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Antioxidative activity of roasted and defatted peanut kernels

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Abstract

Peanut kernels were roasted at 180±2°C for various period of times (0–60 min); then grounded and defatted or further hydrolyzed with proteases to test their antioxidative activity (AOA). Samples roasted for 60 min displayed the most remarkable AOA, determined by Ferric-thiocyanate method, on linoleic acid in emulsions prepared with Tween 20 or 80. In reducing power, the absorbance at 700 nm of enzymatic hydrolysates (1 mg/ml) prepared from a 60-min-roasted sample with Esperase (enzyme/substrate = 1/200, 60°C, pH 8.0) and with Neutrase (enzyme/substrate = 1/200, 50°C, pH 6.0) was 1.24 and 0.81, respectively. The scavenging activity of Esperase and Neutrase hydrolysates on DPPH (α, α'- diphenyl-β-picryldrazyl) radicals was 93 and 89%, respectively, while their chelating activity on Fe^{+2} was 69 and 52%, respectively. Besides, in vitro, Esperase hydrolysates (≥100 μg/ml) exhibited the remarkable antioxidative effect on the oxidation of low-density lipoprotein (LDL) induced by copper by showing a lag time of longer than 6 h.

Keywords: Peanut kernel; Maillard reaction products; Antioxidative activity; Reducing power; Chelating activity

1. Introduction

Rancidification is a problem in food safety and also reduces the shelf life and the nutritional quality of food products. Lipid oxidation can be effectively somehow prevented by using antioxidants. However, some synthetic antioxidants [i.e. butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT)] have been suspected to be responsible for liver damage and carcinogenesis in laboratory animals (Grice, 1988; Witschi, 1986). Thus, efforts have been made to look for potent natural antioxidants.

Maillard reaction products demonstrated to be effective suppressants of rancidity in model systems (Lingnert & Erikson, 1981) as well as in food products (Cho, Miura, Fujimoto, & Lmai, 1988; Tanaka, Sugita, Wenkui, Nagashima, & Taguchi, 1990). Recently, proteins and protein hydrolysates were also reported to be antioxidative in model systems (Hatate, 1996; Taguchi, Iwami, Kawabata, & Ibuki, 1988). Moreover, the sequences of peptides with potent antioxidative activity were elucidated (Chen, Muramoto, Yamauchi, Fujimoto, & Nokihara, 1998).

One third of Taiwan annual production of peanut (around 80 000 t) is used for preparing oil. Roasted peanuts oil is fragrant and brown. That is due to adequate Maillard reactions. However, peanut meals are usually treated as animal feeds and fertilizers; their utilization has to be improved. In addition, the antioxidative effect of Maillard reaction products from a lipid–carbohydrate–protein system in roasted peanut kernels is still unknown.

Therefore, in the present study, roasted and defatted peanut kernels (RDPK) were prepared and tested to investigate their antioxidative activities. These RDPK were later hydrolyzed with proteases to study the increase in value of the antioxidative activity.

2. Materials and method

2.1. Preparation of roasted and defatted peanut kernels (RDPK)

Fresh peanut kernels from a local market were heated at 180±2°C in an oven (FS-420, Advantec, Tokyo,

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Japan) equipped with an air circulation system. Heating times increased from 0 to 10, 20, 30, 40, 50 and 60 min. Roasted peanut kernels were later grounded in a grinder (Model 36BL23,Waring Co., New Hartford, USA) to pass a 20-mesh net (A.S.T.M. E-11, ATM, Wisconsin, USA). n-Hexane (7 v/v) was well mixed with the peanut powder and gently stirred in a magnetic stirrer for 90 min to extract the fat. Next, residual n-hexane in peanut powder was removed under reduced pressure (10 mm Hg) in a vacuum oven (VO-30MIC, Shinch-Jaan Co., Taipei, Taiwan). The same procedure was repeated three times to prepare RDPK (with a yield of 55.8%), which was suspended in distilled water to make a concentration of 20 mg protein/ml prior to AOA determination. Roasted peanut kernels from a local peanut oil factory (Sin-Chen Co., Taipei, Taiwan) were also defatted and prepared using the same procedure to prepare RDPK.

For the observation of the progress of Maillard reaction, absorbance of 0.4 g suspended RDPK in a 50 mM $CaCl_2$/50 mM Tris buffer (pH 7.0) at 420 and 550 nm was monitored with a spectrophotometer (model 7800, Jasco, Tokyo, Japan). Browning index = ($Absorbance_{420\ nm}$ − $Absorbance_{550\ nm}$).

2.2. Hydrolysis of RDPK

Twenty grams of 60-min roasted RDPK were well mixed with Esperase (7.5 KNPU/g; Nova Nordisk, A/S, Bagsvaerd, Denmark; pH 8.0) or Neutrase (1.5 AU/g; Nova Nordisk, A/S, Bagsvaerd, Denmark; pH 6.0) at a ratio of 200:1 (w/w) in 80 ml of distilled water. During a preliminary test, enzymatic hydrolysates were prepared with Esperase, Protease A, Protease N, Neutrase and pepsin to test their antioxidative activities on linoleic acid. A 2-h incubation period in an oven at 60±1°C followed for the Esperase group (with a yield of 56.8%) and a 24-h incubation time at 50±1°C followed for Neutrase group (with a yield of 36.5%) exhibited good antioxidative activity and were chosen for the following experiments. The reaction mixtures were then thermally treated (100°C, 10 min) in boiling water to denature proteases. The reaction mixtures were later centrifuged (10 000×*g*, 15°C, 10 min) and filtrated (Whatman No. 4) to collect the supernatants. Protein concentration was adjusted to 20 mg/ml with distilled water to determine AOA.

