Original paper

Antioxidant Properties of Maillard Reaction Products from Defatted Peanut Meal Hydrolysate-Glucose Syrup and its Application to Sachima

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Antioxidant properties of defatted peanut meal (DPM) hydrolysate-glucose syrup Maillard reaction products (MRPs) were evaluated, and their effects on the oxidative stability and flavour property of Sachima during the storage were studied. DPM hydrolysate-glucose syrup was heated at 120°C for different time. With the heating time increasing, browning and intermediate products increased, free amino group content decreased. MRPs heating for 60 min had the best antioxidant properties, evaluated by 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity, oxygen radical absorbance capacity and inhibition of linoleic acid autoxidation. Sachima added with 1% MRPs showed significantly ($p < 0.05$) lower acidity values and peroxide values than that without MRPs during 5 months storage. Results from GC-MS indicated that MRPs improved the flavour of Sachima. Based on these findings, MRPs derived from DPM hydrolysate-glucose syrup might be used in food lipids stabilization as potent natural antioxidant and flavour enhancer.

Keywords: antioxidant, defatted peanut meal, maillard reaction, lipid peroxidation, sachima

Introduction

Peanut is one of the most popular coarse grains growing worldwide. Majority of the total production is used for oil extraction, leaving a large amount of defatted peanut meal (DPM). DPM contains 50 – 55% high quality protein and has great potential as food protein source. However, its poor protein solubility, low digestibility and other shortcomings limit its application. It is mainly used as animal feed and fertilizer at present. Enzymatic hydrolysis is potentially an effective technique for the recovery of proteins from DPM. Hydrolysate from DPM can be used as valuable protein resource or peptides for Maillard reaction in the presence of sugars. To the best of our knowledge, researches regarding the Maillard reaction products (MRPs) prepared using DPM hydrolysates and their application in food are still limited.

Lipid oxidation is a major cause of food deterioration, which directly results in stale or rancid flavour, and decreased nutritional

quality and safety. Prevention of lipid oxidation has significant importance food industry. Some synthetic antioxidants, such as butylated hydroxytoluene and tertiary butylhydroquinone (TBHQ) have been used in food industry to prevent lipid oxidation. However, the use of synthetic antioxidants is now limited owing to the growing concern over their potential carcinogenic effects (Sun and Fukuhara, 1997). Hence, growing interest is focusing on developing natural antioxidants.

MRPs have been proved to be effective natural antioxidant in model systems and some food (Benjakul *et al.*, 2005; Sun *et al.*, 2010). They attract particular attention of food producer as they play a key role in food process by delaying, retarding, or preventing oxidation processes. Li *et al.* (2013) proved that the MRPs of xylan and chitosan are resultful antioxidative preservatives for lipid food storage in lecithin model system and refrigerated pork meat. In addition, MRPs contribute markedly to the aroma and taste of stored and processed food. Some researchers have added MRPs to

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food for its good flavour and antioxidant activity (Sun *et al.*, 2010).

Sachima is a kind of traditional Chinese pastry. It originates from China's Manchu ethnic group as a sacrifice in ancient times, and now has become more and more popular due to its deliciousness and convenience. Sachima is mainly made from flour and eggs. Deep-frying of dough is a pivotal process as it leads to the loose texture and porous of the product, while it also makes the lipid content up to 20–30%. Prevention of lipid oxidation has become one of the biggest technical challenges for Sachima producing. In this study, the antioxidant properties of MRPs derived from DPM hydrolysate-glucose syrup at different heating time were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, oxygen radical absorbance capacity (ORAC) assay and inhibition of linoleic acid autoxidation. The effects of MRPs on Sachima with regards to lipid oxidation and aroma compounds were investigated.

Materials and Methods

Materials and chemicals Defatted peanut meal was purchased from Shandong Luhua Group Co. Ltd., Shandong, China. It contained 49.1% protein, 31.5% carbohydrate, 6.3% moisture, and 4.5% ash. Glucose syrup (solid content: 80%) was obtained from Hsu Fu Chi International Co. Ltd., Guangdong, China. DPPH, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), fluorescein disodium (FL) and 2,2'-azobis (2-methylpropionamide) dihydrochloride (AAPH), protein standards and amino acid standards were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). All the other chemicals and solvents were of analytical grade.

