Comparation sensory characteristic, non-volatile compounds, volatile compounds and antioxidant activity of MRPs by novel gradient temperature-elevating and traditional isothermal methods

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Abstract A novel gradient temperature-elevating Maillard reaction was developed to effectively produce light-colored MRPs. The main purpose of the present study was to compare the color, taste characteristic, non-volatile compounds, volatile compounds and antioxidant activity of MRPs prepared by the novel gradient temperature-elevating and traditional isothermal methods. The product prepared from soybean peptide, D-xylose and L-cysteine by gradient temperature-elevating method was termed MRP-60. Isothermal method was used to produce light and dark colored MRP, called PXC and PX, respectively. MRP-60 has not only the lowest brown intensity (A420, 0.088), also the highest overall acceptability taste from sensory evaluation. MRP-60 showed the lowest bitterness which could be attributed to the lowest content of bitter amino acids (1.48 mg/ml); MRP-60 also showed indistinctive mouthfulness compared with PXC which elucidated by the percentage of compounds with a molecular weight of 1,000–5,000 Da (15.03 %) and high antioxidant activity. Then, the MRPs prepared by gradient temperature-elevating method successfully attain desirable taste and high antioxidant activity.

Keywords Gradient temperature-elevating method · Light-colored Maillard reaction product · Non-volatile compounds · Volatile compounds · Antioxidant activity

Introduction

Soybean peptide has been recognized as an important flavor potentiate and precursor of the Maillard reaction (Lan et al. 2010). The Maillard reaction, a well-known non-enzymatic browning reaction, is involved in the formation of brown pigments and flavor. It occurs due to the condensation between a carbonyl group of reducing sugars and an amine group of any nitrogenous compound (such as amino acids, peptides and proteins) (Liu et al. 2010). Maillard reaction is a complex reaction that produces a large number of the so-called Maillard reaction products (MRPs), such as aroma compounds (Kim and Lee 2009), ultra-violet absorbing intermediates, and dark-brown polymeric compounds named melanoidins (Wijewickreme et al. 1997). On one hand, color plays an important role in appearance and acceptability of foods (Aguiló-Aguayo et al. 2009). In spite of the longstanding aesthetic and practical interest in Maillard-derived food coloring materials, it is not desirable in some light colored food systems, such as soup and juice. Inhibiting brown color formation involves complex sequential and parallel reactions that are poorly understood. MRPs can also provide distinctive flavor attributes. Strong umami, mouthfulness, continuity, meaty attributes have been demonstrated in cysteine-xylose-soybean peptide model system, as mentioned in our previous study (Huang et al. 2011). Ogasawara et al. (2006a, b) showed that the Maillard peptides with a molecular weight of 1,000–5,000 Da generated

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during the ageing of soybean paste affected not only the basic taste qualities but also enhanced mouthfulness and continuity. The mouthfulness characteristic, therefore, is considered to be associated with molecular weight (MW) distribution of products. On the other hand, it has been reported that MRPs are associated with strong antioxidant activity (Yen and Hsieh 1995; Yoshimura et al. 1997), such as scavenging DPPH radicals in model glucose-lysine system (Morales and Jiménez-Pérez 2001), chelating ferrous ion (Yoshimura et al. 1997), copper ion (Dittrich et al. 2003) and scavenging peroxy radical in glucose-glycine system (Wagner et al. 2002), reducing power and lipid peroxidation inhibiting activity in casein-glucose system (Gu et al. 2009). Lingnert (1980) described that addition of MRPs derived from histidine and glucose to cookie dough successfully inhibited the oxidation of lipids in cookies. Dittrich et al. (2003) for the first time demonstrated clearly that Maillard reaction products derived from D-glucose and amino acids can prevent oxidation of human low-density lipoprotein in vitro. For the pathway of antioxidant activity of MRPs, a lot of studies have been done on the mechanism, but it is still not very clear due to the complexity of MRPs. The potential role of sulfur-containing heterocyclic compounds for inhibiting the oxidative degradation of lipid-rich foods was presented (Eiserich and Shibamoto 1994). It has been shown that there is a relationship between molecular weight of MRPs and antioxidant activity, the high molecular weight melanoidins prepared from xylose and glycine have been found to possess a higher browning, reducing power and antioxidant activity (Rufián-Henares and Morales 2007a; Yamaguchi et al. 1981). Lipid peroxidation reaction can be prevented by crosslinking macromolecules and malondialdehyde, a reactive unsaturated dicarbonyl (Kang et al. 2006), or by reacting with some intermediates of Maillard reaction, such as glyoxal and methylglyoxal, thus producing different compounds than those commonly found (Hidalgo and Zamora 2005). The radical-scavenging activity of MRPs may be related to the composition and structure, because it quenches hydrophilic radicals more efficiently than hydrophobic ones (Jing and Kitts 2002).

