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ORIGINAL PAPER

Influence of pH on the formation and radical scavenging activity of volatile compounds produced by heating glucose with histidine/tyrosine

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Abstract In this study, the effect of pH on the volatile generation over reaction time as well as the DPPH radical scavenging activity of volatile MRPs was investigated in the Maillard reaction of glucose (Glc) with tyrosine (Tyr) and histidine (His). Factor analysis showed clearly that volatiles generated in 5-h open system were of similar composition to those in 0.5-h close system regardless of pH level and amino acid, implying higher pressure in close system could accelerate the volatile formation in Maillard reaction. Besides, all volatiles showed increasing tendency over reaction time under different pH levels with the exception of pyrones which decreased with the extension of reaction time at pH = 7 and pH = 9. Furans, phenols and 2-acetylpyrrole that were favored under acidic condition showed larger increase at pH = 5 over reaction time, while other volatiles except pyrones, furans, phenols and 2-acetylpyrrole, which were favored under neutral and alkaline conditions, showed greater increase at pH = 7 and pH = 9 over reaction time. The measurement of DPPH radical scavenging activity of dichloromethane extract obtained from Maillard reaction mixture revealed that volatile MRPs from Glc–Tyr system showed stronger antioxidant activity under alkaline condition, while volatile MRPs from Glc–His system showed greater antioxidant activity under acidic condition.

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Keywords Maillard reaction · Volatile compounds · Factor analysis · DPPH radical scavenging activity

Introduction

Maillard reaction is one of the important reactions in food chemistry because of its major contribution to the flavor of foods [1–3]. During food processing and storage, the carbonyl-containing compound (such as reducing sugar) could react with amino-containing compound (such as amino acid, peptide and protein) to form aroma compounds. Factors that influence the volatile formation in Maillard reaction include nature of reactants [4–6], reactant ratio [7], temperature [8], pressure [9, 10], pH [11–14], mode of heating [15, 16] and so forth, among which pH was of great importance as the mechanism of Maillard reaction was associated with pH [17]. Generally, furans were formed mainly at acidic pH, while alkaline condition could promote the production of pyrazines [11, 12]. Illmann et al. [18] reported higher pH was favorable to the production of 4-hydroxy-2,5-dimethyl-3(2H)-furanone, an important flavor compound with intense caramel-like odor. Ames et al. [19] reported most compounds generated in cysteine–glucose system showed increasing amount with increasing pH. Yu et al. [13] reported thiophenes and thiazoles were formed mainly at alkaline condition, while thienothiophenes and thienones were formed mainly at acidic pH in L-ascorbic acid-L-cysteine model. Huang et al. [2] reported the ratios of trimethylpyrazine-to-dimethylpyrazine and dimethylpyrazine-to-tetramethylpyrazine increased, while the ratio of tetramethylpyrazine-to-trimethylpyrazine decreased as the pH increased in cocoa liquors. Besides, it was proposed from the literature that pH showed great influence on the amount of volatiles and little influence on the formation

pathway of a particular compound [20]. It was noteworthy that there were some reports implying the existence of interaction between pH and temperature as well as the interaction between pH and reaction time on the volatile generation in Maillard reaction [21, 22]. However, the investigation on the interaction between pH and reaction time was still very limited to the best of our knowledge.

The objective of the present investigation is to study the effect of pH on the volatile generation over reaction time. Besides, volatile generation in close and open systems was also compared. Additionally, the antioxidant activity of volatiles prepared under different reaction conditions was also estimated by DPPH method. Tyrosine (Tyr) and histidine (His) with glucose (Glc) were used as our model systems due to the observation of strong antioxidant activity in such models in our preliminary experiments. Phosphate buffer was employed in this study on account of its higher catalytic activity with comparison to other buffers such as succinate/citrate buffer and acetate buffer [23].

Materials and methods

Chemicals and reagents

D-glucose, L-histidine and L-tyrosine were of biological grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (China). 1,1-diphenyl-2-picryl-hydrazyl (DPPH) was purchased from TCI (Shanghai) Development Co., Ltd. (China). $NaH_2PO_4{\cdot}2H_2O$ and $Na_2HPO_4{\cdot}12H_2O$ were of analytical grade.