Twenty grams of RDPK added with 80 ml (v/w) of 6 N HCl were incubated at 110±2°C for 24 h at a reduced pressure (10 mm Hg) to prepare the acid hydrolysates. Later, hydrolysates were vacuum-dried with the aid of an aspirator in a rotary evaporator (N-N, Rikakikai Co., Tokyo, Japan) and re-dissolved in distilled water. Same procedure was repeated until HCl was completely removed. The yield of acid hydrolysates was 92.2%.

Twenty grams of RDPK were alkaline hydrolyzed with 80 ml of 4 M $Ba(OH)_2$ at 110°C for 24 h. The pH of hydrolysates was adjusted to 7.0 with 0.1 N H_2SO_4 and later centrifuged (1500×*g*, 10 min) to remove the precipitates. The yield of alkaline hydrolysates was 44.1%.

The solid content in both acid and alkaline hydrolysates was adjusted to 30 mg/ml with distilled water prior to AOA determination.

2.3. Determination of AOA

The AOA of RDPK and of enzyme hydrolysates on linoleic acid in emulsions were determined according to the Ferric-thiocyanate method, as described by Mitsuda, Yasumoto, and Iwami (1966). Equal weights (0.56 g) of linoleic acid (Sigma, St. Louis, MO, USA) and Tween 20 or Tween 80 (Sigma, St. Louis, MO, USA) in 100 ml 0.1 M phosphate buffer (pH 7.0) were emulsified using a homogenizer (Polytron PT 3000, Kinematica AG, Lucerne, Switzerland; 6000 rpm) for 20 s to prepare the emulsion containing 50 mM linoleic acid. The emulsion thus obtained (2.5 ml) was well mixed with a 0.5 ml sample solution (20 mg/ml for RDPK, 30 mg/ml for hydrolysates), 0.5 ml distilled water and 1.5 ml 0.1 M phosphate buffer (pH 7.0; RDPK/linoleic acid = 10/35, hydrolysates/linoleic acid = 15/35, w/w). Then it was tightly sealed in a screwed test tube and incubated at 60±1°C in an oven (FS-420, Advantec, Tokyo, Japan). Sample emulsion (0.1 ml) was then mixed well with 75% ethanol, 30% ammonium thiocyanate and ferrous chloride (Sigma, St. Louis, MO, USA) with a Vortex mixer, followed by resting at room temperature for 3 min. Color developed was monitored at 500 nm by a spectrophotometer. The time (h) required for the absorbance of reaction mixture to up to 0.3 was treated as the induction period (Chen, Muramoto, & Yamauchi, 1995; Mitsuda, Yasumoto, & Iwami, 1966). Sampling was done every 4 h to determine the AOA on linoleic acid.

2.4. Reducing power

Reducing power was carried out according to the method described by Oyaizu (1988). Enzymatic hydrolysates (0.5 ml; 0, 0.1, 0.25, 0.5 and 0.75 and 1.0 mg protein/ml) from RDPK were well mixed with 0.5 ml 0.2 M phosphate buffer (pH 6.6) and 0.5 ml 1.0% K-ferricyanide (Sigma, St. Louis, MO, USA). Then the mixtures were incubated at 50±1°C in a water bath for 20 min. Centrifugation (1500×*g*, 10 min) followed the addition of 0.5 ml 10% trichloroacetic acid (Nacalai, Kyoto, Japan) to the sample solution. The supernatant thus collected (1.0 ml) was well mixed with 1.0 ml distilled water and 0.2 ml 0.1% $FeCl_3 \cdot 6H_2O$. Next, it was rested at room temperature (28±2°C) for 10 min. Color changes were monitored at 700 nm by a spectrophotometer.

α-Tocopherol, BHA and ascorbic acid (Sigma, St. Louis, MO, USA) were used to compare the reducing power. The higher the absorbance is, the better the reducing power of the sample is recognized (Oyaizu, 1988).

2.5. Scavenging activity on DPPH radical

With minor modifications, the method described by Blois (1958) was followed to determine the scavenging activity of samples on DPPH radical. RDPK enzymatic hydrolysates (0.3 ml; 0, 0.1, 0.25, 0.5, 0.75 and 1.0 mg/ml) were well mixed with 0.2 ml distilled water and 2.5 ml 75 μM DPPH (α, α′-diphenyl-β-picryldrazyl; Aldrich, Milwaukee, USA)/100% methanol and then let rested at room temperature (28±2°C) for 90 min. Color changes were monitored at 517 nm by a spectrophotometer. α-Tocopherol, BHA and ascorbic acid were used to compare the scavenging activity. Scavenging activity = (1−Absorbance$_{517\ nm\ sample}$)/(Absorbance$_{517\ nm\ control}$)×100%.