In our previous study, we reported the inhibiting effect of cysteine on color formation and the relationship between volatile compounds and the antioxidant activity of the corresponding products (Huang et al. 2011), but the application of isothermal method is still limited in production of desirable light-colored products for the industrial application. Based on the interaction between amadori compounds and cysteine in soybean-xylose Maillard reaction system, a novel gradient temperature-elevating Maillard reaction was developed for effective production of light-colored MRPs (Huang et al. 2012). It has been generally accepted that antioxidant activity of MRPs are related to the brown compounds content, therefore it is quite

necessary to evaluate the antioxidant activities of these light-colored MRPs as well as flavor characteristics. Consequently, the objective of present study was to evaluate the color, taste, non-volatile compounds, volatile compounds and antioxidant activity characteristics of the MRP prepared by the gradient temperature-elevating method compared to those produced under the isothermal condition.

Materials and methods

Materials

Soybean protein isolate (867 g protein.kg⁻¹) was purchased from Hufeng Biotechnology Co. (Shanghai, China), Alcalase (2.4L FG) from Novozymes China Inc. (Wuxi, China), L-cysteine and D-xylose used were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Preparation of Maillard reaction products by gradient temperature-elevating method (MRP-60) and isothermal method (PXC and PX)

Soybean peptide (1 g) prepared according to the method of Huang et al (2011) and xylose (0.15 g) was suspended in 10 ml de-ionized water. pH was adjusted to 6.5. The solution was initially heated at 80 °C for 60 min. After adding L-cysteine (0.1 g), the solution temperature was raised to 120 °C immediately and held for 110 min in oil bath. It was immediately cooled in ice bath after this treatment. The gradient temperature-elevating Maillard reaction product thus produced was termed as MRP-60.

PXC was prepared under isothermal condition. Soybean peptides (1 g) and xylose (0.15 g) were mixed well in 10 ml de-ionized water and pH was adjusted to 6.5. The solution was heated at 120 °C for 10 min, after which L-cysteine was added (0.1 g) and the heating was continued up to 110 min. The mixture was immediately cooled in ice bath after this treatment and used accordingly. PX was prepared without adding cysteine under the same conditions as PXC.

Color measurement

The browning of MRPs with 50-fold dilution was monitored by their absorbance values at 420 nm with a UV-vis spectrophotometer (UV-1600, Meipuda Co., Shanghai, China).

The color changes was also carried out by Hunters parameters using de-ionized water as blank ($L^* = 65.87$; $a^* = 1.96$; $b^* = 2.59$) using a WSC-S color difference meter (Karangwa et al. 2010; Rao et al. 2011) (Shanghai Precision & Scientific Instrument Co., Ltd, Shanghai, China). A 3 mL sample was pipetted into a 2.5 cm diameter test tube for measurement.