Preparation of Maillard reaction products (MRPs)

Glucose, tyrosine and histidine were used to prepare samples. Glucose was dissolved in phosphate buffer (0.5 mol/L, pH = 5, 7, 9) to obtain a final concentration of 0.05 mol/L. For reaction in close system, equimolar amounts of tyrosine (0.4530 g) or histidine (0.3879 g) were accurately weighted in 100 mL Pyrex vial and then added with 50 mL of 0.05 mol/L glucose solution. The vial was sealed with silicone/Teflon septa and metallic cap. The mixture was then heated while stirring at 130 °C for 0.5, 1.5 and 2.5 h in oil bath. For reaction in open system, equimolar amounts of tyrosine (0.9060 g) or histidine (0.7758 g) were accurately weighted in 250 mL round-bottom flask and then added with 100 mL of 0.05 mol/L glucose solution. The flask was equipped with spherical condenser where cold water flew through, and then the flask was heated while stirring at 130 °C for 5 h. The reactions were stopped by cooling under a stream of cold water.

Isolation of volatiles

Aliquots (25 mL) of each reaction mixture were extracted with 3 × 15 mL of dichloromethane, being vibrated at HY-5 maneuver style vibrator (Jintan Zhongda Instrument Factory, China) for 3 × 20 min. Before the extraction, 50 μL of phenyl ethyl acetate in dichloromethane (1 μL/mL) was added to the reaction mixture as internal standard. The extract was dried over anhydrous sodium sulfate, then concentrated to a final volume of 1 mL under vacuum (Buchi rotavapor, model R201, 0.1 atm, water bath temperature 35 °C) and filtered through 0.45 μm micropore film prior to GC–MS analysis.

Gas chromatography–mass spectrometry

Analyses were performed using Trace GC-Ultra gas chromatograph coupled to ISQ mass detector (Thermo fisher scientific, USA). Volatiles were separated using a BP-5MS capillary column (50 m length × 0.2 mm i.d. × 0.33 μm film thickness, General Separation Technologies, USA). The carrier gas was helium at a constant flow rate of 1 mL/min. One μL of the extract was injected into the capillary column with a split ratio of 1:10. The oven temperature was held at 40 °C for 2 min and then programmed to 250 °C at 4 °C/min and held for 5 min. The injector and ion source temperatures were 260 and 220 °C, respectively. The transfer line to the mass spectrometer was maintained at 250 °C. The mass detector was operated in the electron impact mode with ionization energy of 70 eV and a scanning rage of 30–500 a.m.u. Compounds were tentatively identified by comparing their mass spectra with those contained in the Nist mass spectrum database and by comparison of their retention index with those reported in the literatures [24–28]. The quantification of volatile compounds was approximately determined by comparing their characteristic ion peak area with that of internal standard (phenyl ethyl acetate) in GC–MS total ion chromatogram, using a response factor of 1. This allowed investigation into the volatiles as affected by reaction conditions but did not provide absolute concentrations in the solutions.

Determination of DPPH radical scavenging activity

Antioxidant activity of the volatile compounds obtained from the MRPs was measured using the DPPH method [29] with some modifications. A 400 μL aliquot of the volatile extract obtained in part "Isolation of volatiles" without concentration was added with 4 mL of 0.06 mmol/L DPPH ethanolic solution. The mixture was shaken vigorously and then kept at room temperature for 1 h. The absorbance was measured at 517 nm using a spectrophotometer (TU-1901, Beijing purkinje general instrument Co., Ltd., China). The

blank was prepared in the same way with the substitution of ethanol for sample. The percentage of DPPH radical scavenging activity was calculated as $(A_0 - A_S)/A_0 \times 100$, where A_0 and A_S referred to the absorbance of blank and sample, respectively.

Statistical analysis

The results given in the tables and figures were means of two experiments. Results of volatiles formed under different conditions were statistically analyzed by factor analysis using STATISTIC 6.0 (StatSoft Inc., USA).

Results and discussion

Formation of volatile compounds

The volatile MRPs prepared from Glc–Tyr/His systems under different reaction conditions were extracted with dichloromethane that extracted more volatiles than ether and ethyl acetate in our preliminary experiment. Considering much fewer volatiles were detected in open system as being compared with those in close system, longer reaction time was chosen in this study to investigate the volatile generation in open system. The major volatile compounds detected in Glc–Tyr/His systems were listed in Tables 1 and 2, from which it was found that regardless of pH level and amino acid, the composition of volatiles generated in 5-h open system fell in between the volatiles prepared from 0.5-h close system and those from 1.5-h close system, implying higher pressure in close system could accelerate the volatile formation in Maillard reaction. Besides, whatever pH levels and amino acids were employed in this study, volatiles increased with the reaction time in close system. For further study on the effects of pH and reaction time on the volatile generation, the volatiles identified in Tables 1 and 2 were classified as aliphatic compounds, carbocyclic compounds, pyrazines, furans, pyrones, furanones and others, which were discussed later in turn.