Preparation of DPM hydrolysate The DPM hydrolysates were prepared according to the method of Su *et al.* (2011). Five hundred grams of DPM was added into 500 mL of deionised water and heated at 121°C for 15 min using an autoclave (Shanghai Shenan Instrument Co. L td., Shanghai, China), then mixed with 4000 mL of deionised water and homogenised at 10,000 rpm for 1 min using an Ultra Turrax homogeniser (Beijing Jingke Huarui Instrument Co. Ltd., Beijing, China). The pH of homogenate was adjusted to 7.0 with 1 M NaOH. Then the crude protease extract prepared from *Aspergillus oryzae* HN 3.042 (activity of 15,478 U) was added to the homogenate with an enzyme/DPM ratio of 1.0 mg/g. The homogenate was continuously stirred with a mechanical stirrer for 24 h at 60°C. At the end of hydrolysis, the enzyme was inactivated by heating in a boiling water bath for 15 min. The hydrolysate was centrifuged in a GL-21M refrigerated centrifuge (Xiangyi Instrument Co. Ltd., Changsha, China) at 5000 × g for 20 min at 20°C and the supernatants were collected, lyophilized (R2L-100KPS, Kyowa Vacuum Engineering, Tokyo, Japan) and stored in a desiccator for further use.

Preparation of MRPs MRPs were prepared as follows: four grams of DPM hydrolysates were added to one hundred grams glucose syrup (solid content: 80%). They were mixed together and

heated at 120°C for 0min, 10min, 20min, 30min and 60min, namely M0, M1, M2, M3 and M4, respectively. After being autoclaved and cooled, the MRPs were kept at 4°C for further use.

Preparation of Sachima Sachima was produced using an industrial production line in Hsu Fu Chi International Co. Ltd, Guangdong, China. Sachima was produced in 100 kg batch for each treatment. Sachima was prepared according to the following formulation: strong flour (75 kg), egg wash (46.1 kg), defatted milk powder (4.5 kg), salt (0.15 kg), yeast powder and some amount of prepared MRPs. The ratio of MRPs was 1 g/100 g flour dough. After mixing thoroughly, the dough was flatted, divided and sent into the Fermenting Box for fermentation. Then the dough was passed pasta roller at 0.2 cm thickness and cut into squares (2.0 cm × 1.0 cm). The flat square-shaped dough was fried in palm oil with addition of 0.02% TBHQ at 160°C for 30 seconds in a temperature-controlling electronic oil bath. Then the fried dough was blended with syrup (108°C) used in Sachima and moulded, finally cut into square and packed. Samples were normally packed and stored at 25°C in a temperature-controlled chamber for 5 months. The control samples were prepared without MRPs. Samples were periodically taken at 0–5 month for analyses.

Analysis of molecular weight distribution of DPM hydrolysates Molecular weight distribution of DPM hydrolysates was determined by gel permeation chromatography. A protein purification chromatography (Amersham plc, Buckinghamshire, United Kingdom) with a Superdex Peptide 10/300 GL column was used for the analysis. The mobile phase (isocratic elution) was 0.02 M sodium phosphate buffer containing 0.25 M NaCl (pH 7.2), at a flow rate of 0.5 mL/min. Absorbance was monitored at 214 nm. The water-soluble peptide fraction was filtered through a micropore film (0.22 µm of pore size). Six protein standards, Globin III (2512 Da), Globin II (6214 Da), Globin I (8519 Da), Globin I + III (10,700 Da), Globin I + II (14,404 Da) and Globin (16,949 Da) were taken to make reference curve. The molecular weight of peptides was calculated by the elution volume. UNICORN 5.0 software (Amersham Biosciences Co., Piscataway, NJ, USA) was used to analyze the chromatographic data.

