanwhile, the reducing power and TPC were 0.156±0.006 trolox equivalent antioxidant capacity (TEAC) mmol g^{-1} dry weight (DW) and 25.10±0.58 gallic acid equivalent (GAE) µg g^{-1} DW respectively. The Pearson correlation test further revealed a significantly moderate to strong positive relationships between antioxidant properties with protein content and antioxidant properties with TPC. Taken together, both, the crude and precipitated protein obtained from PKC showed substantial amount of antioxidative activities, which could be used as sustainable source of antioxidant peptides in enhancing the quality of cosmeceutical products.*

Keywords: antioxidant properties, cosmeceutical, crude protein, palm kernel cake, precipitated protein.

Date received: 25 April 2018**; Sent for revision:** 21 May 2018**; Received in final form:** 17 July 2018**; Accepted:** 3 January 2019

INTRODUCTION

Palm kernel cake (PKC), also known as palm kernel meal (PKM), is one of the most abundant by-products of edible oils obtained from oil palm (*Elaeis guineensis*) after extraction. According to Malaysian palm oil statistics (MPOB, 2014), PKC is produced annually in large quantities, with about 1.8 million tonnes of PKC left over from the oil extraction process in the oil palm industry. This figure alone has attracted huge potential interest of PKC as a bioresource of raw material in many industries, especially those utilising PKC for its high protein content (Iluyemi *et al.*, 2006) like animal feed (Arifin *et al.*, 2009).

Cosmeceuticals are topical cosmetic-pharmaceutical hybrids which lie on the spectrum between drugs and cosmetics (Lintner *et al.*, 2009). One of the aims of cosmeceutical products is to improve the antioxidant properties of the skin, hence

* Department of Biotechnology, Faculty of Applied Sciences, UCSI University, 1 Jalan Puncak Menara Gading, Taman Connaught, 56000 Kuala Lumpur, Malaysia. E-mail: wsyap@ucsiuniversity.edu.my

** Vice-Chancellor's Office, KDU University College, SS 22/41, Damansara Jaya,47400 Petaling Jaya, Selangor, Malaysia.

‡ University Council, UCSI University, 1 Jalan Puncak Menara Gading, 56000 Kuala Lumpur, Malaysia.

‡‡ Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

retarded the ageing process. Recently, the evolutions of antioxidative peptides which possess low molecular weight, easy absorption and high activity have drawn the attention of many researchers (Nakajima *et al*., 2008; Xie *et al*., 2008). Antioxidant peptides are peptides with antioxidative effect. It has advantages of being simpler in structure and more stable compared to other antioxidative enzymes. In addition, antioxidant peptides can react in human body without dangerous immunoreaction (Xie *et al.*, 2008). These peptides also exhibit numerous bioactivities, such as inhibition of bio-macromolecules peroxidation and elimination of free radicals produced *in vivo* (Xie *et al.*, 2008).

Large quantities of PKC by-product are being produced annually in Malaysia from the oil palm plantations, and because this by-product has a high crude protein content, it would be valuable if this by-product could be transformed into new and non-conventional source of proteins such as cosmeceutical peptides (Arifin *et al*., 2009). Therefore, the aim of this study is to evaluate PKC as a potential valuable source of peptides to be used as cosmeceutical agent.

MATERIALS AND METHODS

PKC Extraction

The dry powder of PKC was purchased from ACE Edible Oil Industries Sdn Bhd, Malaysia. Then, it was ground and passed through 1.18 mm mesh sieve before it was used for extraction. PKC protein extraction was carried out using the method described by Arifin *et al.* (2009) with slight modifications. Approximately 75 g of mashed PKC were mixed with 750 ml of 0.03 M sodium hydroxide (NaOH) pH 12 solution at 25°C, 60°C and 80°C, respectively and stirred continuously at 150 rpm for 4 hr. The NaOH: PKC ratio was 10:1 (v w^{-1}). To precipitate PKC protein, modified method described by Moure *et al.* (2001) was used. Briefly, 50 ml of crude protein was mixed with 12.5 ml of cold ethanol (20%) at 0°C. The mixture was centrifuged at 11 200 g for 7 min. The pellet collected was air-dried and the supernatant was used in subsequent precipitation with 40%, 60% and 80% cold ethanol, in succession. The protein content was further determined using the Bradford method (Bradford, 1976) and the protein yield was calculated as follows: protein yield (%) = (protein obtained after precipitation/initial protein content) x 100%.