Sensory evaluation

To evaluate the taste characteristics of MRPs, the sensory evaluation was performed on MRPs according to the method of Huang et al (2011). The panel was composed of eight trained assessors aged between 23 and 49 years from the School of Food Science and Technology, Jiangnan University (Wuxi, China). The assessors were trained with reference solutions for the odor and the taste attributes: caramel (prepared from broken barley sugar in water), umami (Monosodium glutamate (MSG), 4 mM, pH 5.6), salty taste (NaCl, 12 mM), sourness (lactic acid, 10 mM), bitterness (caffeine, 1.5 mM), meaty, mouthfulness and continuity (bouillon: 10 g bouillon cube (beef flavor consisting of MSG, yeast extract, and beef extract), dissolved in water, filtrated and diluted).

The samples solutions (1.5 g.kg^{-1}) were individually dissolved in consommé soup. Consommé soup consisted of 15 g.kg^{-1} consommé soup premix, comprising 7.5 g.kg^{-1} salt, 1.05 g.kg^{-1} sugar, 0.45 g.kg^{-1} onion extract powder, 0.45 g.kg^{-1} beef extract powder, 0.225 g.kg^{-1} sodium glutamate, 0.18 g.kg^{-1} caramel, 0.03 g.kg^{-1} white pepper, 0.03 g.kg^{-1} celery powder and 5.085 g.kg^{-1} lactose. The taste solution was heated to 60°C in a water bath. A 60 ml sample and 60 ml of control solution (consommé soup added to Dextrin DE 8-10 in stead of the samples) were served at the same time. The samples were coded with 3-digit numbers, served in a randomized order and tasted at $22\pm2^\circ\text{C}$ in separated sensory booths.

Prior to the evaluation of the samples, the panelists tasted the reference solutions and were asked to memorize the respective intensities, which had been set in mutual consensus to 50 % of the assessment scale. The panelists were asked to score the intensities of the solutions using a scale of 1–7, where 3 point was the score given to the control sample. The values given by the panelists were averaged.

Estimation of molecular weight distribution (MWD) of non-volatile compounds

The method used was according to Kim and Lee (2009) with some modifications. The molecular weight (MW) of MRPs was determined using a Waters 600 liquid chromatograph (Waters Co., Milford, MA, USA) equipped with a Waters 2487 UV detector and Empower workstation. A 2000SWXL TSK gel filtration column ($300 \text{ mm}\times 7.8 \text{ mm}$; Tosoh Co., Tokyo, Japan) was used. The mobile phase consisted of acetonitrile/ water/ trifluoroacetic acid (45: 55: 0.1 v/v/v) at a flow rate of 0.5 ml.min^{-1} . The column temperature was 30°C , and $10 \mu\text{l}$ of sample was injected into the high-performance liquid chromatography system. A MW calibration curve was constructed using the following standards from Sigma Chemical Co.:

cytochrome C (12,500 Da), aprotinin (6,500 Da), bacitracin (1,450 Da), tetrapeptide GGYR (451 Da) and tripeptide GGG (189 Da). The results were obtained with the UV detector set at 220 nm. Data analysis was performed using gel permeation chromatography (GPC) software (Waters Co., Milford, US).

Determination of free amino acids

Amino acids were determined according to the method reported by Fekkes et al. (1995). Briefly, the free amino acids in the sample were analyzed using an Agilent liquid chromatograph 1100 with a UV detector operated at 338 nm. The column was ODS Hypersil ($250 \text{ mm}\times 4.6 \text{ mm}$), whilst the mobile phase, consisting of 20 mM sodium acetate and 1:2 (v/v) methanol-acetonitrile, was delivered at a flow rate of 1 ml/min. The column temperature was 40°C . An equivalent volume of trichloroacetic acid (TCA, 5 %) was added to the sample to precipitate peptides or proteins. For calculation, a calibration curve was obtained with standard amino acid mixture (Sigma Chemical Co., St. Louis, MO, USA) and quantification was made on the basis of retention time and peak area of standard compounds.

