2012 International Conference on Environment, Energy and Biotechnology
IPCBEE vol.33 (2012) © (2012) IACSIT Press, Singapore

Palm Kernel Cake Protein and Its Hydrolysate: A Novel and Inexpensive Antioxidant

Ng Khar Ling, Siti Norhazira bt Bosra Abu and Mohd Khan Ayob

School of Chemical Science and Food Technology, Faculty of Science and Technology, University Kebangsaan Malaysia, Bangi, Selangor.

Abstract. The aim of the study was to evaluate the antioxidant activities of palm kernel cake protein (PKCP) and its hydrolysate (PKCH). The protein was isolated using an alkaline extraction method and further hydrolysed by 2.4L alcalase to obtain PKCH. The antioxidant activity of PKCP and PKCH were determined using ferric thiocyanate (FTC) and thiobarbituric acid (TBA) assays while the radical scavenging capacity was measured using 1,1-diphenyl-2-picrylydrazyl (DPPH) method. In addition, the reducing power of PKCP and PKCH was also evaluated. Isolated soy protein (ISP) and its hydrolysate (ISPH) were tested as a comparative study. Meanwhile butylated hydroxytoluene (BHT) and ascorbic acid were used as positive control for each assay. The PKCP and PKCH demonstrated excellent antioxidant activities in all assays. PKCP and PKCH showed as strong antioxidant activities as BHT in FTC assay and the percent of inhibition were 97.68% and 97.84%, respectively. In DPPH assay, the PKCH showed the highest Trolox® equivalent (3165.19 ± 8.31μmol TE/L) compare to PKCP (2369.13 ± 17.56 μmol TE/L), ISPH (616.75 ± 3.34 μmol TE/L) and ISP (390.88 ± 1.96 μmol TE/L). The PKCH also exhibited 10 fold stronger reducing power than the ISPH.

Keywords: palm kernel cake protein, protein hydrolysate, antioxidant

1. Introduction

Consumption of high antioxidant food and supplement is a trend in current human lifestyle. Antioxidant is the key to recover the imbalance circumstance of antioxidant versus pro-oxidant. Free radicals are highly reactive and unstable compounds which produced during metabolic functions or introduced from external environment such as pollution [1]. To overcome this condition, human body has diverse its defense mechanism through antioxidant enzymes (e.g. superoxidase dismutase), free radicals scavengers (e.g. glutathione and vitamin C) and metal chelator (e.g. transferin and ferritin) [2]. When the free radicals are generated excessively, the imbalance might occur and caused cellular damage. The subsequence damage in human body might induce such diseases like atherosis, cardiovascular disease, cancer, neurological degenerative disease and so on [3]. Therefore, beside endogenous antioxidants which self produced under normal condition, human still need an exogenous antioxidant (intake from outside) to balance the excessive amount of free radicals.

An effective antioxidant which extracted from natural plant had paid attention recently due to the safety issues of synthetic antioxidant such as BHT and BHA [4]. However the cost of extracting new antioxidative compound from new plant sources is high and most of the interested compounds (vitamins, phenolics and flavonoids) showed instability during storage. Thus many researchers had changed their target for searching natural antioxidant compounds based on bioactive protein/hydrolysate/peptide. Many of plant proteins had been proven to have comparatively strong antioxidant, such as canola protein [5] and soybean protein [6]. However the costing problem still appeared when come to commercialization stage.

Palm kernel cake (PKC) is one of the abundant co-products of palm kernel oil milling industry. The worldwide production of PKC has achieved 1.514 million tons dry matter/year [7]. In spite of using PKC as animal feedstuff, the abundant amount of production daily still causes environment pollution and waste

management problem. As an alternative to diversify the application of the abundant PKC, further study on the potential of PKC as food ingredient and pharmaceutical product should be carried out. Thus, the objective of this study is to determine the antioxidant properties of palm kernel cake protein and it hydrolysate as a novel and inexpensive antioxidant source.