2.6. Chelating activity on Fe^{+2}

Chelating activity of RDPK hydrolysates (0, 0.1, 0.25, 0.5, 0.75 and 1.0 mg/ml) was determined by the method of Dinis, Madeira, and Almeida (1994). A 0.1 ml sample solution was added with 0.6 ml de-ionized water and 0.1 ml 0.2 mM $FeCl_2 \cdot 4H_2O$ (Wako Co., Osaka, Japan); the mixture was allowed to rest at room temperature (28±2°C) for 30 s. The reaction mixture thus obtained was later added with 0.2 ml 1 mM ferrozine (Sigma, St. Louis, MO, USA) and changes in color were monitored at 562 nm with a spectrophotometer, after a 10-min resting time at room temperature. Ethylene diamine tetraacetate (EDTA, 2 Na-salt; Sigma, St. Louis, MO, USA) was used to compare the chelating activity.

2.7. Inhibition of human LDL oxidation

Three healthy and overnight fasting males (aged 20–30) provided blood (80 ml) which was incubated at 37±1°C in a water bath for 1.5 h for sufficient clotting. After a 1-h incubation period at 4°C, the blood was centrifuged (1200×g, 15min, 4°C) to collected serum. Collected serum (25 ml), was added with 5 ml normal saline (145 mM, $\rho = 1.006$); the mixture was then centrifuged (15 200×g, 16 h, 4°C) to remove with a pipette the VLDL (very low-density lipoprotein) portion (about 10 ml) in the top layer. Then, 10 ml NaBr ($d = 1.22$ g/ml) was added to the serum thus obtained; the homogenous mixture was centrifuged (15 200×g, 16 h, 4°C) to collect the low-density lipoprotein (LDL) portion (d = 1.019–1.063) (about 10 ml). This was sealed in a sterilized plastic test tube filled with N_2 gas and stored at 4°C in darkness. The LDL sample was used within 7 days.

The LDL sample was dialyzed three times against 100 vol of 125 mM NaCl/5 mM phosphate buffer (PBS, pH 7.4) for 3, 3 and 14 h, respectively. That was done in a sealed glass vessel filled with N_2 gas to avoid possible LDL oxidation. Cholesterol content in LDL sample was determined with enzymatic method (Richmond, 1973) using cholesterol enzymatic kit (Merck, Darmstadt, Germany). Dialyzed LDL (10 μl) was well mixed with 1.0 ml kit solution and the change in color was monitored at 500 nm. Various cholesterol solutions (0, 10, 20, 30 and 40 μg/ml) were used to construct the standard curve.

Cholesterol level in dialyzed LDL was adjusted to 100 μg/ml with PBS. Five hundred μl of the thus obtained LDL solution was well mixed with 100 μl Esperase hydrolysates (10–400 μg/ml), 200 μl PBS and 200 μl 25 μM Cu^{+2} (from $CuSO_4 \cdot 5H_2O$). Then, the mixture was monitored at 232 nm by a spectrophotometer every 15 min at room temperature to observe the changes in conjugated dienes in LDL solution (Puhl, Wang, & Esterbauer, 1994). Trolox (Sigma) was used to compare with the hydrolysates.

2.8. Determination of protein content

Protein content was determined by the Bradford method (Bradford, 1976) with Bio-Rad protein assay dye reagent at 595 nm. Bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA) (0.2–1.4 mg/ml) was used as a standard curve to calculate the protein content.

2.9. Statistical analysis

Analysis of variance of results was carried out using General Linear Model Procedure of SAS Statistical Software, Version 6.11 (SAS Institute, 1995). For determining AOA, reducing power, scavenging activity on DPPH radical, chelating activity and inhibition on LDL oxidation induced by copper, three samples were each tested in duplicate.

3. Results and discussion

3.1. AOA of RDPK

Browning index of RDPK was observed to increase with the increasing heating (180±2°C) time (0–60 min) in the oven, indicating that Maillard reaction progressed with the heating time (data not shown). Peanut kernels roasted for up to 60 min showed remarkable AOAs on linoleic acid in emulsions; their antioxidative effect relatively increased with the increasing roasting time (Fig. 1). Peanut kernel oils prepared in Taiwan are mostly from the kernels roasted at about 180°C for 30–60 min, depending on the oil factories.

AOA of RDPK increased with the increasing roasting time (0–60 min; Fig. 1). For 60-min-roasted RDPK, the induction period was determined to be about 200 h in Tween 20 emulsion (Fig. 1A) and about 350 h in Tween 80 emulsion (Fig. 1B). However, the induction period for the control (unroasted and defatted peanut kernel powder) was only 12 h (Fig. 1A) and 47 h (Fig. 1B), though longer than the 6 h of the blank group. The Maillard reaction products apparently developed strong antioxidative effect on the linoleic acid in emulsions.