Antioxidant activity

Considering that the brown compounds of MRPs contribute to antioxidant activity (Delgado-Andrade et al. 2005), it is necessary to evaluate the antioxidant activity of light-colored MRPs. To investigate the antioxidant activity of MRP-60 prepared by improved method, DPPH radical-scavenging activity and reducing power as indicators were evaluated.

Determination of DPPH radical-scavenging activity

MRPs scavenged DPPH radical by donation of hydrogen to form a stable DPPH-H molecule. The color change from purple to yellow by acceptance of hydrogen radical from MRPs and it becomes stable diamagnetic molecule (Benjakul et al. 2005; Shon et al. 2003). DPPH radical-scavenging activity was determined according to the method of Benjakul et al. (2005) with some modifications. An aliquot of MRPs sample (1.0 ml) was added to 2.0 ml of 0.1 mM DPPH in ethanol. The solution was then mixed vigorously and allowed to stand at room temperature in the dark for 20 min. The mixture was centrifuged for 10 min at 750 g. The absorbance of supernatant at 10 min was measured at 517 nm using a UV-vis spectrophotometer (UV-1600, Meipuda Co., Shanghai, China). The blank was prepared in the same manner, except that distilled water was used instead of MRPs samples. For the control, the assay was conducted in the same manner but ethanol was added instead of DPPH solution. The percentage of DPPH radical-scavenging activity was calculated as

follows:

$$\text{Radical - scavenging activity\%} = [1 - (\text{As} - \text{Ac}) / \text{Ab}] \times 100$$

Where As is the absorbance at 517 nm of sample, Ac is the absorbance at 517 nm of the control and Ab is the absorbance at 517 nm of the blank.

Determination of reducing power

During the reducing power assay, the presence of reductant in the test samples result in reducing Fe^{3+} /ferricyanide complex to the ferrous form (Fe^{2+}). The Fe^{2+} can therefore be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Yoshimura et al. 1997). The reducing power of MRPs was determined according to the method of Oyaizu (1988) with some modifications. 1 ml of MRPs sample (100-fold dilution) was mixed with 1.0 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1.0 ml of 1 % potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$). The reaction mixtures were incubated in temperature- controlled water bath at 50 °C for 20 min, followed by addition of 1.0 ml of 10 % trichloroacetic acid after cooling to room temperature. The mixtures were then centrifuged at 750 g (TG16-WS, Xiangyi, Changsha, China) for 10 min at 25 °C. The supernatant obtained (1.0 ml) was treated with 1.0 ml of distilled water and 200 μl of 0.1 % FeCl_3 . The absorbance was measured at 700 nm with UV-vis spectrophotometer (UV-1600, Meipuda Co., Shanghai, China). Reducing power was measured as absorbance at 700 nm and the results were expressed as absorbance units (AU).

Volatile compounds determined by headspace solid phase microextraction/gas chromatography/ mass spectrometry (HS-SPME/GC/MS)

Volatile compounds were determined according to the method of Huang et al. (2011). The analyses were performed using a gas chromatograph/ mass spectrometer (Finnigan Trace GC/MS, Finnigan, California, USA) with DB-Wax capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μm ; J&W Scientific, Folsom, CA, USA). The volatile compounds were sampled with a carboxen/ polydimethylsiloxane (75 μm) SPME fibre (Supelco, Bellefonte, PA, USA), identified by Kovats indices (KI) and quantified by calculating the areas of the peaks.

Data analysis

All measurements were performed in duplicate (analysis of free amino acids and MW distribution) and triplicate (determination of color, sensory evaluation and antioxidant activity). Data was evaluated by analysis of variance (ANOVA) using SPSS 13.0. ANOVA with Duncan's multiple comparison tests were

performed to determine whether there were differences among individual samples.