Aliphatic compounds

The aliphatic compound was formed from the retroaldolization or β-cleavage of deoxyhexosone [30]. It could be found from Tables 1 and 2 that 1-hydroxy-2-propanone was the main aliphatic compound. For both Glc–Tyr and Glc–His systems in close reaction, (1) within the same pH level, aliphatic compounds increased over reaction time in acidic condition, while in neutral and alkaline conditions, aliphatic compounds dropped finally after first increase over reaction time, which might be due to the participation of reductones in the formation of end MRPs during the final stage of Maillard reaction [31]; (2) at the particular reaction time, aliphatic compounds showed the largest quantity in neutral condition, barely above that in alkaline condition and much more than that in acidic condition. Regardless of pH level and amino acid, aliphatic compounds prepared from 5-h open reaction were much less than that from 1.5-h or 2.5-h close reaction with the exception of Glc–Tyr system at pH = 9 where aliphatic compounds showed larger amount than those in every close system. The results demonstrated that difference between open and close systems was related with pH and amino acid.

Carbocyclic compounds

Two kinds of carbocyclic compounds were detected in Glc–Tyr/His systems under different reaction time and pH levels, that is, cyclopentenones and cyclohexanones. Cyclopentenones were of strong caramel-like odors [32] and of much more quantity than cyclohexanones. Thus, the following discussion was focused on the cyclopentenones. It could be demonstrated from Tables 1 and 2 that most cyclopentenones showed increasing tendency with the increase in pH level and the increase in reaction time in close Glc–Tyr/His systems, suggesting the alkaline condition was favorable to the condensation between hydroxyl ketones, which resulted in the formation of cyclopentenones [32, 33]. 2-Hydroxy-3-methyl-2-cyclopenten-1-one was the main cyclopentenone, which was in accordance with 1-hydroxy-2-propanone, the aldolization of which led to the formation of 2-hydroxy-3-methyl-2-cyclopenten-1-one. Compared to 1-hydroxy-2-propanone, lower quantity of 2-hydroxy-3-methyl-2-cyclopenten-1-one was observed in Glc–Tyr system at pH = 9 in open reaction as being compared with close reaction. The result might attribute to the positive effect of high pressure on the condensation of 1-hydroxy-2-propanone in close system.

Pyrazines

In Tables 1 and 2, thirteen pyrazines were detected and 2,5-dimethylpyrazine was the major compound. The result might attribute to the largest quantity of 1-hydroxy-propane which can be converted to methylglyoxal, the intermediate for 2,5-dimethylpyrazine. 2-Acetylpyrazine and 2-acetyl-6-methyl pyrazine might be derived from the condensation of 1-aminobutanedione with aminoacetaldehyde or 2-aminopropionaldehyde based on the hypothesis previously reported [34]. It could be found that pyrazines increased in both quantity and variety with the increase in reaction time and pH level, which was reported in many literatures [2, 12]. Besides, more quantity and variety of pyrazines were observed in Glc–Tyr system, suggesting tyrosine was more active than histidine in the formation of pyrazines under our experimental conditions. In Glc–His system, all pyrazines

Table 1 Influences of pH and reaction time on the volatile formation in the glucose–tyrosine model system