Free amino acid analysis The amino acid profile of DPM hydrolysates was determined according to the method of Bidlingmeyer *et al.* (1987) with a slight modification. Free amino acid composition was determined by high performance liquid chromatography equipped with a PICO. TAG column (Waters, Milford, MA, USA). The following amino acids were used as external standards, including L-alanine, L-arginine, L-aspartic acid, L-cystine, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, L-valine and ammonium chloride (Sigma Co., St. Louis, MO, USA). These standards were used at equal concentration except for ammonium chloride.

Measurement of UV-absorbance and browning The UV-absorbance and browning of MRPs were measured based on the

method of Ajandouz (2001). MRPs was diluted to the concentration of 2% (w/v) using distilled water and the absorbance was measured at 294 nm and 420 nm by a spectrophotometer (UV2100, Unico Instrument Co., Ltd., Shanghai, China) for determining UV-absorbance and browning intensity, respectively.

Measurement of the free amino nitrogen content The amino nitrogen content was determined by formaldehyde titration method (AOAC Methods, 1990).

Analysis of DPPH radical scavenging activity The DPPH radical scavenging activity was measured according to the method of Shimada *et al.* (1992) with some modifications. Two milliliters of MRPs solution with concentration of 1% (w/v) were added into 2 mL of 0.2 mM DPPH in ethanol. The reaction mixture was incubated for 30 min in dark at room temperature. The absorbance of the resulting solution was measured at 517 nm by a spectrophotometer. TBHQ at permitted legal limit of 0.02% was prepared for comparative purpose. A low absorbance of the reaction mixture indicates a high free radical scavenging activity. The scavenging activity was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = 100 \times [1 - (A_{\text{sample}} - A_{\text{control}}) / A_{\text{blank}}] \quad \text{.....Eq. 1}$$

where the A_{blank} is the value of 2 mL of 95% ethanol mixed with DPPH solution, the A_{sample} is the value of 2 mL of sample solution mixed with DPPH solution, and the A_{control} is the value of 2 mL of sample solution mixed with 2 mL of 95% ethanol.

ORAC assay The peroxyl radical scavenging activity of MRPs was measured by ORAC assay as described by Dvålos *et al.* (2004) with some modifications. The reaction was carried out in 75 mM phosphate buffer (pH 7.4), and the final reaction mixture was 200 μL . Antioxidant (20 μL), phosphate buffer (20 μL , 75 mM, final concentration) and fluorescein (20 μL , 70 nM, final concentration) solutions were placed in the well of the microplate. The mixture was incubated for 15 min at 37°C. AAPH solution (140 μL , 12 mM, final concentration) was added rapidly using a multichannel pipettor. The microplate was immediately placed in the reader and the fluorescence recorded every 2 minutes for 120 min. The microplate was automatically shaken prior each reading. A blank (FL + AAPH) using phosphate buffer instead of the antioxidant solution and six calibration solutions using Trolox (10 – 100 μM , final concentration) as antioxidant were also carried out in each assay. Fluorescein filters with an excitation wavelength of 438 nm and an emission wavelength of 520 nm were used. All the reaction mixtures were prepared in duplicate, and at least three independent assays were performed for each sample.

Raw data were exported from the Fluostar Galaxy software to an Excel sheet for further calculations. Antioxidant curves (fluorescence *versus* time) were first normalized to the curve of the blank corresponding to the same assay by multiplying original data by the factor $\text{fluorescence}_{\text{blank}, t=0} / \text{fluorescence}_{\text{sample}, t=0}$. From the

normalized curves, the area under the fluorescence decay curve (AUC) was calculated as

$$\text{AUC} = 1 + \sum_{i=1}^{i=60} f_i / f_0 \quad \text{.....Eq. 3}$$

where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time i .

The net AUC corresponding to a sample was calculated by subtracting the AUC corresponding to the blank. Regression equations between net AUC and antioxidant concentration were calculated for all the samples. ORAC_{FL} values were expressed as Trolox equivalents by using the standard curve calculated for each assay. Final ORAC values were expressed as μM of Trolox equivalent/mL of MRPs solution. Analysis was carried out in triplicate.