Results and discussion

Color characteristics of MRPs

Color properties of PX, PXC and MRP-60 by absorbance at 420 nm and color space parameters (ΔL , Δa , Δb) were compared, as revealed in Table 1. A420 indicates the browning intensity of Maillard reaction (Guan et al. 2010). PX without adding cysteine was taken as control. A420 of MRP-60 was lower (0.088) than that of PXC (0.109), and much lower than that of PX (0.560). L^* is a measurement of the color in the light-dark axis, the rising of L^* value indicates that the samples were turning lighter (Ibarz et al. 1999). Compared to PX (−57.67), ΔL of PXC and MRP-60 were increased to −24.15 and −12.57, respectively. Δa was decreased following the order: PX (12.05) > PXC (7.30) > MRP-60 (4.39), which indicated the decrease of redness. Δb was increased in order: PX (12.11) < PXC (32.08) < MRP-60 (46.60), which demonstrated the increase tendency of yellowness. Cysteine has significant effect in inhibiting the color formation in soybean peptide system as shown in our previous study (Huang et al. 2011). It was observed that the color of MRP-60 was the lightest among the three MRPs. This new method, the gradient temperature-elevating Maillard reaction, successfully inhibited the color formation of MRP.

Taste characteristics of MRPs

To evaluate the taste characteristics of MRPs prepared by the modified method compared with that of isothermal method, sensory evaluation was carried out using a scoring test. The average score of mouthfulness, umami, continuity, sourness, saltiness, bitterness and overall taste are presented in the sensory profiles figure (Fig. 1). Some attributes such as bitterness,

Table 1 Color characteristics of MRPs prepared by isothermal (PX and PXC) and gradient temperature-elevating (MRP-60) method

	MRP-60*	PXC*	PX*
A420	0.088 $\pm$ 0.004 ^a	0.109 $\pm$ 0.001 ^b	0.560 $\pm$ 0.001 ^c
ΔL	−12.57 $\pm$ 0.09 ^a	−24.15 $\pm$ 0.07 ^b	−57.67 $\pm$ 0.10 ^c
Δa	4.39 $\pm$ 0.13 ^a	7.30 $\pm$ 0.08 ^b	12.05 $\pm$ 0.05 ^c
Δb	46.60 $\pm$ 0.30 ^a	32.08 $\pm$ 0.08 ^b	12.11 $\pm$ 0.26 ^c

* The values are the mean $\pm$ standard deviation ($n=3$). Means with different letters within the same line are significantly different ($p<0.05$)

Fig. 1 Taste profile of MRP-60, PXC and PX in umami solution by sensory evaluation. Each value represents the average taste intensity scored on a scale ranging from 1 to 7 by eight panelists

umami and mouthfulness showed significant difference among MRPs. Bitterness of PXC was the highest, followed by PX, and MRP-60. Umami was ranged in the following order: MRP-60 > PXC > PX. PX presented the strongest mouthfulness; no significant difference was found between the mouthfulness of PXC and MRP-60. Continuity, saltiness and sourness attributes had little differences. MRP-60 showed the highest overall taste. The sensory impression of consumer taste acceptance is the result of the simultaneous stimulation of the multiple sensations which is triggered by chemical compounds generated during Maillard reaction.

Molecular weight distribution of MRPs

MWD of the non-volatile compounds of MRPs was illustrated in Fig. 2. MRP-60 and PXC differ significantly from PX in each MW fragment group. PX has the highest percentage of MW portions of above 5,000 Da, 1,000–5,000 Da and the lowest below 1,000 Da. The percentages for the above 5,000 Da, 1,000–5,000 Da and below 1,000 Da MW portions for MRP-60 were not significantly different from that of PXC ($p < 0.05$). The percentages of 1,000–5,000 Da MW portion

Fig. 2 Molecular weight distribution of MRP-60, PXC and PX

for MRP-60 and PXC were 15.03 % and 15.35 %, respectively ($p < 0.05$). Ogasawara et al. (2006a) suggest that the group of a molecular weight of 1,000–5,000 Da (Maillard peptides) enhance taste, which could explain the small different taste of mouthfulness between MRP-60 and PXC.