Yamaguchi and Fujimaki (1974) reported that a glycine-xylose Maillard reaction product exhibited noticeable AOAs, almost equivalent to that of BHA but inferior to that of BHT in a model system. Compared with the ovalbumin or polysaccharide alone, heated ovalbumin–polysaccharide complexes also are reported (Nakamura, Kato, & Kobayashi, 1992) to be effective in increasing the value of the AOAs; so are they with regard to the scavenging activity on free radicals in the food products.

3.2. Effect of hydrolysates on the AOA of RDPK

Fig. 2 illustrates that the acid or alkaline hydrolysates obtained from 60-min-roasted RDPK exhibited lower antioxidative activity on linoleic acid. However, Esperase

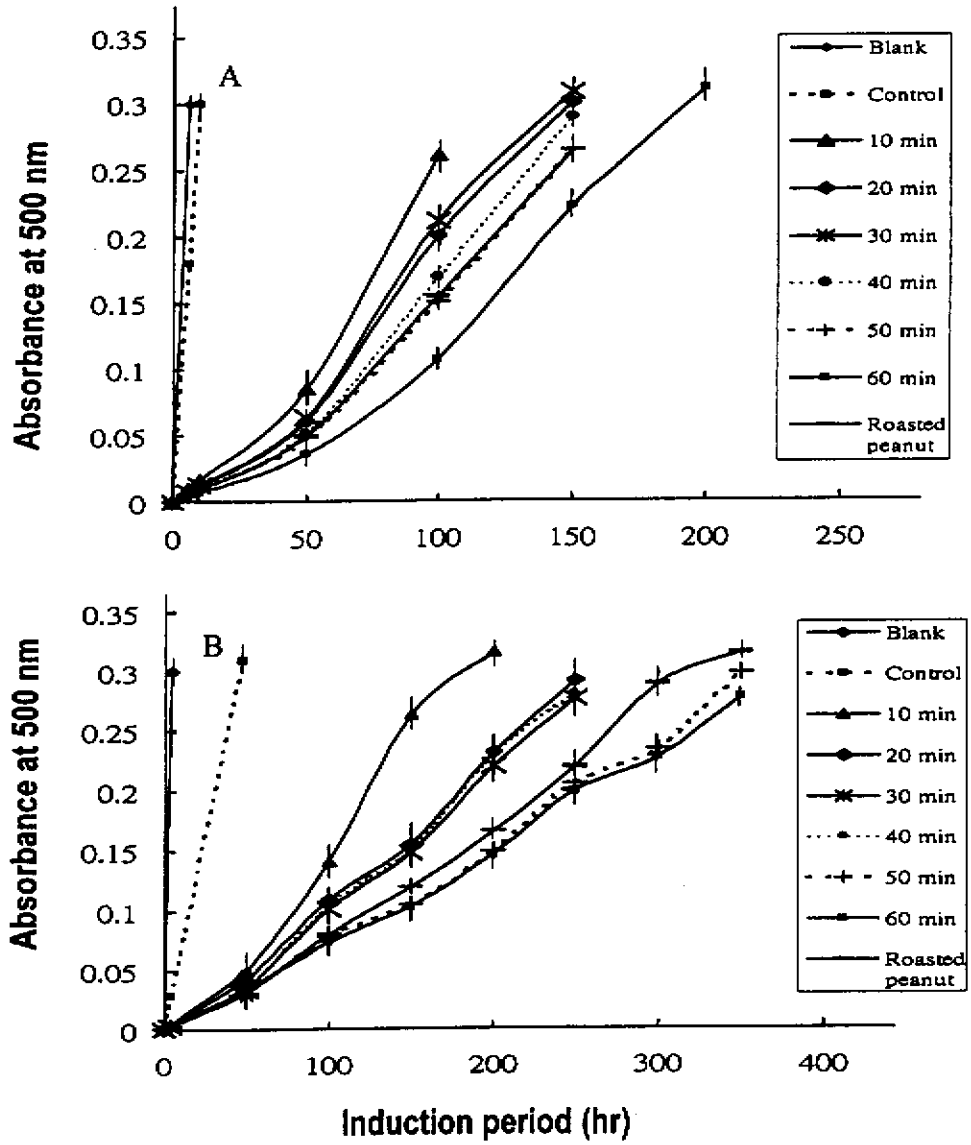

Fig. 1. Antioxidative activity of peanut kernel heated at 180±2°C for up to 60 min on the oxidation of linoleic acid (50 mM/ml) in emulsions prepared with (A) Tween 20 and (B) Tween 80. Heated kernels were defatted and ground to pass a 20-mesh net to make a solution of 20 mg/ml. Roasted peanut is the sample from oil factory. Roasted and defatted peanut kernels (2.0 mg/ml)/linoleic acid = 2/7 (w/w). Each value is the average of three determinations. Bars in the curve refer to standard deviation.