Inhibition of linoleic acid autoxidation MRPs' ability to inhibit oxidation of linoleic acid in emulsions was determined according to the Ferric-thiocyanate method, as described by Dong *et al.* (2008) with some modifications. MRPs were dissolved in deionized water to make the concentration of 8% (w/v) for assessment. Briefly, 2.0 mL of MRPs solution and 2.0 mL of 2.5% (w/v) linoleic acid in ethanol (95%) were mixed in a 20 mL tube, then 4.0 mL of 50 mM phosphate buffer (pH 7.0) was added in the tube, the final volume was adjusted to 10.0 mL with deionized water. In a single experiment, sample was replaced by TBHQ (0.02%) for comparative purposes.

The reaction mixture was incubated in tubes with silicon rubber caps at 40°C in dark and degree of linoleic acid oxidation was spectrophotometrically measured at 24-h intervals. 0.1 mL of reaction mixture was mixed with 75% ethanol (9.7 mL) followed by the addition of 30% ammonium thiocyanate (0.1 mL) and 0.02 M ferrous chloride solution (0.1 mL) in 3.5% HCl. After 3 min, the degree of colour development, which represents the linoleic acid oxidation, was measured at 500 nm by a spectrophotometer. The inhibition activity of MRPs was represented by the inhibition activity at 144 h, it was calculated using the following equation:

$$\text{Inhibition activity (\%)} = 100 \times [1 - (\text{sample } A_{144} - \text{sample } A_0) / (\text{blank } A_{144} - \text{blank } A_0)] \quad \text{.....Eq. 2}$$

where the A_0 is the absorbance value at the initial time ($t = 0$), the A_{144} is the absorbance value at 144 h.

Determination of volatile compounds of Sachima Volatile compounds of Sachima were extracted by a solid-phase microextraction (SPME) device (Supelco, Bellefonte, PA, USA), equipped with 75 μm of carboxen/poly-dimethylsiloxane (CAR/PDMS) fiber. For each experiment, 10 mL of sample with 5% soluble-solid content was weighed into a 20-mL headspace vial and sealed with a Teflon silicone septum. The vial was left at 40°C in a thermal block for 10 min to equilibrate the headspace. Then, the SPME fiber was exposed to the headspace of the vial for 30 min. The fiber were desorbed in the injection port of GC/MS (Thermo Trace DSQ II GC/MS, Palo Alto, CA, USA) for 5 min at 230°C

with a splitless injection mode. The volatile compounds were separated using a 60 m × 0.25 mm (i.d.) DB-5 ms non-polar column, film thickness 0.25 µm, which was equipped with Trace DSQ II GC/MS (Thermo Fisher Co. Ltd., USA). The initial temperature of the column, 40°C, was held for 2 min and then increased at 5°C min⁻¹ to 220°C, which was held for 2 min. Helium was used as carrier gas at a linear velocity of 1.0 mL min⁻¹. The source was kept at 230°C. The transfer line and the detector were maintained at 250°C. Mass spectra in the electron impact (EI) mode were generated at 70 eV and collected from *m/z* 33 to 500. Compounds were identified with standard mass spectra (when available), by comparison with mass spectra libraries (NIST-Gaithersburg MD, INRAMASS-INRA, France). All results have been proved by co-injection of reference compounds, comparing retention times and mass spectra.

Lipolysis and lipid oxidation analysis of Sachima The acidity value was determined according to Fanco *et al.* (2002) for assessing lipolysis of Sachima lipid. Lipid oxidation was evaluated by peroxide value. Peroxide value was determined after extraction of lipids in accordance with the methods reported by Visessanguan *et al.* (2006). It was expressed as milli-equivalent per kg of fat (meq/kg fat).

Statistical analysis All the data were expressed as means ± standard deviations of triplicate determinations. Statistical calculation and between-variable correlation were investigated using the statistical package SPSS 11.5 (SPSS Inc., Chicago, IL, USA). Radar chart was made using the Microsoft Excel 2003 (Microsoft Corporation, USA).