Free amino acids

It is generally accepted that non-volatile compounds, especially for free amino acids, are responsible for the unique taste of MRPs (Solms 1969). To gain further insights into amino acids constituents which are responsible for the taste difference, free amino acids were analyzed. The content of free amino acids available in MRPs were depicted in Fig. 3. The content of asp, glu, ser, gly, thr, val and met was the highest in MRP-60 ($p < 0.05$). Arg, tyr, phe, leu and lys were the lowest among MRPs. Hydrophobic amino acids, are generally known to represent the bitter taste amino acids, and may reflect the bitter properties of final products (Lan et al. 2010). Among 17 tested amino acids, there were 8 bitter amino acids: ser, his, arg, val, met, phe, ile and leu, including three with a strong bitter perception: arg, his and met (Yang and Liu 1983). The total content of bitter amino acids in MRP-60, PX and PXC were 1.48, 2.07 and 2.37 mg/ml, respectively. The content of the strongest bitter taste amino acids in MRP-60, PX and PXC were 0.33, 0.61 and 0.81 mg/ml, respectively. These results are in agreement with those of bitter characteristics. While asp and glu have umami taste, the total content of asp and glu in MRP-60, PX and PXC were 0.74, 0.45 and 0.27 mg/ml, respectively. This could explain why MRP-60 has the highest umami taste. Overall taste of MRP-60 was higher than that of PXC and PX, the balance of various compounds could play an important role in the overall taste. According to the “components balance theory” described by Mulder (1952), taste is not related to single components, but to a mixture of nonvolatile compounds, including amino acids, which must occur in a certain specific balance. Moreover, certain compounds act as taste potentiators and synergists; and all compounds together form then the integrated taste profile.

DPPH radical-scavenging activity

DPPH radical-scavenging activity of MRPs was depicted in Fig. 4. DPPH radical-scavenging activity increased with the increasing concentration, and it reached above 91 % when the concentration of MRP-60 was at 2.42 mg/ml. DPPH radical-scavenging activity of MRP-60 was significantly higher than that of PX and PXC. The reason could be related to the composition and properties such as hydrogen donation of MRPs. Either intermediates or the final brown polymer can

Fig. 3 Free amino acids content of MRP-60, PXC and PX

function as hydrogen donors, sugar caramelization can also contribute to scavenging DPPH radical activity. Furthermore, based upon experiments of Amarowicz (2009), the marked scavenging capacity observed towards DPPH radical is also attributed to the sulfur compounds formation during Maillard reaction.

Reducing power

In the three systems, the reducing power of MRPs increased as concentration increasing (Fig. 5). PXC showed the highest reducing power, followed by PX and MRP-60. This result could be explained with the research reported by Rufián-Henares and

Morales (2007b) who pointed out that the ferric reducing ability was related to the amount of melanoidins. Melanoidin formation was effectively inhibited during the gradient-temperature elevating Maillard reaction. Compounds responsible for reducing activity are formed during the thermolysis of Amadori products in the primary phase of Maillard reaction (Hwang et al. 2001) or they could be formed as heterocyclic products of the Maillard reaction or caramelization of sugars (Charurin et al. 2002). It was revealed that the hydroxyl groups of MRPs play an important role in reducing activity as electron donors (Yoshimura et al. 1997). Additionally, the intermediate reductone compounds of MRPs were reported to break the radical chain by donation of a hydrogen atom (Eichner 1981).

Fig. 4 DPPH radical scavenging activity of MRP-60, PXC and PX **Fig. 5** Reducing power of MRP-60, PXC and PX