LRI[a]	Compound	Identification[b]	m/z[c]	Approximate concentration, mg/mol[d]											
				pH = 5				pH = 7				pH = 9			
				O-5 h[e]	C-0.5 h[e]	C-1.5 h[e]	C-2.5 h[e]	O-5 h[e]	C-0.5 h[e]	C-1.5 h[e]	C-2.5 h[e]	O-5 h[e]	C-0.5 h[e]	C-1.5 h[e]	C-2.5 h[e]
	Aliphatic compounds														
<700	1-Hydroxy-2-propanone	MS	74	0.17	0.69	8.12	20.49	47.68	23.65	169.48	138.57	179.86	89.24	130.73	110.52
<700	2,3-Pentanedione	MS	100	nd	0.01	0.16	0.47	0.05	0.09	0.32	0.53	0.07	0.07	0.24	0.29
708	3-Hydroxy-2-butanone	MS + LRI	88	nd	0.04	0.57	0.79	0.58	0.41	5.14	7.33	3.61	1.86	7.70	8.55
765	1-Hydroxy-2-butanone	MS	88	nd	nd	nd	nd	nd	0.08	3.28	3.97	1.22	0.84	3.62	3.18
786	4-Hydroxy-2-butanone	MS	88	nd	nd	nd	nd	nd	nd	0.12	nd	nd	nd	nd	nd
807	2-Hydroxy-3-pentanone	MS + LRI	102	nd	nd	nd	nd	nd	nd	0.02	0.06	nd	nd	0.05	0.03
927	2,5-Hexanedione	MS*	114	nd	nd	nd	nd	nd	nd	0.15	0.22	0.12	0.22	0.52	0.54
1034	4,5-Dimethyl-1,3-dioxol-2-one	MS	114	nd	nd	3.67	5.77	6.07	1.51	2.04	0.40	0.51	0.91	0.44	0.19
	Pyrazines														
731	Pyrazine	MS*	80	nd	nd	0.11	0.30	0.22	0.59	8.71	14.44	5.97	5.29	18.36	22.38
822	Methylpyrazine	MS + LRI*	94	nd	nd	nd	nd	0.14	0.39	14.23	35.16	8.72	6.71	30.73	45.33
911	2,5-Dimethylpyrazine	MS + LRI*	108	nd	nd	nd	nd	0.17	1.37	42.19	66.07	23.17	18.18	43.76	68.99
916	Ethylpyrazine	MS + LRI*	80	nd	nd	nd	nd	nd	nd	nd	1.61	0.19	nd	nd	1.62
920	2,3-Dimethylpyrazine	MS + LRI*	67	nd	nd	nd	nd	nd	nd	0.12	0.35	0.08	0.09	0.32	0.65
999	2-Ethyl-6-methylpyrazine	MS + LRI	122	nd	nd	nd	nd	nd	nd	0.16	0.68	0.05	nd	0.30	1.01
1002	2-Ethyl-5-methylpyrazine	MS + LRI	122	nd	nd	nd	nd	nd	0.13	6.68	11.84	1.05	1.17	4.83	7.23
1024	2-Acetylpyrazine	MS + LRI	122	nd	nd	nd	nd	nd	nd	0.15	0.25	0.02	0.00	0.05	0.13
1082	3-Ethyl-2,5-dimethylpyrazine	MS + LRI	136	nd	nd	nd	nd	nd	nd	0.11	0.56	0.02	0.03	0.29	0.50
1087	2-Ethyl-3,5-dimethylpyrazine	MS	136	nd	nd	nd	nd	nd	nd	0.05	0.12	nd	nd	0.06	0.08
1090	5-Ethyl-2,3-dimethylpyrazine	MS	136	nd	nd	nd	nd	nd	nd	0.06	0.18	nd	nd	0.08	0.13
1093	2,5-Diethylpyrazine	MS + LRI	136	nd	nd	nd	nd	nd	nd	0.20	0.40	0.01	nd	0.07	0.11
1124	2-Acetyl-6-methylpyrazine	MS + LRI	136	nd	nd	nd	nd	nd	nd	0.07	0.16	0.02	0.01	0.07	0.11
	Furanones														
806	Dihydro-2-methyl-3(2H)-furanone	MS + LRI	100	nd	0.01	0.24	0.76	nd	nd	0.09	0.16	nd	0.03	0.09	0.10
948	Dihydro-3-methlene-5-methyl-2-furanone	MS + LRI	112	nd	nd	nd	nd	0.08	0.09	2.23	2.33	1.09	1.22	2.21	2.58
1055	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	MS	128	nd	nd	0.40	2.10	5.35	2.60	32.91	42.44	9.33	7.35	26.32	25.74
1129	2,5-Dimethyl-4-methoxy-3(2H)-furanone	MS	142	nd	nd	nd	nd	nd	nd	0.46	0.67	0.36	0.43	1.26	1.20
1140	2-Ethyl-4-ethoxy-5-methyl-3(2H)-furanone	MS	142	nd	nd	nd	nd	0.22	0.04	2.21	3.74	0.31	0.15	1.43	1.63

