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Inhibiting the color formation by gradient temperature-elevating Maillard reaction of soybean peptide-xylose system based on interaction of L-cysteine and Amadori compounds

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Light color and savory flavor enhancer are attractive for consumers and food producers. The effect of addition time of L-cysteine on inhibiting color formation was investigated in soybean peptide-xylose system, and the possible pathway was explored. Once dicarbonyl compounds were formed during the Maillard reaction, the addition of L-cysteine had no color-inhibiting effect; if L-cysteine was added immediately after the Amadori compound was formed, the extraordinary color-inhibiting effect was observed. Therefore, an improved way to inhibit color formation was proposed on the basis of the interaction of L-cysteine and Amadori compounds by controlling the addition time of L-cysteine through gradient temperature-elevating Maillard reaction. The system was heated at 80 °C for 60 min to form Amadori compounds, followed by the addition of L-cysteine, and the temperature was raised to 120 °C and held for 110 min. Compared with traditional products, the lightest color product was found desirable by GC/MS analysis and sensory evaluation. The novel method proposed can be a guide for the industrial preparation of light-colored products. Copyright © 2012 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: Maillard reaction; color-inhibiting effect; gradient temperature- elevating method; L-cysteine; Amadori compound

Introduction

The Maillard reaction (nonenzymatic glycation reaction), a well-known nonenzymatic browning reaction involved in the formation of brown pigments and flavor, occurs because of the condensation between a carbonyl group of reducing sugars and an amine group of free amino acids or any nitrogenous compound (such as amino acids, peptides, and proteins)[1]. During the formation of brown pigments, Maillard reaction typically leads to characteristic absorbance changes in both UV and visible (brown) ranges [2]. In spite of the longstanding aesthetic and practical interest in Maillard-derived food coloring materials, it is not desirable for some special food systems, such as soup and juice. Inhibiting brown color formation involves complex sequential and parallel reactions that are poorly understood. Rizzi [3] reviewed the chemical structure of colored compounds formed in the Maillard reaction, such as chemically well-defined products of molecular weight less than 500 Da, heterocyclic aldehydes and furanones, polymeric products, and caramel colors. However, most structural information are limited to compounds of molecular weight less than 500 Da. The structures of high molecular weight Maillard pigments are still undefined; only studies about their degradation have yielded identifiable fragments with chromogenic properties.

For us to know the color inhibition pathway, the scheme of the Maillard reaction should be well understood. Generally, Maillard reaction follows four steps: (i) the nonenzymatic condensation of a reducing sugar, aldehyde, or ketose with a free amino group of protein to form a glycosylamine (a reversible step), (ii) the rearrangement of glycosylamine to Amadori products, (iii) the degradation and dehydration of Amadori products into amino or carbonyl intermediates, and (iv) the reaction of carbonyl intermediates with other amine groups, as well as the subsequent rearrangement to form advanced glycosylation end-products (AGE-products). During the Maillard reaction, Amadori compounds and the highly reactive α-dicarbonyls initiate a cascade of further reactions that finally result in the formation of a complex mixture containing colored compounds [4]. Glycoxidation of the Amadori products, autoxidations of reducing sugars, or the corresponding Schiff base and the Amadori products, give rise to α-dicarbonyl compounds, such as methylglyoxal and glyoxal [5]. The formation of reactive C2, C3 intermediate dicarbonyls are essential to generate color substances [6]. Some researchers demonstrated that methylglyoxal was the key intermediate during the degradation of Amadori compounds, especially in the formation of protein cross-linking products, such as melanodins [7], whereas others considered glyoxal, or both, as the important intermediates [8]. They could crosslink proteins at different rates, resulting in the formation of polymeric substances [9].

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L-cysteine, as a flavor precursor, is widely documented in meat and other foods [10,11], but little attention has been given to the mechanism of the color-inhibiting effect of L-cysteine. The good color-inhibiting effect of L-cysteine has been observed at A_{420} for brown pigment formation in the final stage of the Maillard reaction [12]. The main objective of the present study was to find out how L-cysteine inhibited the color formation in the Maillard reaction. It is hypothesized that L-cysteine reacts with the degradation and dehydration products of Amadori compounds or possibly by quenching the rearrangement of Amadori products in the initial stages of the reaction. Therefore, the reaction of methylglyoxal or glyoxal with L-cysteine was first investigated. Amadori compounds of D-xylose and reducing glutathione (GSH) were prepared, and the color formation was compared in the absence/presence of L-cysteine in subsequent the Maillard reaction. When the possible color-inhibiting pathway was explored, an improved method to control the color formation was proposed by precisely controlling the addition time of L-cysteine and gradient temperature-elevating Maillard reaction in the soybean peptide-xylose system.

Materials and Methods

Materials

L-cysteine, GSH, and D-xylose were supplied by Huzhou Longs Biochem Co. Ltd. (Zhejiang, China). Glyoxal and methylglyoxal were obtained from Shanghai D&R Finechem Co. Ltd. (Shanghai, China). Soybean protein isolate (containing 86.7% protein) was purchased from Hufeng Biotechnology Co. (Shanghai, China). Alcalase 2.4 l was obtained from Novozymes China Inc. (Wuxi, China).