and Neutrase hydrolysates displayed more remarkable antioxidative effects (Fig. 2). The induction time for Esperase hydrolysates was almost 400 h for Tween 20 emulsion (Fig. 2A) and 500 h for Tween 80 emulsion (Fig. 2B). Compared to Esperase hydrolysates, AOA of Neutrase hydrolysates was less noticeable; yet much stronger than that of the unhydrolyzed RDPK (Fig. 1). Proteins showed increased AOA when treated with proteases (Chen et al., 1998; Hatate, 1996; Taguchi, Iwami, Kawabata, & Ibuki, 1988). Nonetheless, their antioxidative activities were significantly reduced when proteins were completely hydrolyzed to amino acids (Fig. 2). Thus, it is obvious that the AOA of protein does not completely depend upon the compositional amino acid. Appropriate degree of hydrolysis enhanced the exposure of amino acids and or/peptides (functional groups) to the oil and thus, increased the activity to inhibit the oil oxidation (Chen et al., 1998). In addition, enzymatic hydrolysates used in the present study came from roasted peanut kernels, which exhibited much stronger AOA than protein hydrolysates from soy protein isolate (Chen et al., 1998).

3.3. Reducing power of hydrolysates

Although not so remarkable, two enzymatic hydrolysates showed reducing powers (Absorbance 700 nm = 1.24 and 0.81) at a concentration of 1.0 mg

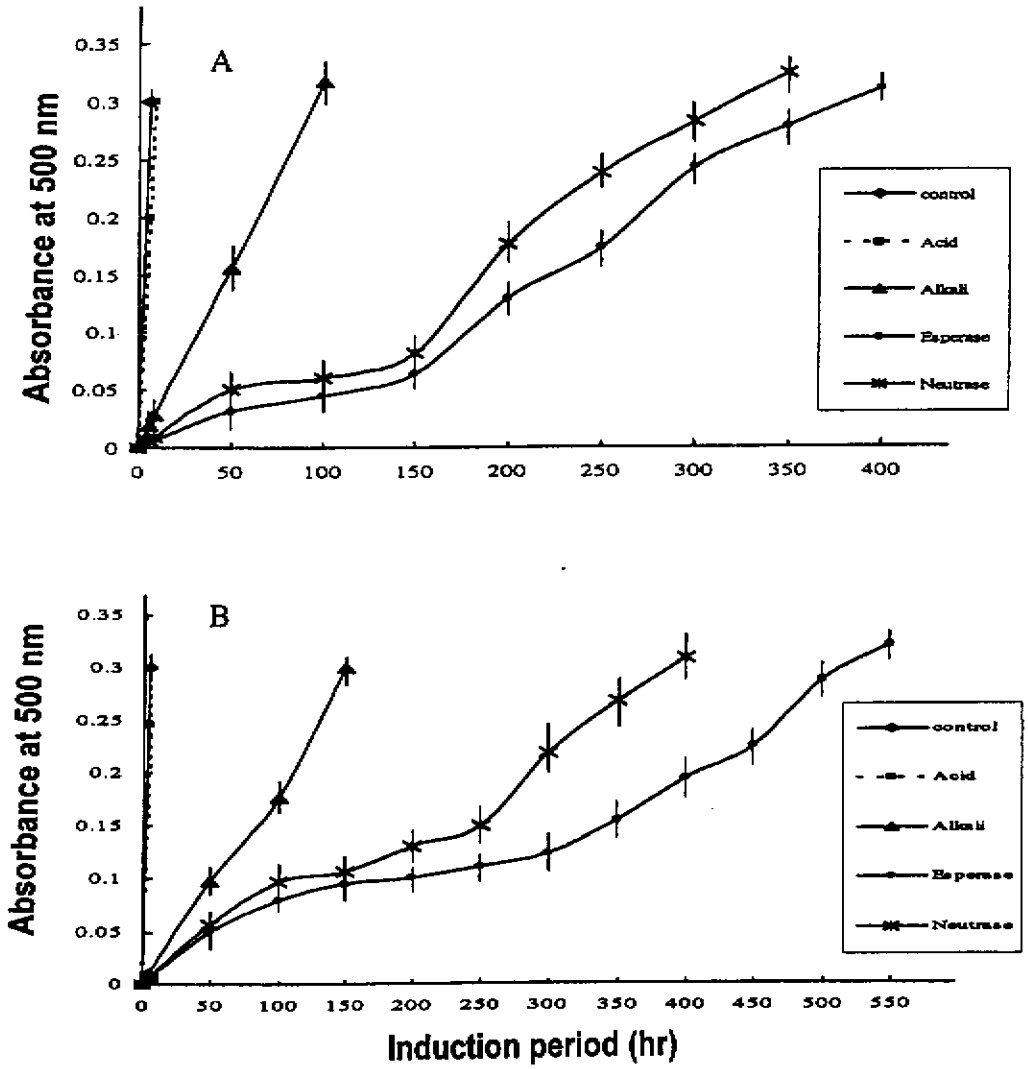

Fig. 2. Inhibitory effect of various hydrolysates on the autoxidation of linoleic acid (50 mM/ml) in emulsions prepared with (A) Tween 20 and (B) Tween 80. Roasted and defatted peanut kernel powder was hydrolyzed with 6 N HCl, 4 M $Ba(OH)_2$ or proteases (Esperase, E/S = 1/200 (w/w), pH 8.0, 60°C, 2 h; Neutrase, E/S = 1/200 (w/w), pH 6.0, 50°C, 24 h). hydrolysates (3.0 mg/ml)/linoleic acid = 3/7 (w/w). Each value is the average of three determinations. Bars in the curve refer to standard deviation.

protein/ml, almost equivalent to the effect of 0.02 mg BHA or α-tocopherol/ml, when reacted with K-ferricyanide and $FeCl_3$ (Fig. 3).