Table 1 continued

LRI[a]	Compound	Identification[b]	m/z[c]	Approximate concentration, mg/mol[d]											
				pH = 5				pH = 7				pH = 9			
				O-5 h[e]	C-0.5 h[e]	C-1.5 h[e]	C-2.5 h[e]	O-5 h[e]	C-0.5 h[e]	C-1.5 h[e]	C-2.5 h[e]	O-5 h[e]	C-0.5 h[e]	C-1.5 h[e]	C-2.5 h[e]
	Furans														
833	Furfural	MS + LRI*	96	0.87	1.98	26.68	42.45	0.10	nd	0.56	0.24	0.09	nd	0.10	0.07
853	2-Furanmethanol	MS + LRI*	98	nd	0.05	1.03	4.68	0.52	0.45	19.77	32.41	2.32	0.54	5.07	8.97
953	5-Methyl-2-furanmethanol	MS + LRI	112	nd	nd	nd	nd	nd	nd	1.68	4.12	0.81	nd	0.95	3.02
1229	5-Hydroxymethyl-2-furaldehyde	MS	126	nd	4.62	75.15	176.74	nd	nd	nd	nd	nd	nd	nd	nd
	Carbocyclic compounds														
908	2-Methyl-2-cyclopentenone	MS*	96	nd	nd	nd	nd	nd	q nd	0.12	0.48	0.05	nd	0.21	0.54
923	2-Hydroxy-2-cyclopenten-1-one	MS + LRI	98	nd	0.03	0.72	1.30	nd	nd	0.26	0.22	nd	nd	0.03	0.09
967	3-Methyl-2-cyclopenten-1-one	MS*	96	nd	nd	nd	nd	nd	nd	nd	0.41	0.08	0.00	0.24	0.82
1029	2-Hydroxy-3-methyl-2-cyclopenten-1-one	MS*	112	nd	nd	0.18	0.63	0.06	0.17	5.60	11.23	3.13	4.69	15.80	18.05
1123	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	MS	126	nd	nd	nd	nd	nd	nd	1.33	2.64	0.12	0.14	1.45	2.26
1112	2,5-Dimethyl-2,5-cyclohexadiene-1,4-dione	MS	136	nd	nd	nd	nd	nd	nd	0.16	0.30	0.08	0.04	0.26	0.36
1120	2,6-Dimethyl-2,5-cyclohexadiene-1,4-dione	MS + LRI	136	nd	nd	nd	nd	nd	nd	0.34	0.54	0.10	0.05	0.45	0.56
	Pyrones														
1149	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	MS	144	0.35	3.32	7.76	13.27	10.37	8.68	3.26	0.16	1.54	5.27	0.48	0.05
1187	3,5-Dihydroxy-2-methyl-4H-pyran-4-one	MS + LRI	142	nd	nd	1.36	1.69	nd	nd	0.10	0.11	nd	nd	nd	nd
	Others														
1049	Benzeneacetaldehyde	MS + LRI*	120	0.06	0.32	1.43	1.44	0.02	0.04	0.20	0.12	0.06	0.10	0.12	0.10
1061	2-Acetylpyrrole	MS + LRI*	109	nd	0.88	7.69	17.58	nd	nd	0.23	0.33	nd	nd	nd	nd
1218	2,3-Dihydro-benzofuran	MS	120	nd	0.02	0.24	0.29	nd	0.03	0.30	0.21	0.03	0.08	0.25	0.14
1360	4-Hydroxy-benzaldehyde	MS	122	nd	2.93	9.71	7.48	nd	nd	2.94	4.88	nd	nd	2.58	3.12
1373	4-Propylphenol	MS	136	81.14	131.14	259.12	276.01	16.09	6.84	15.79	6.75	4.43	7.32	4.98	4.22
1553	1,6-Naphthalenediol	MS	160	nd	0.07	1.16	4.34	1.18	1.10	15.04	40.03	1.09	5.05	29.94	37.28
1655	3-Acetyl-1,6-dimethyl-2,4(1H,3H) pyridinedione	MS	181	nd	nd	nd	nd	nd	0.10	2.49	3.19	0.60	3.84	6.88	6.99
1677	2-Hydroxy-3-(4-hydroxyphenyl)propanoic acid	MS	107	nd	nd	nd	nd	nd	0.11	3.42	10.28	1.21	0.56	4.88	8.89
1779	2-(4-Hydroxyphenyl)ethyl formamide	MS	107	nd	nd	nd	nd	nd	0.00	1.75	9.05	0.28	0.45	4.95	8.83
1921	2,4′-Methylenediphenol	MS	107	nd	nd	nd	nd	nd	nd	0.33	1.72	nd	0.21	1.92	3.92
	Total			82.61	146.09	405.47	578.59	88.90	48.46	366.86	461.67	251.75	162.13	355.05	411.09