Biochemical Assay

The total phenolic content of crude protein and ethanol precipitated samples were determined using a Folin-Ciocalteu (FC) assay as described by Li *et al.* (2008). Antioxidants properties were determined based on previously described methods of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging capacity (Cai *et al*., 2006), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical-scavenging capacity (Guimaraes *et al*., 2007) and ferric reducing ability of plasma (FRAP) assay (Benzie and Strain, 1996). The ferrous ion-chelating (FIC) assay reported by Chan *et al.* (2008) was adopted. The ability of the extracts to chelate ferrous ions was calculated as follows: chelating effect % = $(1- A_{sample}/A_{control})$ x 100.

Statistical Analysis

All crude PKC extractions and ethanol precipitation of the crude PKC extracts were carried out in three independent experiments. The results were presented as mean ± SEM (standard error mean). GraphPad Prism (Version 5, GraphPad Software Inc., USA) was used to calculate the inhibitory concentration 50% (IC_{50}) values of the DPPH, ABTS and FIC assays, whereas IBM SPSS Statistics software (Version 19, IBM Corporation, USA) was used to perform an analysis of variance (ANOVA) and Tukey's Multiple Comparison (TMC) test at 95% significance level. A Pearson correlation test was then conducted to determine the strength of correlation between the different antioxidant activities and both with (i) the protein yield and (ii) the total phenolic content (TPC).

RESULTS

The protein content of PKC increased when the extraction temperature used increased (*Table 1*). The protein content of PKC ranged between 0.177±0.007 mg g^{-1} dry weight (DW) and 0.502±0.006 mg g^{-1} DW. A 1.5-fold increase in protein was observed when the extraction temperature was raised from 25°C to 60°C, while a 2.8-fold increase was observed when the temperature was raised from 25°C to 80°C.

The antioxidant properties of PKC extracts were assessed using DPPH and ABTS scavenging assays, metal chelating assay and ferric reducing power. Of all the three extraction temperatures used, 80°C yielded the best results for all antioxidant assays [(IC_{50} value of DPPH assay = 0.774±0.019 mg ml^{-1}; IC_{50} value of ABTS assay = 0.055±0.001 mg ml^{-1} and FRAP assay = 0.67±0.02 trolox equivalent antioxidant capacity (TEAC) mmol g^{-1}] except for metal chelating effect (IC_{50} value of FIC assay = 5.04 ±0.15 mg ml^{-1}) (*Table 1*). On the other hand, TPC was recorded between 54.62±0.98 and 67.70±0.72 gallic acid equivalent (GAE) μg g^{-1} DW. The PKC extracted at 80°C had the highest TPC. No significant difference in TPC

TABLE 1. EFFECTS OF EXTRACTION TEMPERATURE ON PROTEIN CONTENT, ANTIOXIDANT PROPERTIES AND TOTAL PHENOLIC CONTENT FROM PALM KERNEL CAKE

Extraction temperature (°C)	Protein content (mg g^{-1} DW)	IC_{50} of DPPH assay (mg ml^{-1})	IC_{50} of ABTS assay (mg ml^{-1})	IC_{50} of FIC assay (mg ml^{-1})	FRA assay (TEAC mmol g^{-1} DW)	Total phenolic content (GAE µg g^{-1} DW)
25	0.177±0.007§	1.676±0.046*	0.086±0.002*	2.38±0.03‡	0.52±0.02‡	57.16±0.66‡
60	0.266±0.007‡	1.412±0.022‡	0.068±0.003‡	4.18±0.11*	0.47±0.02‡	54.62±0.98‡
80	0.502±0.006*	0.774±0.019§	0.055±0.001§	5.04±0.15*	0.67±0.02*	67.70±0.72*

Note: Mean ± SEM, * ‡ § Within a column, values shown with different superscript symbols are significantly different ($p < 0.05$).
DW - dry weight.
DPPH - 1-diphenyl-2-picrylhydrazyl.
ABTS - 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid).
FIC - ferrous ion-chelating
TEAC - trolox equivalent antioxidant capacity.
GAE - gallic acid equivalent.
FRAP - ferric reducing ability of plasma.

was found between extraction temperatures of 25°C and 60°C, whereas an increase of about 24% was observed at 80°C. Further comparison between the protein yield and TPC of PKC extracted at 80°C demonstrated that the protein yield was about 6.4-fold higher than the TPC. This suggests that protein which is abundantly found in PKC sample extract is responsible for most of the antioxidant activities occurring in this study. Since PKC sample extracted at 80°C demonstrated the highest protein yield and high antioxidant activities, this condition was used for the subsequent precipitation process using cold ethanol.