Preparation of Amadori Compound

The preparation of the GSH-xylose Amadori compound (GX-AMD) was carried out according to the method described in [13]. Solution of GSH (0.31 g) and D-xylose (2.25 g) was incubated in MeOH-AcOH (9:1) (300 ml) for 5 days at 37 °C. The solvent was evaporated; the residue was dissolved in 10 ml water and applied to a column (12 mm × 80 cm, Shanghai Xiamei Biochemical Science Technique Development Ltd., Inc. Shanghai, China) of Amberlite IR-120 in H^+-form. The column was first washed with water and then with 10% formic acid. The fractions containing Amadori compound were further purified using Atlantis T3 RP-HPLC (5 μm, 10 × 150 mm) at 3.0 ml/min by linear gradient from 10 to 40% MeOH/0.1% TFA over 15 min. UV detection was performed at 215 nm with the use of a photodiode-array detector 2996 (Waters, USA). The Atlantis C18 RP-HPLC (5 μm, 3.9 × 150 mm) was used for the analysis.

GSH-xylose-cysteine Amadori compound (GXC-AMD) was prepared and purified at the same conditions as that of GX-AMD. The only difference was the addition of L-cysteine (0.18 g) in the reaction system.

Mass Spectrometry

The LC-ESI-MS mass spectra were obtained by mass spectrometry (Waters Synapt MALDI Q-TOF MS, USA) with positive ESI mode, and the ionization conditions were as follows: capillary voltage 3.5 kV, cone voltage 30 V, and extractor voltage 7 V. The source block temperature was 100 °C, and the desolvation temperature was 250 °C. The cone gas flow was adjusted to 50 ml/min. The scanning of the sample was recorded over the range of m/z 50–1000 with a scan time 1 s and an interscan delay of 0.1 s. Analytical Ultra Performance Liquid Chromatography (UPLC) conditions on LC-MS: column, C18, 2.1 mm × 100 mm, 1.7 μm; injection volume, 0.2 μl; flow rate, 0.3 ml/min; and UV detector was set at a range of 200–600 nm. The samples were analyzed by linear gradient from 5 to 60% MeOH/0.1% TFA over 30 min by directly injecting the sample. Data were acquired using MassLynx software (version 4.1, Milford, MA, USA).

General Maillard Reaction

Soybean peptide (1.00 g), prepared according to the method described in [12], and xylose (0.15 g) was mixed well in 10 ml of de-ionized water. The solution was adjusted to pH 6.5 with 6 N HCl or NaOH if necessary. The solution was initially heated at 120 °C for 2 h. L-cysteine (0.10 g) was added at reaction time of 0, 5, 10, 15, 20, and 25 min. The mixture was immediately cooled in ice water and stored at 4 °C in the fridge for further use. The Maillard reaction products (MRP) prepared by adding L-cysteine at 0 min was termed as PXC.

For the reaction of the L-cysteine and the Amadori compound, a solution of GSH-xylose Amadori compound (30 mmol/l) and L-cysteine (80 mmol/l) was mixed well. The solutions were adjusted to pH 6.5 with 6 N NaOH or 1 N HCl, and the tubes were tightly capped and heated in a thermostatic oil bath with magnetic stirring at 120 °C. After heating for 30 min, the mixture was immediately cooled in ice water. The sample without added L-cysteine was taken as control.

Gradient Temperature-elevating Maillard Reaction

Soybean peptide (1.00 g), prepared according to the method reported in [12], and xylose (0.15 g) was mixed well in 10 ml de-ionized water. The pH was adjusted to 6.5 with 6 N NaOH or 1 N HCl. The solution was initially heated at different temperatures (80, 90, 100, 110, and 120 °C) for different times (0–100 min). After each added L-cysteine (0.10 g), the solution temperature was raised to 120 °C immediately and held for 110 min. The mixture was immediately cooled in ice water for immediate use. The corresponding products heated at 80 °C for 20, 40, 60, 80, and 100 min were used for sensory evaluation and termed as MRP-20, MRP-40, MRP-60, MRP-80, and MRP-100, respectively.

Absorption Spectra Measurements

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

Color Measurements

The color measurement was carried out by Hunters parameters using de-ionized water as blank ($L0^* = 65.87$; $a0^* = 1.96$; $b0^* = 2.59$) with a WSC-S color difference meter [14,15] (Shanghai Precision & Scientific Instrument Co., Ltd, Shanghai, China). A 3-ml sample was pipetted into a 2.5-cm diameter test tube for measurement. The CIEL*a*b* system provides the values of three color components: L^* (black/white component, a higher value indicating higher lightness), a^* (red (+)/green (−) component), and b^* (yellow (+)/blue (−) component). Total color difference (ΔE) was calculated from the Hunter–Scotfield equation $\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$.