Table 2 Volatile compounds with high correlation to antioxidant activity

KI ^a	ID ^b	compounds	PXC ^c (μg/g)	PXC ^c (μg/g)	MRP-60 ^c (μg/g)
Nitrogen compounds					
1216	B	pyrazine	0.002±0.000	0.170±0.012	0.394±0.027
1280	B	methylpyrazine	0.015±0.002	0.736±0.084	0.710±0.047
1326	AB	2,5-dimethylpyrazine	0.027±0.007	0.200±0.073	0.133±0.014
1410	B	2,3,5-trimethyl pyrazine	0.009±0.001	0.152±0.015	0.299±0.026
1441	B	pyrrole	N.D	0.135±0.011	0.576±0.092
1512	B	iso-amylpyrazine	0.002±0.000	0.422±0.027	N.D
1525	AB	iso-propenyl-pyrazine	0.009±0.000	0.167±0.018	N.D
1560	B	3-acetoxypyridine	N.D	0.979±0.083	N.D
Sulfur compounds					
1439	B	2-furanmethanethiol	N.D	1.034±0.021	2.045±0.007
1501	AB	2-thiophenethiol	N.D	0.252±0.014	3.281±0.128
1721	AB	methyl furfuryl disulfide	0.003±0.001	0.186±0.011	0.469±0.062
2406	B	bis(2-methyl-3-furyl)-disulfide	N.D	0.954±0.018	0.020±0.003
1256	B	2-formyl-2,3-dihydro-thiophene	N.D	2.213±0.192	1.093±0.184
2001	B	2-(1-propenylthio)thiophene	N.D	0.303±0.016	1.012±0.290
1826	B	dimethylformylthiophene	N.D	2.397±0.212	0.770±0.017
Furans					
1540	B	tetrahydro-2,2-dimethyl-5-(3-methylethyl)-furan	0.019±0.004	0.290±0.019	0.321±0.024
1727	B	2-octanoylfuran	0.035±0.001	0.033±0.003	N.D
1763	B	3-phenylfuran	0.054±0.001	0.113±0.011	0.901±0.076
Esters					
1244	B	S-ethylethanethioate	N.D	0.190±0.017	0.412±0.026
1998	B	5-ethoxy-3,4-dihydro-2H-pyrrole-2-carboxylic acid ethylester	0.007±0.000	0.352±0.049	0.461±0.077
2422	B	1,2-benzenedicarboxylic acid,bis(2-methylpropyl)ester	0.007±0.000	0.497±0.018	0.703±0.082
Alcohols					
1436	B	1-undecanol	0.010±0.001	0.254±0.038	N.D
1555	B	1-octanol	0.005±0.000	0.375±0.006	0.304±0.007
1619	AB	furfuryl alcohol	0.044±0.001	0.106±0.011	N.D
1654	AB	1-nonanol	0.008±0.000	0.097±0.007	0.115±0.011
1814	B	1-hexadecanol	N.D	0.053±0.003	N.D
1984	B	4-methyl-phenol	N.D	0.199±0.025	0.035±0.005
Acids					
1698	AB	pentanoic acid	N.D	0.102±0.009	1.203±0.028
1797	AB	hexanoic acid	0.027±0.005	0.701±0.006	0.176±0.005
2065	AB	octanoic acid	0.011±0.004	0.449±0.026	0.070±0.003
2119	B	parabanic acid	0.042±0.001	0.796±0.032	0.086±0.003
2169	B	nonanoic acid	0	0.105±0.083	0.886±0.172
2217	B	hexadecanoic acid	0.003±0.000	0.193±0.061	0.795±0.118
2042	B	3-methylcyclohexane-1-carboxylic acid	0.017±0.002	0.223±0.023	0.720±0.071
2322	B	benzoic acid	0.002±0.000	0.179±0.013	3.211±0.308
2410	B	4-methyl-benzoic acid	0.010±0.001	0.198±0.042	0.232±0.026
Ketones					
1285	B	10-ethyl-8-phenyl-9H-pyrrolo[3',4',3,4]pyrrolo[2,1-A]phthalazine-9,11(10H)-dione	0.007±0.001	0.059±0.017	N.D
1435	B	1-(2-furanyl)-ethnone	N.D	0.323±0.073	0.129±0.019
1457	B	dihydro-2-methyl-3(2H)-thiophenone	N.D	0.128±0.021	0.287±0.026
1528	B	1-(2-pyridinyl)ethanone	N.D	0.115±0.031	0.221±0.029
1573	B	acetophenone	0.016±0.003	0.155±0.012	0.017±0.007
1668	B	2(5H)-furanone	0.021±0.003	0.171±0.007	0.204±0.048