Results and Discussion

Molecular weight distribution and free amino acid composition of hydrolysates The molecular weight distribution of DPM hydrolysates is showed in Fig. 1. It was dominated by the fractions with molecular weight 1–3 kDa (34.94%) and 3–6 kDa (44.66%). The fractions with molecular weight less than 1 kDa, 6–10 kDa and more than 10 kDa only accounted for 17.22%, 2.99% and 0.18%, respectively. Peptide chain length often had important influence on the antioxidant activity of MRPs (Kim and Lee, 2009). Ogasawara *et al.* (2006) found that the key material of the flavour enhancement in MRPs was peptides between 1000 and 5000 Da, which were generally called Maillard peptides. At this point, DPM hydrolysate could be suitable for Maillard reaction.

The free amino acid composition of DPM hydrolysates is shown in Table 1. Glu (576.7 mg/100 g) was the major free amino acid in the hydrolysate. Arg, Asp, Lue, Ser and Phe also had higher concentrations than others. The total free amino acid content was 2652.0 mg/100 g, which indicated that DPM hydrolysates were rich source of free amino acids. The specific free amino acid composition may affect the composition of MRPs and then influence the antioxidant properties and flavour of the MRPs. The peptide and free amino acid themselves had antioxidant effect, they

Fig. 1. The molecular weight distribution of hydrolysate of DPM.

Table 1. Free amino acid concentration (mg/100 g) of DPM hydrolysate.

Amino acid	Concentration	Amino acid	Concentration
Asp	216.9	Ala	123.2
Glu	576.7	Tyr	123.2
Lys	83.8	Thr	103.5
His	59.1	Met	54.2
Arg	300.7	Pro	103.5
Val	128.2	Cys	4.9
Ile	108.5	Ser	142.9
Leu	202.1	Gly	93.7
Phe	142.9	Trp	83.8
Total	2652.0		

might partly contributed to antioxidant properties of the MRPs.

Changes of absorbance at 294 nm and 420 nm and free amino group content The browning colour intensity is often used as an indicator of the extent to which the Maillard reaction took place in foods and it symbolizes an advanced stage of the Maillard reaction (Morales and Jiménez-Pérez, 2001). As shown in Table 2, an increase in browning of DPM hydrolysate-glucose MRPs was observed as the heating time increased ($p < 0.05$). The similar results were obtained by Kim and Lee (2009). Absorbance at 294 nm could be used to determine the intermediate compounds of the Maillard reaction (Benjakul *et al.*, 2005). Continuous increase in absorbance at 294 nm was observed as the heating time increased up to 60 min ($p < 0.05$). Compared with the changes of browning colour intensity, the statistic test showed that there was a good linear correlation between them ($r = 0.956$). The similar relationship between the increase in UV-absorbance and browning (absorbance at 420 nm) suggested that a large proportion of the intermediate product was converted to a brown polymer (Ajandouz *et al.*, 2001).

The free amino group content continuously decreased as the heating time increased up to 60 min. This result suggested that a α - or ϵ -NH₂ group of amino acids or proteins covalently attached to a sugar to form glycated proteins to a greater extent, particularly

Fig. 2. DPPH radical scavenging activities of MRPs (1%, w/v) prepared by heating at 120°C for various time. *Values in a column followed by the different letter are significantly different ($p < 0.05$), the concentration of TBHQ is 0.02%.

Fig. 3. Oxygen radical absorbance capacity (ORAC) of MRPs prepared by heating at 120°C for various time. *Values in a column followed by the different letter are significantly different ($p < 0.05$).

when the heating time increased. From the above result, the decreases in free amino group were in accordance with the increase in browning and absorbance at 294 nm. The similar results were also reported by Gu *et al.* (2009), who investigated Maillard reaction products from a casein-glucose model system.

DPPH scavenging activity DPPH is one of the compounds that possess a proton free radical with a characteristic absorption, which decreases significantly on the exposure to proton radical scavengers (Yamaguchi *et al.*, 1998). The DPPH radical was scavenged by donation of hydrogen to form a stable DPPH-H molecule (Matthaus, 2002). The colour changed from purple to yellow by acceptance of a hydrogen atom from MRPs and it became a stable diamagnetic molecule. As shown in Fig. 2, DPPH radical scavenging activity of MRPs (1%, w/v) increased as the heating time increased ($p < 0.05$). M4 showed the highest DPPH radical scavenging activity with 79.73%, which was higher than that of TBHQ (0.02%). Kirigaya *et al.* (1968) found that antioxidant activity increased with increasing colour intensity. In the present study, DPPH radical scavenging activity correlated well