Headspace Solid Phase Microextraction/Gas Chromatography/Mass Spectrometry (HS-SPME-GC/MS) Analysis

A DB-Wax capillary column (30 m × 0.25 mm × 0.25 μm; J&W Scientific, Folsom, CA, USA) was used to analyze volatile compounds. The analyses were performed using a gas chromatograph/mass spectrometer (Finnigan Trace GC/MS, Finnigan, California, USA). The volatile compounds were sampled with a carboxen/polydimethylsiloxane (75 μm) SPME fiber (Supelco, Bellefonte, PA, USA). Each MRP sample (3 g) was placed in a 15-ml vial, and an internal standard of 1,2-dichlorobenzene (50 μg in 1 ml of methanol) was added prior to trapping. The vial was sealed with a polytetrafluoroethylene/butyl septum and equilibrated at 50 °C for 30 min in the presence of the SPME fiber. After the equilibration time, the injection of the sample was conducted in the splitless mode at 250 °C for 3 min. The gas chromatograph (GC) temperature was initially held at 40 °C for 3 min, then raised to 80 °C at 5 °C min^{-1}, raised to 160 °C at 10 °C min^{-1}, held at 160 °C for 0.5 min, raised to 175 °C at 2 °C min^{-1}, raised to 230 °C at 10 °C min^{-1}, and finally held at 230 °C for 7 min. Helium was used as carrier gas at a linear velocity of 1.8 ml min^{-1}. Mass spectra were obtained in electron impact mode at an energy voltage of 70 eV and an emission current of 35 μA. The detector was set to scan the range m/z 35–450 at a rate of 4.45 scans s^{-1}.

The identification of the volatile compounds was carried out by the comparison of their mass spectra with the Wiley, NIST, and Replib libraries and also by comparing their Kovats indices (KIs) with those of standard compounds and data from the literature. Linear KIs of the compounds were calculated using a series of n-alkanes injected under the same chromatographic conditions and compared with available literature data. The identified volatile compounds of MRPs were quantified by GC/MS. The areas of the peaks were measured by calculating the total ion current.

Sensory Evaluation

To evaluate the flavor characteristics of MRPs, we performed an evaluation on MRPs according to the method described in [16] with some modifications. 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 MRPs (MRP-20, MRP-40, MRP-60, MRP-80, MRP-100, and PXC) solutions (1.0%) were individually dissolved in an umami solution that consisted of 1.0% monosodium glutamate (MSG) and 0.5% sodium chloride (NaCl). The resulting solutions were heated to 60 °C in a water bath. Both 60 ml of sample solution and 60 ml of umami solution (control) were served at the same time. The samples were coded with three-digit numbers, served in a randomized order, and tasted at 22 ± 2 °C in individual sensory booths. The panelists were asked to score the intensities of the solutions by using a scale of 1–7, where 3 were the score assigned to the control sample. The values given by the panelists were averaged.

Statistical Analysis

The experiments were made in triplicate. Experimental data were subjected to analysis of variance (ANOVA), and the Student–Newman–Keuls test was applied with a level of significance of 95%. All statistical analyses were performed using SPSS Version 13.0 (SPSS Inc., Chicago, IL, USA).

Result and Discussion

Effect of Addition Time of L-cysteine on Inhibiting Color Formation

The color parameters of the MRPs were investigated to assess the effect of the addition time of L-cysteine on inhibiting the color formation in the Maillard reaction of soybean peptide-xylose system. Color was measured in terms of lightness (L^*), redness (a^*) and yellowness (b^*). The total color differences (ΔE) were then calculated to estimate the overall browning [17]. The decrease of browning is related to the increase of lightness, decrease of redness, and yellowness. These color parameters (A420, L^*, a^*, b^*, ΔE), which reflected visual changes of MRPs, were significantly affected by the addition time of L-cysteine (Table 1). The strongest color-inhibiting effect was observed at the L-cysteine addition time of 10 min ($p < 0.05$). Color-inhibiting effect of L-cysteine may be due to its interaction with a preformed Amadori compound in the initial stage of Maillard reaction.

Possible Mechanism of Color-inhibiting Effect of L-cysteine

Reaction of L-cysteine and α-dicarbornyl compounds

During the Maillard reaction, retro-aldolization of the intermediate deoxyglycosones from Amadori products give rise to reactive α-dicarbornyl compounds, such as methylglyoxal and glyoxal. The α-dicarbornyl compounds play an important role in the color formation. When methylglyoxal or glyoxal were heated together with L-cysteine for 30 min at a mild condition (25 °C), the absorbance at 420 nm was 0.540 and 0.221, respectively, which was much stronger than that of methylglyoxal (0.039) or glyoxal (0.007) heated alone. Absorbance at 420 nm is used to measure the extent of brown compound formation [18]. The origin of visual color has occasionally been attributed to the presence of paramagnetic species, that is, free radicals in Maillard polymers [3]. Moreover, methylglyoxal and glyoxal can be further condensed

Table 1. Color changes with addition time of L-cysteine in soybean peptide-xylose system heated at 120 °C