When the precipitation was carried out using 40% cold ethanol, no precipitate was obtained. Therefore, no data was tabulated for PKC precipitated at this percentage. With regard to protein yield (*Table 2*), 80% cold ethanol precipitation gave the highest protein yield of about 57%, which was 27.5-fold and 5.3-fold higher than 20% and 60% cold ethanol precipitation, respectively. Meanwhile, antioxidant activities of PKC protein precipitated at 80% cold ethanol showed lower IC_{50} for DPPH and ABTS assays and higher FRAP activity as compared to 20% and 60% cold ethanol precipitates. On the other hand, no significant difference was observed in terms of FIC assay for 80% and 60% cold ethanol precipitates. Antioxidant activities of precipitated PKC protein at 80% was improved as compare to crude protein with reduction of 33% (DPPH), 15% (ABTS) and 56% (FIC) of IC_{50} respectively. The FRAP assay also showed an approximate reduction of 76% of antioxidant activity for 80% cold ethanol precipitation as compared to crude extract, revealing the loss of antioxidant protein towards the ferric reducing antioxidant power.

Besides, an increase in the TPC was obtained when crude PKC protein was precipitated with increasing concentrations of cold ethanol, where the highest amount was 25.10±0.58 GAE µg g^{-1} DW. This result demonstrated that besides protein, a part of the phenolic compound was also being precipitated using cold ethanol. However, when compared with crude protein extract, a reduction of 63% of TPC was observed. Subsequent analysis revealed a significant moderate to strong positive correlations (from 0.519

TABLE 2. PROTEIN YIELD, ANTIOXIDANT PROPERTIES AND TOTAL PHENOLIC CONTENT FROM COLD ETHANOL PRECIPITATED PROTEIN OF PALM KERNEL CAKE EXTRACTED AT 80°C

Cold ethanol precipitated protein (%)	Protein yield (%)	IC_{50} of DPPH assay (mg ml^{-1})	IC_{50} of ABTS assay (mg ml^{-1})	IC_{50} of FIC assay (mg ml^{-1})	FRAP assay (TEAC mmol g^{-1} DW)	Total phenolic content (GAE µg g^{-1} DW)
20	2.0±0.00§	5.407±0.194*	0.743±0.030*	NA	0.006±0.001‡	1.02±0.03‡
60	8.8±0.02‡	1.493±0.074‡	0.248±0.009‡	2.19±0.08*	0.025±0.001‡	3.54±0.09‡
80	56.6±0.06*	0.517±0.016§	0.047±0.009§	2.21±0.13*	0.156±0.006*	25.10±0.58*

Note: Mean ± SEM, * ‡ § Within the column, the same superscript symbols indicates no significant difference ($p < 0.05$), NA - not available.
DW - dry weight.
DPPH - 1-diphenyl-2- picrylhydrazyl.
ABTS - 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid).
FIC - ferrous ion-chelating.
TEAC - trolox equivalent antioxidant capacity.
GAE - gallic acid equivalent.

to 0.995) for both protein yield and TPC with the antioxidant activities measured ($p<0.01$) (*Table 3*). Moderate positive correlation was observed in FIC assay [0.545 for bovine serum albumin (BSA) while 0.519 for TPC]; whereas strong positive correlations were observed for DPPH (0.721 for BSA while 0.707 for TPC), ABTS (0.783 for BSA while 0.770 for TPC) and FRAP (0.989 for BSA while 0.995 for TPC) (*Table 3*). Positive correlations were also demonstrated for both protein content (BSA) and TPC obtained from cold ethanol precipitated PKC with antioxidant activities, whereby their *r* values were very close to each other.

or non-protein substances with chelating abilities in the PKC sample extracts. According to Zhu *et al.* (2014), high temperature may lead to the change of protein secondary structure, thus leading to lower metal chelating activity. Meanwhile, TPC increases when the extraction temperature increases. When higher temperature was used, the cell wall integrity of the samples might weaken, resulting in more polyphenols migrating into the solvent (Spigno *et al.*, 2007).

Further protein precipitation using various percentage of cold ethanol revealed that protein yield improved as the ethanol percentage increased.