Fig. 4. Effect of MRPs (8%) on inhibition of linoleic acid autoxidation: (A) Linoleic acid autoxidation with different MRPs and TBHQ (0.02%) determined as described in the text during 144 h and (B) their antioxidant activities after 144 h *Values in a column followed by the different letters are significantly different ($p < 0.05$).

with browning intensity ($r = 0.925$) and absorbance at 294 nm ($r = 0.991$). Similar result was also reported by Benjakul *et al.* (2005). Either intermediates or the final brown polymer can function as hydrogen donors (Benjakul *et al.*, 2005), so the browning intensity and absorbance at 294 nm well indicated the antiradical activity by DPPH test.

ORAC assay ORAC assay is one of the few methods that combines both inhibition percentage and inhibition time of the reactive species action by antioxidants into a single quantity (Dvalos *et al.*, 2004). An improvement in quantitation is achieved in the ORAC assay by taking the reaction between substrate and free radicals to completion and using an area-under-curve technique for quantitation compared to the assays that measure a lag phase.

The ORAC assay has been largely applied to the assessment of free radical scavenging capacity of human plasma, proteins, DNA, pure antioxidant compounds and antioxidant plant/food extracts (Prior and Gao, 1999). As shown in Fig. 3, ORAC values of MRPs per mL of solution increased from 20.71 to 54.70 μM Trolox equivalent/mL as the heating time increased. MRPs prepared at

Table 2. Changes in Maillard intermediate level (absorbance at 295 nm), browning intensity (absorbance at 420 nm) and free amino nitrogen content (%) of MRPs during heating to 60 min.

Heating time (min)	Absorbance at 294 nm	Absorbance at 420 nm	Free amino nitrogen content (%)
0	1.109 ± 0.002 ^a	0.228 ± 0.006 ^a	0.121 ± 0.0002 ^a
10	1.438 ± 0.003 ^b	0.345 ± 0.004 ^b	0.100 ± 0.0011 ^b
20	1.870 ± 0.002 ^c	0.384 ± 0.004 ^c	0.088 ± 0.0027 ^c
30	2.111 ± 0.004 ^d	0.402 ± 0.002 ^d	0.083 ± 0.0018 ^d
60	3.010 ± 0.001 ^e	0.499 ± 0.001 ^e	0.083 ± 0.0029 ^d

^{a-e} Different letter superscripts denote significant difference ($p < 0.05$).

Table 3. Changes in the acidity value (mg KOH/g fat) and peroxide value (meq/kg fat) of Sachima lipid during storage with the addition of MRPs (1%).

Treatments	Storage time (month)					
	0	1	2	3	4	5
Acidity value(mg KOH/g fat)						
Control	0.686 ± 0.003 ^{Xa}	0.724 ± 0.053 ^{Xa}	0.747 ± 0.009 ^{Xa}	0.762 ± 0.130 ^{Xa}	0.793 ± 0.001 ^{Xab}	0.923 ± 0.008 ^{Xb}
1%	0.627 ± 0.041 ^{Xa}	0.656 ± 0.006 ^{Ya}	0.664 ± 0.006 ^{Xa}	0.673 ± 0.001 ^{Ya}	0.783 ± 0.056 ^{Xb}	0.820 ± 0.004 ^{Yb}
Peroxide value (meq/kg fat)						
Control	298 ± 12 ^{X,a}	730 ± 15 ^{X,c}	690 ± 16 ^{X,c}	543 ± 78 ^{X,b}	516 ± 70 ^{X,b}	467 ± 33 ^{X,b}
1%	278 ± 3 ^{Y,a}	285 ± 2 ^{Y,a}	289 ± 1 ^{Y,a}	294 ± 19 ^{Y,a}	268 ± 4 ^{Y,a}	237 ± 18 ^{Y,b}

^{a-c} Different letter superscripts denote significant differences between the storage months;

^{X-Y} Different letters reflect significant differences between the control sample and sample with 1% MRPs ($p < 0.05$).