Time[a]/min	A420	L^*	a^*	b^*	ΔE
0	0.241 ± 0.001^{adb}	41.72 ± 0.07^{a}	19.26 ± 0.08^{a}	61.92 ± 0.09^{a}	66.35 ± 0.08^{a}
5	0.236 ± 0.001^{ab}	43.05 ± 0.13^{b}	17.71 ± 0.15^{b}	59.21 ± 0.16^{b}	63.04 ± 0.15^{b}
10	0.215 ± 0.005^{c}	46.17 ± 0.08^{c}	15.24 ± 0.08^{c}	57.00 ± 0.12^{c}	59.38 ± 0.11^{c}
15	0.231 ± 0.003^{b}	44.24 ± 0.15^{d}	16.39 ± 0.18^{d}	59.03 ± 0.14^{b}	62.20 ± 0.06^{d}
20	0.246 ± 0.001^{d}	40.74 ± 0.11^{e}	20.61 ± 0.16^{e}	63.40 ± 0.35^{d}	68.39 ± 0.31^{e}
25	0.260 ± 0.002^{e}	36.37 ± 0.31^{f}	24.02 ± 0.15^{f}	65.76 ± 0.40^{e}	73.13 ± 0.40^{f}

[a]Data presented are the color changes of the MRPs prepared by different addition time of L-cysteine.
[b]The values are the mean ± standard deviation ($n = 3$). Means with different letters within the same column are significantly different ($p < 0.05$).

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or cross-linked to form a color precursor. Zeng and Davies [19] reported that a clear major stable product was formed in the reaction of L-cysteine residues with glyoxal and glucose. The increased electrophilicity of these dicarbonyl compounds results in their relatively fast reactions with L-cysteine, which has the thiol group with a powerful nucleophile. Meade *et al.* [9] reported that methylglyoxal and glyoxal can crosslink amine compounds at vastly different rates to form premelanoidins or more advanced low molecular weight compounds [20].

Therefore, it was clear that α-dicarbonyl compounds of reducing sugar derivatives reacted rapidly and irreversibly with L-cysteine. This represented an interesting situation; once dicarbonyl compounds were produced in Maillard reaction, L-cysteine had no effect on inhibiting the color formation. It was speculated that it would be effective in inhibiting color formation before dicarbonyl compounds were formed.

Preparation and identification of GX-AMD

Because the constituents of soybean peptide are complex, GSH was firstly subjected to react with xylose to prepare the Amadori compound (GX-AMD) in order to investigate the color-inhibiting pathway of L-cysteine. It is known that glycosylamines, intermediates of the Amadori rearrangement, are often too unstable to be isolated and characterized [21,22]. Among the four stages of the Maillard reaction, the inhibiting effect of L-cysteine on color formation could not occur in advanced stages. Additionally, as mentioned previously, once dicarbonyl compounds produced in the Maillard reaction, L-cysteine had no effect on inhibiting the color formation. It was assumed that L-cysteine might react with the Amadori compound to inhibit color formation.

As presented in Figure 1, the MS/MS spectrum of the molecular $[M+H]^+$ ion of the compound with m/z 440 shows loss of one glutamic acid molecule (129 Da loss), giving rise to an ion at m/z 311 which indicates the presence of glutamic acid. The spectrum exhibits the presence of ions originating from the consecutive loss of 18 Da (m/z 293, 275), corresponding to the elimination of one water molecular. In analogy to the pentose-derived Amadori products reported in [23], the fragment ions were most likely formed by consecutive losses of one, two, or three water molecules with the completely dehydrated sugar moiety dominating the spectra. Cleavage of glutamic acid from the L-cysteine residue generated abundant ions at m/z 177. The tandem mass spectrum of the molecular $[M+H]^+$ ion of the compound with m/z 440 was considered as the structure of the Amadori-type of compound. The MS/MS spectrum information of the Amadori compound observed were consistent with a previous report in [13].

Role of L-cysteine in inhibiting color formation

When GX-AMD was heated in the absence of L-cysteine, yellow-green color was formed at 30 min, and when GX-AMD was heated together with L-cysteine (GX-AMD + Cys, Table 2), the absorbance at 420 nm and overall color difference (Δ*E*) were significantly decreased, which indicates that the visible color formation was significantly inhibited by the interaction of L-cysteine with the Amadori compound. This phenomenon might be attributed to the fact that polymerization of low molecular weight colored MRPs were suppressed [24]. Additionally, the decrease of absorbance in the UV region showed that the formation of the noncolored low molecular weight substances was also suppressed by L-cysteine during the intermediate stage (data not shown). Absorbance in the UV region has been used to determine the noncolored and/or the fluorescent low molecular weight intermediate compounds from the sugar fragment and the Strecker degradation [25]. Amadori compounds might be reactive (electrophilic); as a result, they react even more rapidly with amines (powerful nucleophilic) to form low molecular weight and double-bond unsaturated products [3].