Glucosamine directly heated at 90°C for 1 h exhibited high absorbance (0.45) at 700 nm (Oyaizu, 1988). Reductones from the thermolysis of Amadori products in the primary stage of Maillard reaction were mainly responsible for the production of reducing power in Maillard reaction products (Yamaguchi & Yoshito, 1967). Thus, reductone-related products appeared to be present in the hydrolyzed RDPK.

3.4. Scavenging activity on DPPH radical

Increased levels (0.1–0.5 mg/ml) of enzymatic hydrolysates in the reaction mixtures exhibited proportionally higher scavenging effects (Fig. 4). At a concentration of 0.5 mg/ml, Esperase hydrolysates showed about 90% scavenging effect on the DPPH radicals, closely to the effect (91%) of 0.01 mg BHA/ml. Maillard reaction products prepared with xylose and lysine at 100°C for 5 h displayed about 50% scavenging activity on DPPH radicals (Yen & Hsieh, 1995). Ascorbic acid is an antioxidant with an enediol structure conjugated with the carbonyl group in a lactone ring and it is capable of terminating the chain reactions of free radicals by providing hydrogen atoms to the radicals (Shimada, Fujikawa, Yahara, & Nakamura, 1992). Therefore, the scavenging effect of protein hydrolysates from RDPK could be due to their reductone-like structures as a result of the roasting.

3.5. Chelating activity on Fe^{+2}

Protein hydrolysates also showed increased chelating activity on Fe^{+2} at an increased concentration (Fig. 5). At a concentration of 1.0 mg/ml, Esperase and Neutrase hydrolysates showed 69 and 53% chelating effect on Fe^{+2} in the present system, almost equal to the effect of EDTA at a concentration of 0.0075 mg/ml. Maillard reaction products (glucose–casein) are reported (Miller, 1979) to be capable of chelating divalent cations and result in the decrease in Fe^{+2} absorption from foods in animal model. The formation of melanoidins in the late stage of Maillard reaction was responsible for the chelating ability (Rendleman, 1987), which was dependent on the sugar sources as well as on the heating conditions e.g. glucose was most favorable for the formation of copper chelating affinity (Wijewickreme, Kitts, & Durance, 1997).

3.6. Effect of hydrolysates on the copper-induced LDL oxidation

Various levels (0–400 μg/ml) of Esperase hydrolysates were well mixed with LDL (50 μg cholesterol/ml) from human source in the presence of 5 mM copper and the changes in absorbance at 232 nm were then recorded.

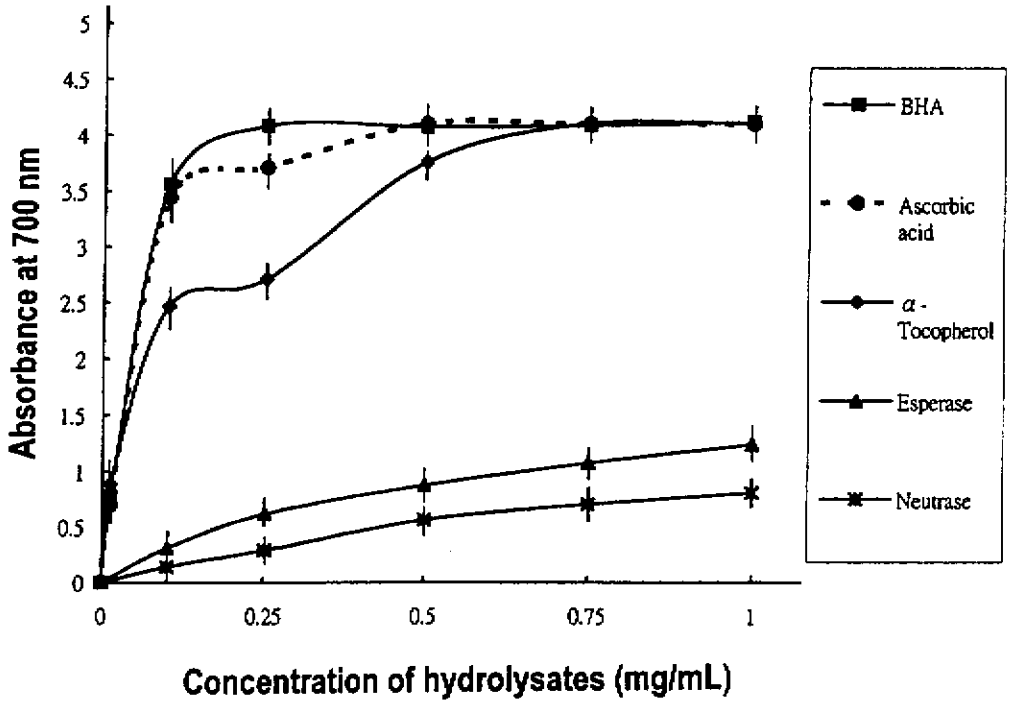

Fig. 3. Reducing power of various levels of enzyme hydrolysates (20 mg/ml). Preparation of hydrolysates sees the footnote in Fig. 2. Each value is the average of three determinations. Bars in the curve refer to standard deviation.