[a] Linear retention indices were calculated according to Mailat et al. (1974)

[b] Identification was performed as follows: MS + LRI, mass spectrum was identical with that in Nist mass spectral database, and retention index was consistent with that reported in the literatures; MS, mass spectrum was consistent with that of Wiley mass spectrum database; *, mass spectrum and retention index were consistent with that of authentic compound

[c] characteristic ion used for quantification

[d] mg of volatile/mol of amino acid, means of duplicate experiments were shown, nd, not detected

[e] O-5 h, C-0.5 h, C-1.5 h and C-2.5 h represented four reaction conditions, which were 5-h reaction with reactor unsealed, 0.5-h reaction with reactor sealed, 1.5-h reaction with reactor sealed and 2.5-h reaction with reactor sealed, respectively

Table 2 Influences of pH and reaction time on the volatile formation in the glucose–histidine model system

LRI[a]	Compound	Identification[b]	m/z[c]	Approximate concentration, mg/mol[d]											
				pH = 5				pH = 7				pH = 9			
				O-5h[e]	C-0.5h[e]	C-1.5h[e]	C-2.5h[e]	O-5h[e]	C-0.5h[e]	C-1.5h[e]	C-2.5h[e]	O-5h[e]	C-0.5h[e]	C-1.5h[e]	C-2.5h[e]
	Aliphatic compounds														
<700	1-Hydroxy-2-propane	MS	74	10.84	9.18	50.14	47.87	72.07	46.18	223.51	205.99	89.86	51.33	197.42	181.26
<700	2,3-Pentanedione	MS	100	0.06	nd	0.51	0.84	nd	0.08	0.10	0.17	nd	nd	0.08	0.11
708	3-Hydroxy-2-butanone	MS + LRI	88	nd	nd	nd	0.28	nd	nd	1.20	2.49	nd	0.32	3.90	6.40
765	1-Hydroxy-2-butanone	MS	88	nd	nd	nd	nd	nd	nd	1.12	2.54	nd	0.17	3.21	4.34
927	2,5-Hexanedione	MS*	114	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.14	0.13
1034	4,5-Dimethyl-1,3-dioxol-2-one	MS	114	2.37	nd	2.50	15.34	nd	nd	nd	nd	nd	nd	nd	nd
	Pyrazines														
731	Pyrazine	MS*	80	nd	nd	nd	0.01	nd	nd	nd	0.12	nd	nd	0.04	0.27
822	2-Methylpyrazine	MS + LRI*	94	nd	nd	nd	0.09	nd	nd	0.43	3.14	nd	0.13	2.02	8.65
911	2,5-Dimethylpyrazine	MS + LRI*	108	nd	nd	0.06	0.21	0.37	0.11	5.45	21.19	2.45	3.02	17.46	37.20
916	2-Ethylpyrazine	MS + LRI*	80	nd	nd	nd	nd	nd	nd	nd	0.11	nd	nd	0.05	0.17
999	2-Ethyl-6-methylpyrazine	MS + LRI	122	nd	nd	nd	nd	nd	nd	0.03	0.25	nd	nd	0.10	0.42
1002	2-Ethyl-5-methylpyrazine	MS + LRI	122	nd	nd	nd	nd	nd	nd	0.49	2.21	nd	0.08	1.01	2.25
1093	2,5-Diethylpyrazine	MS + LRI	136	nd	nd	nd	nd	nd	nd	0.02	0.10	nd	nd	0.03	0.05
	Furanones														
806	Dihydro-2-methyl-3(2H)-furanone	MS + LRI	100	0.06	nd	0.63	1.10	nd	nd	0.05	0.10	nd	nd	0.02	0.05
948	Dihydro-3-methlene-5-methyl-3-furanone	MS + LRI	112	nd	nd	nd	nd	nd	nd	0.17	0.46	nd	0.12	0.77	1.03
1055	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	MS	128	0.99	0.45	11.69	21.02	5.17	3.96	65.55	51.83	15.67	26.01	54.15	36.65
1140	2-Ethyl-4-ethoxy-5-methyl-3(2H)-furanone	MS	142	nd	nd	nd	nd	nd	nd	0.66	1.38	nd	nd	0.79	0.96
	Furans														
833	Furfural	MS + LRI*	96	0.61	nd	2.71	4.24	nd	nd	nd	0.06	nd	nd	nd	nd
853	2-Furanmethanol	MS + LRI*	98	nd	nd	1.27	3.74	nd	nd	10.06	28.33	nd	nd	7.51	13.36
953	5-Methyl-2-furanmethanol	MS + LRI	112	nd	nd	0.15	0.09	nd	nd	0.37	2.59	nd	nd	nd	1.00
1229	5-Hydroxymethyl-2-furaldehyde	MS	126	1.42	nd	24.45	77.57	nd	nd	nd	nd	nd	nd	nd	nd