To further investigate the color-inhibiting mechanism, L-cysteine was added at the beginning of the reaction. The Amadori compound, GXC-AMD, was prepared and heated alone at 120 °C

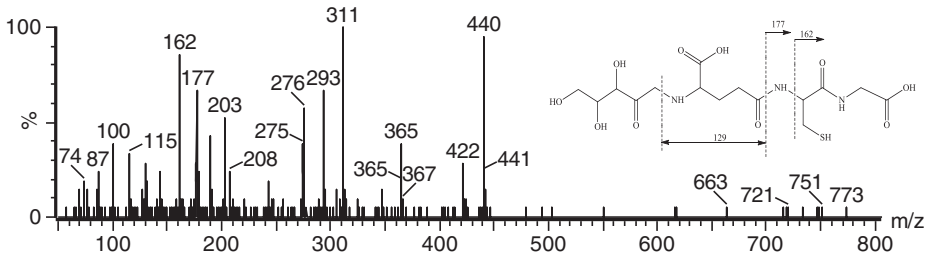

Figure 1. LC-MS/MS spectra of the peak at m/z 440 and the proposed chemical structure and fragmentation assignments of ions observed in the MS/MS spectra of the molecular $[M+H]^+$ ions of the Amadori compound (N-1-deoxy-xylulos-yl GSH).

Table 2. Color changes of GX-AMD, GXC-AMD, and the mixture of GX-AMD and cysteine heated at 120 °C for 30 min

Samples[a]	A420	*L**	*a**	*b**	Δ*E*
GX-AMD	0.614 ± 0.00[b]	48.69 ± 0.11	16.00 ± 0.15	58.13 ± 0.24	59.16 ± 0.27
GX-AMD + Cys	0.197 ± 0.00	61.18 ± 0.03	−1.05 ± 0.03	30.41 ± 0.11	27.27 ± 0.12
GXC-AMD	0.564 ± 0.02	30.65 ± 0.15	26.96 ± 0.21	36.48 ± 1.38	54.86 ± 1.02

[a]Samples are denoted by the reaction systems of related Amadori compounds, the system of GX-AMD heated alone (GX-AMD), GX-AMD heated together with L-cysteine (GX-AMD + Cys), the system of GXC-AMD heated alone (GXC-AMD).

[b]The values are the mean ± standard deviation ($n = 3$).

for 30 min. Red-colored substances were observed (Table 2). It indicates that the color-inhibiting effect was decreased if the L-cysteine was added at the beginning of reaction. The possible reason is that L-cysteine might affect or interfere with the original formation of the Amadori compounds. Actually, the Maillard reaction was a cascade of events, and the intermediates would react with each other or with the amine group, proceeding to the advanced stage to generate colored substances. It is possible that L-cysteine played a double role subsequently; one was the interaction with the Amadori compound; the other was the nonenzymatic condensation with reducing sugar as the amine group provider.

Color Changes of MRPs through Gradient Temperature-elevating Method at Different Temperatures

The addition time of L-cysteine was vital to inhibit the color formation during reaction. Once the Amadori compounds are formed, L-cysteine was added immediately, and the extraordinary color-inhibiting effect could be observed. If L-cysteine is added at the beginning, its own Amadori compound would be formed; if L-cysteine is added later during the reaction, the Amadori compound would have undergone subsequent Maillard reaction, such as forming α-dicarbonyl compounds. The most significant color-inhibiting effect was achieved when L-cysteine was added