2. Methodology

2.1 Palm kernel cake protein extraction

The crude protein of PKC was extracted according to [8]. Fine PKC powder was suspend in alkaline solution at pH11 and extracted in water bath shaker at 50°C, 150rpm for 30 min. The solution was then centrifuged, 4000 rpm at room temperature for 20 min to obtain the supernatant. The protein in supernatant was precipitated using 3M HCl at pH range 4.5. The precipitate was centrifuged and defatted by re-suspending it in ethanol and centrifuged again at 10 000 rpm for 20min. The obtained protein pellet was collected and lyophilized at -40°C for 24h used as PKCP in further investigation.

2.2 PKCP and ISP protein hydrolysis

The optimized enzymatic hydrolysis where achieved the highest degree of hydrolysis at 70% was carried out using alcalase (according to our unpublished data). Briefly, lyophilized PKCP (0.05g) was re-suspend in 10mL phosphate buffer (pH 8.5) and 1µL of 2.4L alcalase was added to hydrolyze the protein. The solution was hydrolyzed in water bath shaker at 51.5°C, 150rpm for 60min. After 1 h enzymatic hydrolysis, the solution was centrifuged. The supernatant was lyophilized at -40°C for 24h and used as PKCH for further analysis.

For ISP hydrolysis, the optimized parameters were also studied and achieved degree of hydrolysis at 100% (according to our unpublished data). Dry ISP (0.1g) was re-dissolved in phosphate buffer (pH 8.5) together with 3µL of 2.4L alcalase and incubated in water bath shaker at 150rpm shaking speed, and at 60°C incubation temperature for 6h. The resultant solution was centrifuged and protein pellet was collected for lyophilization. The lyophilized hydrolysate was used as ISPH for further analysis.

2.3 Ferric Thiocyanate (FTC) Assay

The FTC assay was employed to determine the amount of peroxide at the initial stage of lipid peroxidation [1]. The experiment was carried out according to Kikuzaki and Nakatani [9]. All samples (4mg) were added in a mixture of 4ml absolute ethanol, 4.1ml 2.52% linoleic acid (in ethanol), 8ml 0.02M phosphate buffer (pH 7) and 3.9ml distilled water in a screw-capped vial and placed in an oven at 40°C and dark condition. The above mixture (0.1ml) was added to 9.7ml 75% (v/v) ethanol, followed by 0.1ml of 30% (w/v) ammonium thiocyanate. After 5 min, 0.1ml 0.02M ferrous chloride (v/v) (in 3.5% HCl solution) was added into the mixture and mix thoroughly. The absorbance of reacted mixture was measured at 500nm. The measurement was taken every 24 h until the absorbance achieved the maximum value. BHT was used as a positive control.

2.4 Thiobarbituric Acid (TBA) Assay

The TBA assay was carried out according to Kikuzaki and Nakatani [9]. The 2ml 0.67% TBA and 2ml 20% trichloroacetic acid (TCA) were mixed with resultant mixtures (1ml) from FTC assay. The mixture was boiled in a water bath for 10min, cooled, and centrifuged at 3000rpm for 20min. The absorbance was measured at 552nm. BHT was used as a positive control.

2.5 DPPH Assay

The DPPH radical scavenging capacity was determined according to the method described by Yu [10] with minor modifications. Briefly, 100µl of DPPH solution (0.15mM, in methanol) was reacted with 100 µl of the PKCH dissolved in deionized water. The reaction was incubated at room temperature for 20 minutes, and the absorbance was read at 517nm against blank. The results were expressed as micromoles Trolox® Equivalent per liter (µmol TE/L).

2.6 Reducing Power Assay

The reducing power of PKEP hydrolysate was determined based on the method described by Moure, Domingueuez, and Parajo [11] with minor modifications. All samples were prepared at 0.1mg/ml and 1 mL of the sample was mixed with 2.5mL 0.2M phosphate buffer (pH6.6) and 2.5mL 1% potassium ferricyanide. The mixtures were then incubated in a water bath at 50°C for 20 minutes. Then, 2.5mL trichloroacetic acid was added to the mixture and centrifuged for 10 minutes at 3000rpm. The 2.5mL supernatants were diluted in 2.5mL distilled water and followed by adding 0.5mL 0.1% ferric chloride. The absorbance was measured at 700nm. Ascorbic acid was used as a positive control.