Table 2 continued

LRI[a]	Compound	Identification[b]	m/z[c]	Approximate concentration, mg/mol[d]											
				pH = 5				pH = 7				pH = 9			
				O-5h[e]	C-0.5h[e]	C-1.5h[e]	C-2.5h[e]	O-5h[e]	C-0.5h[e]	C-1.5h[e]	C-2.5h[e]	O-5h[e]	C-0.5h[e]	C-1.5h[e]	C-2.5h[e]
	Carbocyclic compounds														
885	2-Cyclopentene-1,4-dione	MS + LRI*	96	nd	nd	0.39	0.44	nd	nd	nd	nd	nd	nd	nd	nd
908	2-Methyl-2-cyclopenten-1-one	MS*	96	nd	nd	nd	nd	nd	nd	nd	0.12	nd	nd	0.11	0.25
923	2-Hydroxy-2-cyclopenten-1-one	MS + LRI	98	nd	nd	0.26	0.67	nd	nd	nd	nd	nd	nd	nd	nd
967	3-Methyl-2-cyclopenten-1-one	MS*	96	nd	nd	nd	nd	nd	nd	nd	0.07	nd	nd	0.13	0.29
1029	2-Hydroxy-3-methyl-2-cyclopenten-1-one	MS*	112	nd	nd	0.46	0.96	nd	0.06	2.34	4.26	0.27	1.47	16.93	21.50
1123	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	MS	126	nd	nd	nd	nd	nd	0.14	6.75	4.45	nd	0.30	2.96	2.60
1120	2,6-Dimethyl-2,5-cyclohexadiene-1,4-dione	MS + LRI	136	nd	nd	nd	nd	nd	nd	0.03	0.09	nd	nd	0.10	0.13
	Pyrones														
1149	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	MS	144	22.27	22.57	41.12	44.58	9.34	4.35	4.43	0.81	2.05	2.53	0.24	0.04
1187	3,5-Dihydroxy-2-methyl-4H-pyran-4-one	MS + LRI	142	0.12	nd	0.30	16.32	nd	nd	0.24	0.18	nd	nd	nd	nd
	Others														
1061	2-Acetylpyrrole	MS + LRI*	109	4.17	1.47	15.71	27.31	nd	nd	nd	0.13	nd	nd	nd	0.11
1557	2-Acetylpyrido[3,4-d]imidazole	MS + LRI	161	1.18	nd	11.19	120.93	113.84	6.24	246.96	301.95	481.32	91.06	540.11	568.08
1663	1H-Benzimidazol-2-ylmethanamine	MS	147	0.06	nd	3.40	16.92	2.78	1.06	44.92	46.14	10.31	4.04	51.34	51.50
	Total			44.14	33.66	166.93	400.55	167.35	62.16	614.87	681.26	601.94	180.60	900.61	938.78

[a, b, c, d, e] of the same meaning with those shown in Table 1

showed maximal quantity at pH = 9 in 2.5-h close reaction except for 2,5-diethylpyrazine which was produced more at pH = 7 in 2.5-h close reaction. In Glc–Tyr system, pyrazine, 2-methylpyrazine and C2-pyrazine (side chain containing two carbons in total) reached maximum at pH = 9 under 2.5-h close reaction, while the largest content of C4-pyrazine was observed at pH = 7 under 2.5-h close reaction, and C3-pyrazine showed maximal quantity at pH = 7 or pH = 9. The above observation demonstrated that the alkaline condition was favorable to the formation of pyrazines with short-side chain, and the generation of pyrazines with long-side chain was favored under neutral condition.