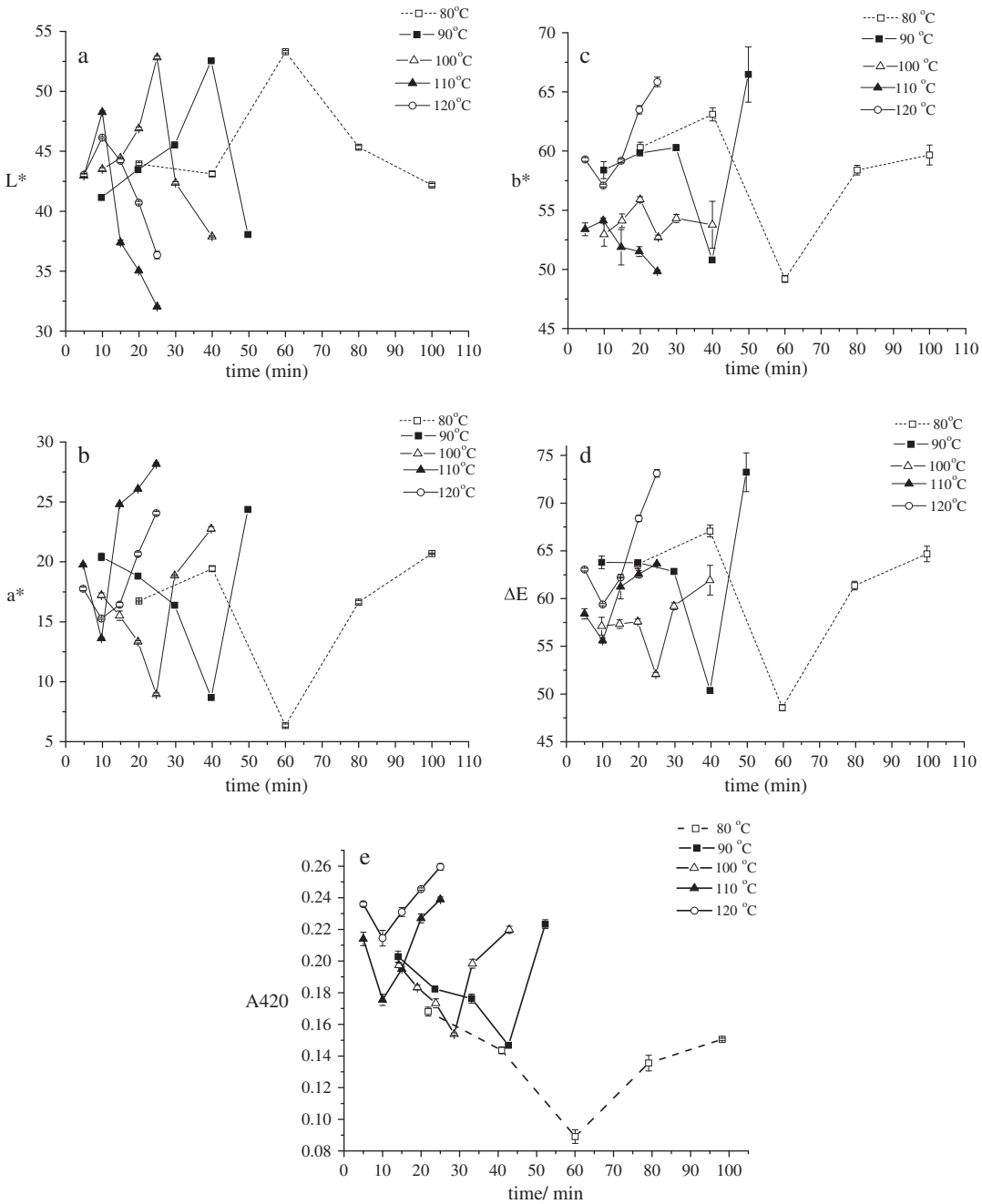

Figure 2. Color space parameters and absorbance at 420 nm (A420) of MRPs prepared using the gradient temperature-elevating method.

Table 3. Major volatile compounds found in the MRPs by SPME-GC/MS

Compounds	KI[p]	ID[q]	MRP-20 (μg/g)	MRP-40 (μg/g)	MRP-60 (μg/g)	MRP-80 (μg/g)	MRP-100 (μg/g)	PXC (μg/g)
Hexanal	1084	A	0.2389 ± 0.002^{a}	0.1988 ± 0.002^{b}	0.1019 ± 0.005^{c}	0.2031 ± 0.013^{b}	0.2415 ± 0.007^{a}	0.3147 ± 0.008^{d}
Heptanal	1193	A	0.0751 ± 0.004^{a}	0.1011 ± 0.002^{b}	0.1126 ± 0.015^{b}	0.0694 ± 0.002^{a}	0.0444 ± 0.001^{c}	0.334 ± 0.015^{d}
Pyrazine	1216	B	0.0905 ± 0.012^{a}	0.0518 ± 0.009^{b}	N.D	0.0284 ± 0.006^{c}	0.1505 ± 0.010^{d}	0.1696 ± 0.012^{d}
Methylpyrazine	1280	B	0.0429 ± 0.005^{a}	0.0315 ± 0.007^{a}	N.D	0.0115 ± 0.001^{a}	0.068 ± 0.006^{a}	0.7385 ± 0.084^{b}
2-Methyl-3-furanthiol	1304	A	0.0036 ± 0.001^{a}	0.0048 ± 0.0014^{a}	0.0508 ± 0.009^{a}	0.0197 ± 0.003^{a}	0.1091 ± 0.020^{a}	0.7393 ± 0.083^{b}
Hexanol	1354	B	N.D	0.0776 ± 0.005^{a}	0.0585 ± 0.004^{a}	0.4660 ± 0.005^{a}	N.D	1.956 ± 0.078^{b}
Nonanal	1399	A	0.6107 ± 0.016^{a}	0.7587 ± 0.022^{b}	1.3912 ± 0.099^{c}	1.0191 ± 0.1198^{d}	0.8033 ± 0.030^{b}	1.3849 ± 0.036^{c}
2-Furanmethanthiol	1439	B	N.D[g]	N.D	0.0453 ± 0.007^{b}	N.D	N.D	1.0338 ± 0.021^{c}
Acetic acid	1460	A	N.D	0.2469 ± 0.047^{a}	0.2098 ± 0.002^{ab}	0.1659 ± 0.007^{b}	N.D	2.0640 ± 0.064^{c}
4-Nitrophthalamide	1462	B	N.D	N.D	0.0686 ± 0.004^{a}	N.D	N.D	0.1101 ± 0.010^{b}
Furfural	1473	B	0.1970 ± 0.007^{a}	0.1279 ± 0.003^{b}	0.0967 ± 0.005^{c}	0.1478 ± 0.005^{d}	0.3001 ± 0.001^{e}	0.380 ± 0.002^{f}
Decanal	1495	A	0.1948 ± 0.008^{a}	0.2521 ± 0.016^{b}	0.3748 ± 0.011^{c}	0.1877 ± 0.007^{a}	0.1695 ± 0.015^{a}	0.3694 ± 0.013^{c}
Benzaldehyde	1541	A	1.2642 ± 0.064^{a}	1.1394 ± 0.005^{b}	1.0920 ± 0.039^{b}	1.0829 ± 0.053^{b}	1.4735 ± 0.060^{c}	0.7977 ± 0.012^{d}
(E)-2-nonenal	1542	B	0.1351 ± 0.010^{a}	0.0447 ± 0.021^{b}	N.D	0.0419 ± 0.016^{b}	0.1956 ± 0.012^{c}	0.2372 ± 0.020^{c}
1-Octanol	1557	B	N.D	0.0746 ± 0.006^{a}	0.3039 ± 0.007^{b}	0.1241 ± 0.005^{c}	N.D	0.375 ± 0.006^{d}
1-Nonanol	1602	A	N.D	0.0730 ± 0.004^{a}	0.1149 ± 0.011^{b}	0.0488 ± 0.003^{c}	N.D	0.965 ± 0.007^{d}
Dodecanal	1718	A	N.D	N.D	0.0161 ± 0.001^{a}	0.01592 ± 0.001^{a}	N.D	0.118 ± 0.004^{b}
Hexanoic acid	1853	A	N.D	0.2579 ± 0.004^{a}	0.1763 ± 0.005^{b}	0.2577 ± 0.003^{a}	N.D	1.255 ± 0.006^{c}
3-Hydroxy-2-methyl-4H-pyran-4-one	1969	B	0.0745 ± 0.007^{ab}	0.0577 ± 0.005^{bc}	0.0306 ± 0.004^{d}	0.0472 ± 0.007^{c}	0.0664 ± 0.009^{b}	0.0251 ± 0.001^{d}
Octanoic acid	2067	A	N.D	0.1005 ± 0.03^{a}	0.0699 ± 0.003^{b}	0.1073 ± 0.007^{a}	N.D	0.4490 ± 0.026^{c}
Parabanic acid	2119	B	N.D	0.1181 ± 0.003^{a}	0.0857 ± 0.003^{a}	0.1014 ± 0.005^{ba}	N.D	0.7960 ± 0.032^{b}
Bis(2-methyl-3-furyl) Disulfide	2163	A	0.1887 ± 0.015^{a}	0.1003 ± 0.003^{b}	0.0198 ± 0.003^{c}	0.0637 ± 0.019^{b}	0.3208 ± 0.029^{d}	0.9542 ± 0.018^{e}