2.7 Statistical analysis

All data obtained from triplicate experiments or analyses and recorded as mean ± standard deviation and analyzed by SAS package. One-way analysis of variance was performed using ANOVA procedures while significant differences between means were determined by Duncan's Multiple Range test ($p<0.05$).

3. Results and discussion

Exogenous antioxidant has plays important roles in human health. It is because reactive oxygen species from both endogenous and exogenous sources had been proven as the etiologies of diverse human diseases [12]. Antioxidant may be useful in preventing the deleterious consequences of oxidative stress [13]. Furthermore the increasing interest in the protective biochemical functions of natural plant-based protein or peptide has brings a great impact in research finding. Thus PKC had been targeted as an inexpensive antioxidant in Malaysia which produced bulky PKC daily.

Alkaline extraction is a common and effective way in protein extraction. It is important to increase the recovery yield or the extractability of protein from the PKC. As describe by [8], PKC is one of the by-products obtained from expelling process. Due to the heat generated in expelling process, the protein quality might partially been denatured. However, the quality of PKCP can be improved through hydrolysis process. In this study, ISP had been used a comparative protein and hydrolysate. This is because ISP is by-product and well knows as good protein sources in food industry, thus ISP and its hydrolysate may contribute great antioxidant activity as others study [5,6].

Table 1: Mean (n=3) results for FTC, TBA, DPPH and reducing power of protein and their hydrolysates.

	FTC (% of inhibition)	TBA (% of inhibition)	DPPH (µmol TE/L)	Reducing power (Absorbance)
PKCP	69.57 ± 0.00^{a}	97.68 ± 0.00^{b}	2369.13 ± 17.56^{b}	0.68 ± 0.01^{b}
PKCH	68.70 ± 0.00^{ab}	97.84 ± 0.00^{b}	3165.19 ± 8.31^{a}	0.76 ± 0.00^{b}
ISP	32.02 ± 0.03^{d}	66.52 ± 0.01^{d}	390.88 ± 1.96^{d}	0.07 ± 0.01^{c}
ISPH	51.54 ± 0.05^{c}	75.11 ± 0.01^{c}	616.75 ± 3.34^{c}	0.08 ± 0.01^{c}
Positive control				
BHT	67.86 ± 0.00^{b}	99.67 ± 0.00^{a}	-	-
Ascorbic acid	-	-	-	3.52 ± 0.25^{a}

[a-d] Significant differences ($p<0.05$) with different letters in same column.

Table 1 shows the antioxidant activities of PKCP, ISP and their hydrolysates (PKCH and ISPH). In the FTC and TBA assay, the percent of inhibition were determined. It was found that PKCP and PKCH had shown excellent antioxidant activities compared to ISP and its hydrolysate. In FTC assay, PKCP and PKCH significantly ($p<0.05$) inhibited most peroxyl in the early stage of lipid peroxidation and reached 69.57% and 68.70% of the inhibiton, repectively. BHT is a synthetic antioxidant which commercially used to inhibit lipid peroxidation. Thus, BHT was chosen as a positive control in FTC assay and the % of inhibition showed that it is lower than PKCP and PKCH significantly. Furthermore, PKCP and PKCH were also able to inhibit almost 100% carbonyl compounds formed in TBA assay just as good as BHT. If compared to ISP and its hydrolysate which inhibited only 66.52% and 75.11% of carbonyl compounds that decomposition in a later stage of lipid peroxidation. Thus, the palm-based extracted protein and hydrolysates showed the highest antioxidant activities.