120°C for 60 min showed the highest ORAC value. ORAC values of different MRPs correlated well with DPPH scavenging activity. The complexity in MRPs structures limits the determination of antioxidant activity for each compound in the whole group of MRPs. Therefore, the ORAC assay could be used to determine the total antioxidant capacity of MRPs (Yilmaz and Toledo, 2005).

Inhibition of linoleic acid autoxidation *In vitro* lipid peroxidation inhibition activity of MRPs was determined by assessing their ability to inhibit oxidation of linoleic acid in an emulsified model system. As shown in Fig. 4A, all MRPs could act as significant retarders ($p < 0.05$) of lipid peroxidation. Overall, their antioxidant effects relatively increased with the increasing heating time. DPM hydrolysate-glucose heated for up to 60 min (M4) showed remarkable effect of inhibiting linoleic acid autoxidation. It had better effect than synthetic antioxidant TBHQ (0.02%) until the incubation time up to more than 4 days (Figure 4B). while after 4 days, the effect of M4 was not as good as TBHQ. It might be due to the fact that MRPs with water solubility couldn't play a full part in inhibiting lipid oxidation as the emulsification systems disappear.

Effect of MRPs on the oxidative stability of Sachima The lipolysis changes of Sachima during the storage are shown in Table 3. The acidity value increased during storage for both the treatments, indicating that lipolysis of Sachima lipids occurred during storage. The acidity value of control sample was 0.686 mg KOH/kg fat at 0 month, which increased to mg KOH/kg fat after 5 months of storage ($p < 0.05$). The acidity value of sample addition

with of MRPs (1%) increased from 0.627 to 0.820 mg KOH/kg fat during the 5 months storage. The difference between control sample and MRPs addition expanded from 0.059 to 0.103 mg KOH/kg fat after 5-month storage. It showed that the addition of MRPs had some effect on inhibiting lipid hydrolysis, while it was not that significant. MRPs might retard lipid oxidation by scavenging free radicals.

Peroxide value measures the formation of hydroperoxide groups that are initial products of lipid oxidation (Azeredo *et al.*, 2004). The peroxide value results of Sachima lipids are shown in Table 3. The peroxide value of control sample increased from 298 to 730 meq/kg fat during the first month storage ($p < 0.05$), and decreased during 2 – 5 months ($p < 0.05$). In comparison with the control, MRPs prevented peroxidation by extending the induction period.

The peroxide value of samples with MRPs increased at quite low rate, from 278 to 294 meq/kg fat during the first three months of storage, which indicated that MRPs added to Sachima showed good antioxidant properties. MRPs were reported to have the capability of forming stable free radicals thus causing the inhibition of lipid oxidation. The results of present study were similar to that of Jayathilakan and Sharma (2006) regarding the effects of MRPs in a methyl linoleate model system. We noticed that TBHQ was introduced to Sachima from the palm oil in the process. So the improved oxidative stability of Sachima with 1% addition of MRPs may not totally due to the antioxidant properties of MRPs, it may also include the cooperative or synergistic effect of MRPs with

Table 4. Volatile compounds of Sachima with / without the addition of MRP (M4, 1%).