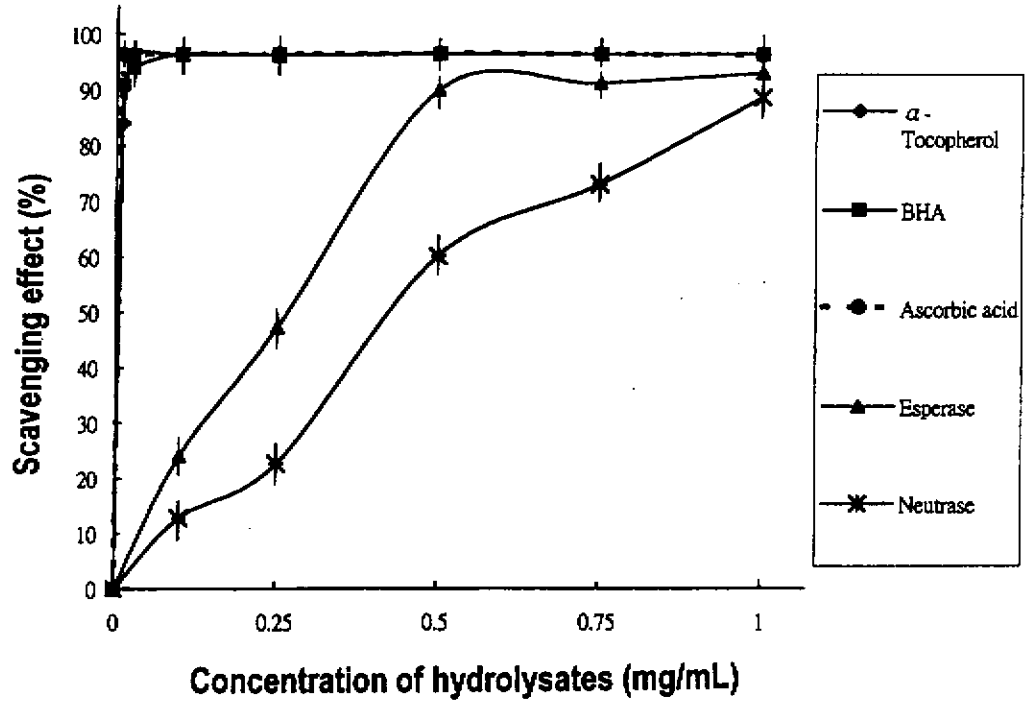

Fig. 4. Scavenging effect of various levels of enzyme hydrolysates (20 mg/ml). Preparation of hydrolysates sees the footnote in Fig. 2. Each value is the average of three determinations. Bars in the curve refer to standard deviation.

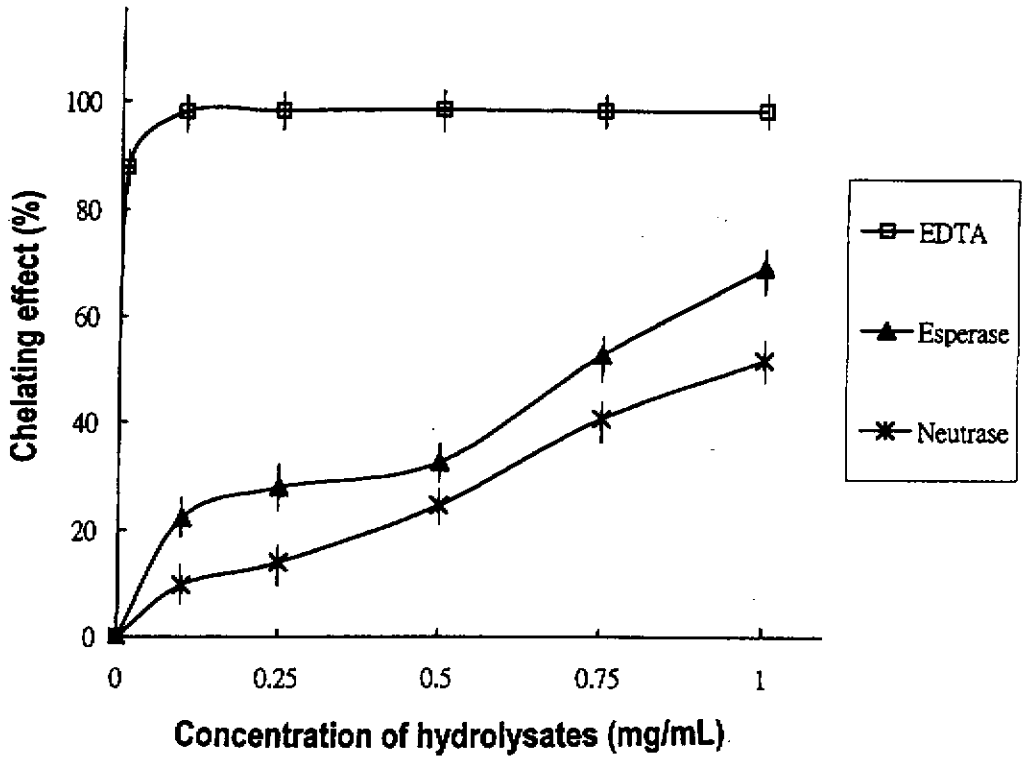

Fig. 5. Chelating activity of various levels of enzyme hydrolysates (20 mg/ml). Preparation of hydrolysates sees the footnote in Fig. 2. Each value is the average of three determinations. Bars in the curve refer to standard deviation.