In DPPH assay, all the samples were expressed in μmol Trolox® equivalent/L. Trolox® is a lipo-soluble derivative of Vitamin E and used in biochemical application to reduce oxidative stress. Trolox® equivalent is used as a standard measurement and benchmark of antioxidant strength. Thus, the higher the value of Trolox® equivalent represents greater antioxidant strength of the compounds. As showed in Table 1, PKCH exhibited the highest antioxidant capacity (3165.19μmol TE/L) and 5-fold higher and stronger than ISPH (613.78 μmol TE/L) significantly ($p<0.05$). In this study, ISP was used as a comparative protein to PKCP due to its similarity in amino acid profile. However, ISP and ISPH showed lower ability in scavenging free radicals compare to PKCP and PKCH.

Table 1 also shows the reducing power of all samples and ascorbic acid. As a positive control, ascorbic acid exhibited the highest reducing power with the absorbance value of 3.52. This was followed by PKCP and PKCH. However the absorbance of ISP and ISPH were 10 fold lower than PKCP and its hydrolysates. In another words, ISP and it hydrolysates failed to reduce any Fe (III) to Fe (II).

In conclusion, the PKCP and its hydrolysates had shown excellent potential as antioxidant. Furthermore the market value of PKC is low compared to natural plant product. This study demonstrates that PKCP and PKCH are a novel and inexpensive source of plant antioxidant.

4. Acknowledgements

This study was sponsored by Sciencefund Grant of Ministry of Agricultue and Agro-idustry (MOA), 05-01-02-SF1006. Authors kindly acknowledge FELDA Kernel Products Sdn. Bhd., Malaysia for supplying PKC sample.

5. References

[1] S. A. Sharipah Ruzaina, M. Sunalti, A. Norizan, M. J. Faridahanim, and A. Rohaya. Phenolic content and antioxidant activity of fruits of Ficus deltoidera var *Angustifolia sp*. *The Malaysian Jour. Anal. Sci*. 2009, 13(2): 146-150.

[2] N. Noguchi, A. Watanabe, and H. Shi. Diverse functions of antioxidants. *Free Radical Res.* 2000, 33: 809-817.

[3] Y. Wei, and H. Lee. Oxidative stress, mitochondrial DNA mutation, and impairment of antioxidant enzymes in aging. *Exp. Biol. Med.* 2002, 227(9):671-682.

[4] R. Kahl, and H. Kappus. Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. *Europ. Food Res. Technol*. 1993, 196 (4):329 – 338.

[5] C. Nichole, Z. Ying, N. Marian, and S. Fereidoon. Antioxidant activity and water-holding capacity of canola protein hydrolysates. *Food Chem*. 2008, 109:144-148.

[6] M.D. Mesa, J.M. Silvan, J. Olza, A. Gil, and M.D. del Castillo. Antioxidant properties of soy proteins-fructoologosaccharide glycation systems and its hydrolysates. *Food Res. Int.* 2008, 41(6):606-615.

[7] Oil world annual. 2002. Available at www.gpfeeds.co.uk/analysis/palmkern.htm.

[8] K.L. Ng, and M.K. Ayob. Enzymatic preparation of palm kernel expeller protein hydrolysate (PKEPH). *Int. Food. Res. Jour.* 2012, 19(2):721-725.

[9] H. Kikuzaki, and N. Nakatani. Antioxidant effects of some ginger constituents. *Jour. Food Sci*. 1993, 58:1407-1410.

[10] L. Yu. *Wheat Antioxidants*. John Wiley & Sons, 2008.

[11] A. Moure, H. Domingueuez, and J.C. Parajo. Antioxidant properties of ultra filtration recovered soy protein fractions from industrial effluents and their hydrolysates. *Process Biochem*. 2006, 41: 447-456.

[12] B. Halliwell, J.M.C. Gutteridge. *Free radical in biology and medicine.* Oxford University Press, 1998.

[13] F. Aqil, I. Ahamd and Z. Mehmood. Antioxidant and free radical scavenging properties of twelve tradiotionally used Indian medicinal plants. *Turk J. Biol.* 2006, 30:177-183.