Table 2 (continued)

KI ^a	ID ^b	compounds	PX ^c (μg/g)	PXC ^c (μg/g)	MRP-60 ^c (μg/g)
Aldehydes					
1088	AB	hexanal	0.007±0.007	0.315±0.008	0.102±0.005
1291	AB	octanal	0.006±0.001	0.032±0.002	0.275±0.023
1397	AB	nonanal	0.029±0.003	1.385±0.036	1.391±0.099
1536	B	benzaldehyde	0.353±0.011	0.798±0.012	1.092±0.039
1610	B	2-thiophenecarboxaldehyde	0.014±0.008	0.129±0.009	0.051±0.007
1630	B	dodecyl aldehyde	0.009±0.002	0.051±0.005	N.D
1671	B	2-docecen-1-al	N.D	0.019±0.003	N.D
1729	B	2,5-dimethyl-benzaldehyde	0.009±0.001	0.069±0.007	1.045±0.042
1836	B	2,4,5-trimethylbenzaldehyde	N.D	1.458±0.054	0.023±0.006
2034	B	2-pyrrolicarbaldehyde	0.101±0.082	0.163±0.007	N.D

^a Kovats index calculated for DB-Wax capillary column

^b Reliability of the identification proposal is indicated by the following: A, mass spectrum and Kovats index according to literature data; B, mass spectrum compared with mass spectral database in Wiley, NIST and Replib libraries

^c Values are mean± standard deviation ($n=3$). It dedicates content of compounds identified

Comparison of active volatile compounds with high correlation to antioxidant activity

To elucidate the highest antioxidant activity of MRP-60 among the three MRPs, volatile compounds which were significantly correlated with antioxidant activity were listed in Table 2, according to the researches (Amarowicz 2009; Eiserich and Shibamoto 1994; Gomyo and Horikoshi 1976) and our previous study (Huang et al. 2011). The content of pyrrole was 0.5763 μg/g in MRP-60 and 0.1354 μg/g in PXC, as reflecting the high positive correlations with antioxidant activity. Methylpyrazine and 2,3,5-trimethylpyrazine were characterized with positive correlations to ferrous ion-chelating activity. The ferrous ion-chelating activity of MRPs decreased in the following order: MRP-60> PXC> PX (details are not listed). The content of methylpyrazine was 0.0149 μg/g, 0.7385 μg/g and 0.7102 μg/g in PX, PXC and MRP-60, respectively; and the content of 2,3,5-trimethylpyrazine was 0.0085 μg/g, 0.1519 μg/g and 0.2987 μg/g in PX, PXC and MRP-60, respectively. 2-furanmethanethiol and methyl furfuryl disulfide were significantly correlated with both ferrous ion-chelating activity. The content of 2-furanmethanethiol and methyl furfuryl disulfide was highly rated in MRP-60 (2.0453 μg/g and 0.4689 μg/g). Sulfur-containing compounds were reported to have salience attribution to scavenging DPPH activity (Amarowicz 2009). The sum content of sulfur-containing compounds was 0.0031 μg/g, 7.3463 μg/g and 7.6781 μg/g in PX, PXC and MRP-60, respectively. Some other compounds, such as esters, ketones, and alcohols were also significantly different in the three MRPs, as can be seen in Table 2. The noticeable difference of volatile compounds of MRP-60 to other MRPs was in agreement with prominent antioxidant activity.

Conclusions

The gradient temperature-elevating Maillard reaction successfully helped to attain savory taste and light color MRPs. The product prepared by the modified method can also improve its antioxidant activity; such method may be useful in enlarging the application field in food industries for operable and feasible preparations of tasty and light-colored flavor enhancers with antioxidant activity in food products.

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