Values are mean ± standard deviation ($n = 3$).

MRP-20, MRP-40, MRP-60, MRP-80, and MRP-100 were Maillard reaction products prepared with the gradient temperature-elevating method. The solution was heated at 80 °C for 20, 40, 60, 80, and 100 min, respectively. Then, each was added with L-cysteine (0.1000 g), and the solution temperature was raised to 120 °C immediately and held for 110 min. PXC was the Maillard reaction product. The mixture of soybean peptid, xylose, and L-cysteine was heated at 120 °C for 120 min.

KI[p], Kovats index calculated for the DB-Wax capillary column.

ID[q], reliability of the identification proposal is indicated by the following: A, mass spectrum and Kovats index according to literature data; and B, mass spectrum compared with Wiley mass spectral database.

[a]Means with different letters within the same row are significantly different ($p < 0.05$).

[b]Means with different letters within the same row are significantly different ($p < 0.05$).

[c]Means with different letters within the same row are significantly different ($p < 0.05$).

[d]Means with different letters within the same row are significantly different ($p < 0.05$).

[e]Means with different letters within the same row are significantly different ($p < 0.05$).

[f]Means with different letters within the same row are significantly different ($p < 0.05$).

[g]N.D, not determined.

10 min later in the Maillard reaction at 120 °C. However, it is very difficult to realize in practical production because the formation time of Amadori compounds was too short at a higher temperature (120 °C). According to the Arrhenuis equation of general reaction, the formation time of Amadori compounds depends on temperature. In general, the lower the temperature, the longer the formation time of Amadori compounds. Therefore, the gradient temperature-elevating Maillard reaction was applied. The lightest color of MRPs at each given temperature, that is 80, 90, 100, 110, and 120 °C was formed when heated for 60, 40, 25, 10, and 10 min, respectively (Figure 2(a–d)). Similar bell-shape or inverted bell-shape curves were found for all color parameters; a turning point of the time course can be observed at each temperature. The changes of the L^* value followed a typical bell-shape curve. However, a^*, b^*, and ΔE value followed an inverted bell-shape curve. This result was in agreement with our previous study that the color-inhibiting effect of L-cysteine was because of its interaction with the Amadori compound.

From all the color parameters, the most significant color-inhibiting effect was observed when soybean peptide-xylose system was heated at 80 °C for 60 min, followed by the instant addition of L-cysteine. The highest 'L^*' value (53.30) of MRPs was observed when heated at 80 °C for 60 min (Figure 2(a)), which indicated the formation of the lightest color. Hunter 'a^*' 'b^*' value of MRPs were both significantly affected by the addition time of L-cysteine ($p < 0.05$) (Figure 2(b and c)). The decrease in ΔE (Figure 2(d)) corresponded to the changes in the parameters 'L^*', 'a^*', and 'b^*' values.