TABLE 3. CORRELATION COEFFICIENT (R) OF PROTEIN YIELD AND TOTAL PHENOLIC CONTENT WITH DIFFERENT ANTIOXIDANT PROPERTIES FROM COLD ETHANOL PRECIPITATED PROTEIN OF PALM KERNEL CAKE EXTRACTED AT 80°C

Assay	DPPH	ABTS	FIC	FRAP	Protein yield	Total phenolic content
Protein yield	0.721**	0.783**	0.545**	0.989**	1.00	0.998**
Total phenolic content	0.707**	0.770**	0.519**	0.995**	0.998**	1.00

Note: ** Correlation is significant at the 0.01 level (2-tailed).
DPPH - 1-diphenyl-2- picrylhydrazyl.
ABTS - 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid).
FIC - ferrous ion-chelating.
FRAP - ferric reducing ability of plasma.

DISCUSSION

This study showed that the protein yield is correlated with the increase in extraction temperature. At higher temperature, the degradation of protein macromolecules might take place leading to smaller molecular weight peptides with higher solubility, thus increasing the protein yield (Ortiz and Wagner, 2002). In the cosmeceutical field, the smaller molecular weight peptides have a major advantage in that these molecules tend to have a better diffusivity in skin, especially those peptides with 500 Da or lesser (Gorouhi and Maibach, 2009). In addition, more small molecules are believed to be present (indicated by high protein yield) at 80°C, which leads to a higher probability of these molecules having the ability to access the binding sites of the free radicals in DPPH and ABTS assays, compared to those proteins extracted at 25°C and 60°C, respectively. The results are also consistent with the study done by Teixeira *et al.* (2012) on *Mentha pulegium* whereby hot water extract gave the highest DPPH free radical scavenging activity (EC_{50} = 16.3 ± 0.4 µg ml^{-1}). In contrast, a lower chelating ability (FIC) was observed on PKC samples extracted at 80°C. This might be due to the degradation of protein

At high ethanol concentrations, more proteins are precipitated due to a decrease in solvating power of water for a charged hydrophilic protein molecule. On the ethanol solvent itself, this can be explained in terms of the reduction of the dielectric constant, or simply the bulk displacement of water owing to partial immobilisation of water molecules through hydration of the organic solvent (Scopes 1994). Moreover, higher antioxidative activity was also detected on PKC protein precipitated at 80% cold ethanol.

Analysis of correlation coefficient (*r*) of protein yield and TPC with different antioxidant activities showed significantly moderate to strong positive correlation. Previous studies have shown that the antioxidant activities measured depended mainly on the TPC (Kiselova *et al.*, 2006; Silva *et al.*, 2007) which is in agreement with the result obtained from our analysis. In addition, recent study by Chang *et al.* (2014) on protein hydrolysates produced from oil palm kernel showed that significant positive correlations were found between the protein content of oil palm kernel hydrolysates (OPKH) and the $ABTS^{\bullet +}$ scavenging activities and OPKH with FRAP, which is similar with this study. The size and amino acid sequence of peptides contributed to the unique

chemical composition and physical properties of proteins, which play an important role to increase the antioxidant capacity (Elias *et al.,* 2008). Thus, it can be deduced that the cold ethanol precipitated protein from PKC in this study could contain such proteins or peptides which conferred the antioxidant activities. Although both protein content and TPC showed positive correlations with the antioxidant activities measured, nevertheless further research on purification is needed to eliminate or minimise the interference of other potentially antioxidative constituents.

CONCLUSION

In summary, alkaline extraction of PKC at 80°C gave the highest protein content as well as antioxidative activities. Using cold ethanol precipitation, protein pellet precipitated at 80% demonstrated satisfactory protein recovery of about 56%, and better antioxidative properties for most assays, except FRAP when compared to crude protein. Our study showed significant positive correlations between protein content and TPC with antioxidant activities, thus depicting PKC as a promising source of proteins as well as non-protein based substances with highly effective antioxidative components to be used in cosmeceutical products. This study revealed the potential uses of PKC as a valuable and sustainable source of cosmeceutical additive or component for cosmeceutical industry.

ACKNOWLEDGEMENT

The authors would like to express their sincere appreciation for the grant provided by UCSI University Research Grant (Proj-in-FAS-002) to carry out this project.