Compound name	t _R ^a (min)	Relative peak area (%)	
		Control	1%
Aldehydes			
methylglyoxal	1.82	3.65	4.67
2-methylpropanal	2.09	3.81	4.11
3-methylbutanal	2.98	9.80	11.1
2-methylbutanal	3.09	6.41	6.40
hexanal	5.83	2.82	2.38
heptanal	8.77	0.33	0.57
benzaldehyde	11.05	0.59	0.64
nonanal	15.05	1.07	1.20
Ketones			
2,3-butadione	2.30	9.76	10.30
1-hydroxy-2-propanon	3.24	9.90	13.10
2,3-pentanedione	3.60	1.18	1.26
3-hydroxy-2-butanon	4.00	3.14	1.58
Esters			
acetic ether	2.47	9.28	3.60
1-propyl acetate	3.82	16.80	-
1-butyl acetate	6.14	1.50	8.42
1,4-butanolide	9.89	0.23	2.14
Furans			
furan-3-carboxaldehyde	7.10	1.61	2.97
2-furanmethanol	7.75	5.11	7.04
2-pentylfuran	11.30	0.40	0.42
Nitrogen Compounds			
pyrazine	4.57	1.83	3.25
2-methyl pyrazine	6.89	1.66	2.38
2-ethylpyrazine	9.57	0.60	-
2,3-dimethyl-pyrazine	9.73	0.17	0.59
Acids			
acetic acid	2.79	0.71	0.87
4-hydroxybutanoic acid	9.83	1.03	1.11
Hydrocarbons			
hexane	2.23	0.20	-
methyl-benzen	4.90	2.79	1.04
2,4-dimethyl-1-heptene	6.47	-	1.26
ethyl-benzen	7.38	0.58	0.29
1,2-dimethyl-benzene	7.66	0.49	0.30
Miscellaneous			
ethyl alcohol	1.69	2.20	5.76
dimethyl disulfide	4.45	0.20	0.11
pentanol	5.21	-	0.74
maltol	16.30	0.17	0.46

^a Retention time of the compound.

TBHQ. Whatever the reason, the addition of 1% MRPs had positive effect on the oxidative stability of Sachima.

Effect of MRPs on the flavour of Sachima Maillard reaction is complex and provides a large number of compounds which contribute to flavour. Maillard reaction can improve palatability and consumer acceptance as in roasting of coffee or meat and baking of bread (Cho *et al.*, 2010). Since Ruckdeschel reported

aroma generation by Maillard pathways in 1914, the aroma compounds from Maillard reaction became the focus of research. Some aroma compounds such as potato-like aromas, meat-like aromas have been produced by the Maillard reaction. MRPs could be added to Sachima as flavour enhancers to improve the flavour of it. The flavour profiles of Sachima with/without MRPs (M4, 1%) are shown in Table 4.

A total of 34 volatile compounds were identified in Sachima, mainly including aldehydes (8 compounds), ketones (4 compounds), esters (4 compounds), furans (3 compounds) and nitrogen compounds (4 compounds). The content of aldehydes increased with the addition of MRPs. Aldehydes has a great impact on the aroma of food due to their low odor threshold values. They exhibit characteristic aroma notes, such as butter, sweet, floral, toasted, or green odors. Strecker aldehydes, such as 2-methylpropanal and 3-methylbutanal were significantly increased for Sachima with MRPs compared with control. Huang *et al.* indicated that they may derive from valine and leucine, respectively (Huang *et al.*, 2004). About ketones and esters, the content changes were quite different depending on different compounds. For example, the content of 1-hydroxy-2-propanone, 1-butyl acetate and 1,4-butanediol improved a lot, while acetic ether and 1-propyl acetate decreased sharply. These could be due to the antioxidant properties of the MRPs that affected the conversion of intermediates. More furans and furan derivatives were identified from Sachima with MRPs compared with the control sample, which exhibiting sweet, fruity, and caramel-like odor notes. *N*-containing volatile flavour compounds increased in Sachima with MRPs. They originated from the breakdown of proteins, free amino acids, and nucleic acids, and their characteristic aroma notes have been described as nutty, meaty, green, potato-like, and vegetable-like (Wettasinghe *et al.*, 2001). In addition, maltol in Sachima with MRPs improved four times. It was an important flavoring substance. These results indicated that MRPs could act as flavour enhancer and improve the sensory properties of Sachima.

Conclusion

The present study clearly showed that MRPs could be used in food lipids stabilization as potent natural antioxidants and flavour enhancer. MRPs derived from DPM hydrolysate-glucose exhibited good antioxidant activity evaluated by DPPH radical scavenging activity, the inhibition of linoleic acid autoxidation and ORAC, while it might be partly due to the antioxidant effect of peptides and free amino acid in DPM hydrolysates. Antioxidant activity of MRPs increased with extended heating time, and MRPs prepared by heating 60 min showed the highest antioxidant activity. The application of MRPs in Sachima effectively inhibited lipid oxidation during extended storage at 25°C and improved the flavour of Sachima. Further work on the identification of the structure of the active compounds in Maillard reaction will be conducted.

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