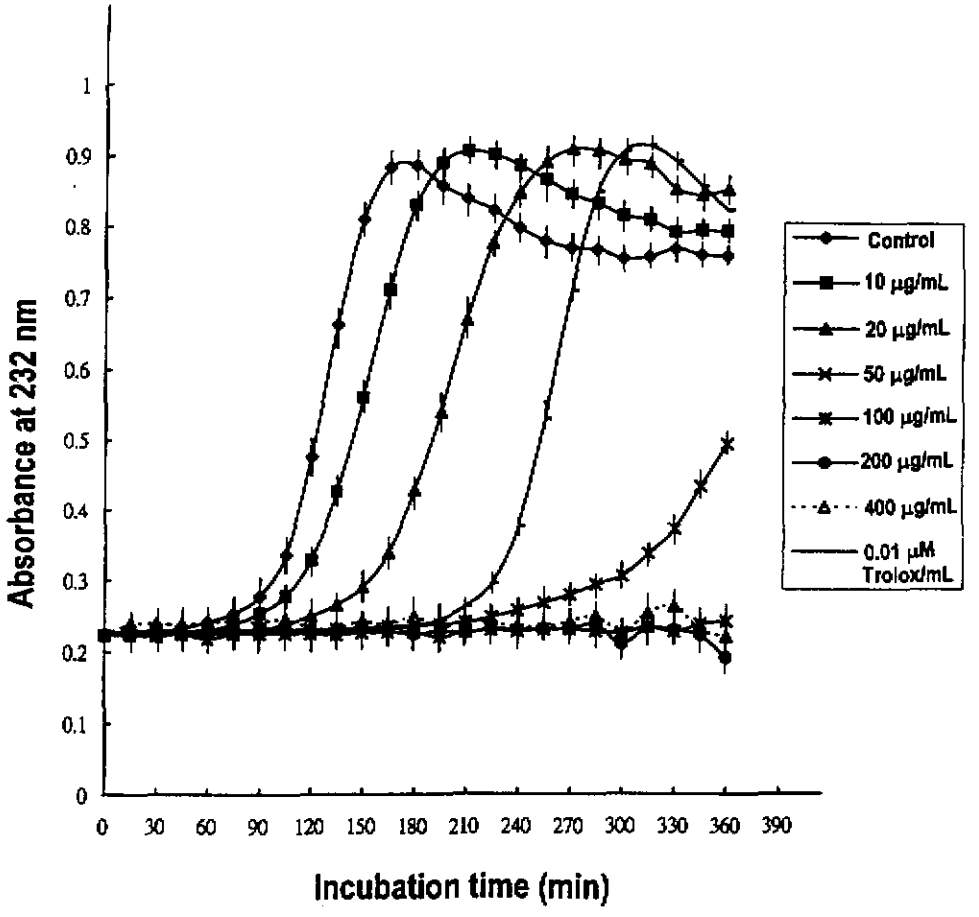

Fig. 6. Effect of Esperase hydrolysates levels (10–400 μg/ml) on the oxidation of LDL induced by copper ion (5 μM/ml). LDL prepared from human blood was diluted to 100 mg cholesterol/ml with PBS (125 mM NaCl/5 mM phosphate, pH 7.4). Preparation of hydrolysates sees the footnote in Fig. 2. Trolox (0.01 μM) was used to compare with the hydrolysates. Each value is the average of three determinations. Bars in the curve refer to standard deviation.

As shown in Fig. 6, the lag time of the absorbance curve was apparently prolonged at the increased hydrolysates level. At concentrations higher than 100 μg/ml, Esperase hydrolysates showed remarkable inhibitory effects on the oxidation of LDL during the 6-h experiment. Therefore, it is obvious that the use of enzymatically hydrolyzed RDPK effectively suppresses the oxidation of LDL in vitro, which produces cytotoxic products when attacked by free radicals in vivo (Hwang, 1991). The oxidized LDL is likely to cause atherosclerosis (Hwang, 1991). Therefore, inhibition on the LDL oxidation is considered to be effective in preventing atherosclerosis.

4. Conclusion

The roasting process was effective in enhancing the AOA and, furthermore, roasted products exhibited much stronger AOA when hydrolyzed by proteases. Peanut meals are side-products during the peanut oil preparation. Although further tests are needed, the strong AOA of enzymatic hydrolyzed RDPK reveals the possibility of it being used as an antioxidant in food products.

Acknowledgements

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