The lowest browning intensity was also found at 80 °C for 60 min, followed by 90, 100, 110, and 120 °C for 40, 25, 10, and 10 min, respectively(Figure 2(e)), which was highly consistent with the result of the color parameters measurement. The addition time of the L-cysteine for effectively inhibiting color formation shortened with increasing temperature. The result confirmed that the color of MRPs could be adjusted through the subsequent method. Amadori compounds were first formed at a relatively lower temperature and then L-cysteine was instantly added to produce a desirable lighter product at a suitable higher temperature.

Volatile Compounds and Aroma Profile of Light-colored MRP Prepared by Gradient temperature-elevating Method

A total of 75 compounds of MRPs were detected by SPME-GC/MS. For us to qualify major compounds and clearly compare the changes of volatile compounds, principle component (PC) analysis was conducted between the scores of six odor attributes and all identified compounds. Compounds which were significantly correlated with the odor attributes among MRPs (PC1 55%, PC2 41%, data not shown) are listed in Table 3.

The average score of odor attributes can be seen in the profiling in Figure 3. The profiling shows the distinctive difference between PXC and light-colored MRPs for meaty, roasted, sulphuric, and malty, whereas the difference for the caramel was less clear. Specific aroma compounds can be related to the odor attributes. For example, pyrazine and methylpyrazine were reported as responsible for the roasted note [26]. The amount of pyrazine and methylpyrazine of light-colored MRPs were significantly lower than that of PXC (Table. 3). It might be because of the formation of pyrazine precursors being suppressed by further reaction of Amadori compounds. The α-amino carbonyls can be formed from the reactions between dicarbonyl compounds and amino acids during Strecker degradation and are generally considered as

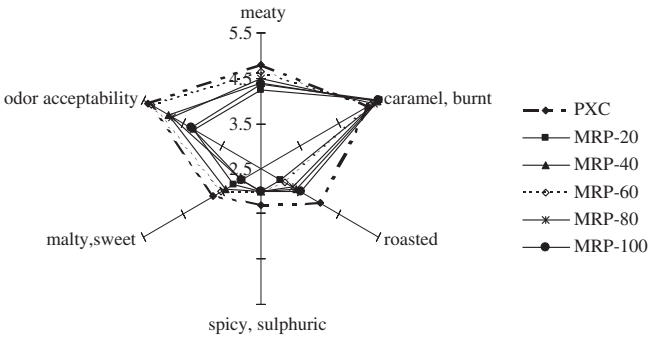

Figure 3. Aroma profile of MRPs through sensory evaluation.

pyrazine precursors [11]. The dicarbonyl compounds such as glyoxal, ethylglyoxal, and butanedione can be produced by sequential reaction of Amadori compounds. When L-cysteine is added at the time of the Amadori compound's formation, to a large extent, L-cysteine participates in subsequent reactions related to Amadori compounds. Consequently, reactions of both L-cysteine releasing ammonia and the formation of dicarbonyl compounds could be suppressed [27].

Volatile thiols are generated by a heating process (the sulfur amino-carbonyl reaction) and yield a strong-smelling flavor [28]. These flavors play an important role in contributing meaty, savory, and roast flavors to the cooked and roasted foods because of their low odor thresholds [29]. Besides, 2-methyl-3-furanthiol content in PXC and MRP-60 were much higher than that in other samples, whereas 2-furanmethanthiol was only found in PXC and MRP-60. That could be the reason why meaty aroma in the sensory profile is distinctly different (Figure 3).

For the caramel and burnt attributes, 3-hydroxy-2-methyl-4H-pyran-4-one (maltol) was described as a caramel-like, cotton candy odor. The content of maltol in PXC and MRP-60 was not significant (Table.3). This could cause less differences in the caramel odor of MRPs by sensory evaluation (Figure 3). Moreover, some acids were statistically significant in correlation with odor attributes by the PC analysis, such as octanoic acid, hexanoic acid, and so on, as can be seen in Table 3. It was reported that they contributed to sour odor formation [30]. Hexanol, 1-octanol, and 1-nonanol were also found with significant differences in MRPs. These compounds do not have a specific aroma, but as substrate, they play an important role in the flavor, which could lead to the change of overall odor profile of MRPs. Odor acceptability of PXC was the highest among all the MRPs, followed by MRP-60, MRP-40, MRP-80, and MRP-100. The lowest was MRP-20 (Figure 3). The aforementioned results indicated that the overall aroma of light-colored MRP-60 was also acceptable.

Conclusion

The present work deals with an important issue related to the food industry, that is, how to effectively control the Maillard reaction browning in preparing light-colored tasty products. Through the manipulation of the color-inhibiting mechanism of L-cysteine, an effective method was developed to control the color formation through gradient-elevating temperature in the soybean peptide-xylose reaction system. Additionally, by adjusting the addition time of L-cysteine, a desirable aroma was developed. Other sensory characteristics of light-colored MRPs need to be investigated. This

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product could be useful in food industries for the preparation of savory and light-colored food products.

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