REFERENCES

Arifin, B; Bono, A; Farm, Y Y; Ling, A L L and Fui, S Y (2009). Protein extraction from palm kernel meal. *J. Appl. Sci., 9*: 2996-3004.

Benzie, I F F and Strain, J J (1996). The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay. *Anal. Biochem., 239*: 70-76.

Bradford, M (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem., 72*: 248-254.

Cai, Y Z; Sun, M; Xing, J; Luo, Q and Corke, H (2006). Structure-radical scavenging activity relationship of phenolic compounds from traditional Chinese medicinal plants. *Life Sci., 78*: 2872-2888.

Chan, E W C; Lim, Y Y; Wong, L F; Lianto, F S; Wong, S K; Lim, K K; Joe, C E and Lim, T Y (2008). Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species. *Food Chem., 109*: 477-483.

Chang, S K; Hamajima, H; Ismail, A; Yanagita, T; Esa, M N and Baharuldin, M T H (2014). Health promoting properties of protein hydrolysates produced from oil palm (*Elaeis guineensis*) kernel. *Food Sci. Biotechnol., 23*: 1279-1285.

Elias, R J; Kellerby, S S and Decker, E A (2008). Antioxidant activity of proteins and peptides. *Crit. Rev. Food Sci. Nutr., 48*: 430-441.

Gorouhi, F and Maibach, H I (2009). Role of topical peptides in preventing or treating aged skin. *Int. J. Cosmetic Sci., 31*: 327-345.

Guimaraes, C M; Giao, M S; Martinez, S S; Pintado, A I; Pintado, M E and Bento, L S (2007). Antioxidant activity of sugar molasses, including protective effect against DNA oxidative damage. *Food Chem. Toxicol., 72*: 39-43.

Iluyemi, F B; Hanafi, M M; Radziah, O and Kamarudin, M S (2006). Fungal solid state culture of palm kernel cake. *Bioresource Techno., 97*: 477-482.

Kiselova, Y; Ivanova, D; Chervenkov, T; Gerova, D; Galunska, B and Yankova, T (2006). Correlation between the *in vitro* antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. *Phytother. Res., 20*: 961-965.

Li, H B; Wong, C C; Cheng, K W and Chen, F (2008). Antioxidant properties *in vitro* and total phenolic contents in methanol extracts from medicinal plants. *Lebensmittel-Wissenschaft and-Technol., 41*: 385-390.

Lintner, K; Mas-Chamberlin, C; Mondon, P; Peschard, O and Lamy, L (2009). Cosmeceuticals and active ingredients. *Clin. Dermatol., 27*: 461-468.

Moure, F; Rendueles, M and Diaz, M (2001). Coupling process for plasma protein fractionation using ethanol precipitation and ion exchange chromatography. *Meat Sci., 64*: 391-398.

MPOB (2014). Economics and Industry Development Division. Accessed on 25 June 2017.

Nakajima, K; Yoshie-Stark, Y and Ogushi, M (2008). Comparison of ACE inhibitory and DPPH radical scavenging activities of fish muscle hydrolysates. *Food Chem., 114*: 844-851.

Ortiz, S E M and Wagner, J R (2002). Hydrolysates of native and modified soy protein isolates: Structural characteristics, solubility and foaming properties. *Food Res. Int., 35*: 511-518.

Scopes, R K (1994). Separation by precipitation. *Protein Purification: Principles and Practice* (Scopes, R K ed.). Springer-Verlag, New York. p. 71-101.

Silva, E M; Souza, J N S; Rogez, H; Rees, J F and Larondelle, Y (2007). Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. *Food Chem., 101*: 1012-1018.

Spigno, G; Tramelli, L and De Faveri, D M (2007). Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *J. Food Eng., 81*: 200-208.

Teixeira, B; Marques, A; Ramos, C; Batista, I; Serrano, C; Matos, O; Neng, N R; Nogueira, J M F; Saraiva, J A and Nunes, M L (2012). European pennyroyal (*Mentha pulegium*) from Portugal: Chemical composition of essential oil and antioxidant and antimicrobial properties of extracts and essential oil. *Ind. Crop Prod., 36*: 81-87.

Xie, Z J; Huang, J R; Xu, X M and Jin, Z Y (2008). Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. *Food Chem., 111*: 370-376.

Zhu, C Z; Zhang, W G; Kang, Z L; Zhou, G H and Xu, X L (2014). Stability of an antioxidant peptide extracted from Jinhua ham. *Meat Sci., 96*: 783-789.