Furans

Four furan compounds were detected in this study, and 5-hydroxymethyl-2-furaldehyde was the main compound (Tables 1, 2). 5-Hydroxymethyl-2-furaldehyde was formed from the cyclization of 3-deoxyhexose, which was generated from the 1,2-enolization of Amadori compound in acidic condition [31]. It was noteworthy that acidic condition was not favorable to the generation of all furan compounds. Tables 1 and 2 showed that furfural and 5-hydroxymethyl-2-furaldehyde reached maximum under acidic condition, while 2-furanmethanol and 5-methyl-2-furanmethanol showed the largest quantity under neutral condition and the least quantity under acidic condition. Besides, total furans increased over reaction time regardless of pH level, and larger quantity of furans was observed in Glc–Tyr system, similar with pyrazines.

Pyrones

3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one was considered to be an effective antioxidant [35] and was the major pyrone under our experimental conditions. As shown in Tables 1 and 2, pyrones reached maximal content at pH = 5 in 2.5-h close reaction for both Glc–Tyr and Glc–His systems, with higher amount observed in Glc–Tyr system, contrary to pyrazines and furans. Besides, in both Glc–Tyr and Glc–His systems, pyrones showed increasing tendency over reaction time in acidic conditions, while opposite tendency was observed in neutral and alkaline conditions, implying pyrones might undergo different pathways at different pH levels.

Furanones

2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF), the major furanone identified in Tables 1 and 2, was an important flavor compound with an intense caramel-like aroma and generally formed from 1-deoxyosone via acetylformoin reduction [36]. 1-Deoxyosone was derived from the 2,3-enolization of Amadori compound at pH $\geq$7 [31]. Thus, larger amount of DMHF was detected in neutral and alkaline conditions. The data shown in Tables 1 and 2 illustrated that neutral condition was most favorable to the formation of furanone at particular reaction time. Additionally, there was more production of furanones in Glc–His system than that in Glc–Tyr system, especially under acidic condition, suggesting the reactivity of amino acid in furanone generation was related with pH.

Others

Phenols were tyrosine-specific reaction products, and different phenol compounds showed maximal quantity at different pH levels, such as 4-propylphenol at pH = 5, 2-hydroxy-3-(4-hydroxyphenyl)propanoic acid at pH = 7 and 2,4′-methylenediphenol at pH = 9 (Table 1). Taking into account the major part of 4-propylphenol in phenols, total phenols showed maximal content in acidic condition. Equivalent content of phenols was observed under neutral and alkaline conditions. 2-Acetylpyrido[3,4-d]imidazole and 1H-benzimidazol-2-ylmethanamine were histidine-specific reaction products (Table 2), which showed increasing tendency with increasing pH level and increasing heating time. 2-Acetylpyrido[3,4-d]imidazole was formed via the condensation of pyruvic aldehyde with the Strecker degradation product of histidine [37]. 2-Acetylpyrrole was detected in both Glc–Tyr and Glc–His systems, and its production was favored under acidic condition.

For overall analysis of all volatiles formed under different conditions, factor analysis was employed in this paper with the result shown in Fig. 1. In both Glc–Tyr and Glc–His systems, factor 1 and factor 2 expressed most of the total variance, implying two factors were enough to interpret all data. It was directly observed from Fig. 1c, d that with the extension of reaction time, volatiles in neutral condition followed the similar tendency with those in alkaline condition which was quite different from that in acidic condition. This observation was coherent with the mechanism of Maillard reaction [17]. Besides, based on the observation that the points representing 1.5-h close reaction were closer to the points representing 2.5-h close reaction than to the points representing 0.5-h close reaction, it could be demonstrated that the change of volatile composition decreased with increasing time, especially under neutral and alkaline conditions.

In Glc–Tyr system, pyrazines, cyclopentenones, pyridines, furanones and aliphatics, which were of higher loadings at factor 1, represented most important information of factor 1, while phenols, furans and 2-acetylpyrrole of higher loading at factor 2 represented most important information of factor 2 (Fig. 1a). Based on the interpretation of factor 1&2, the following conclusions could be given from Fig. 1c. Dash lines

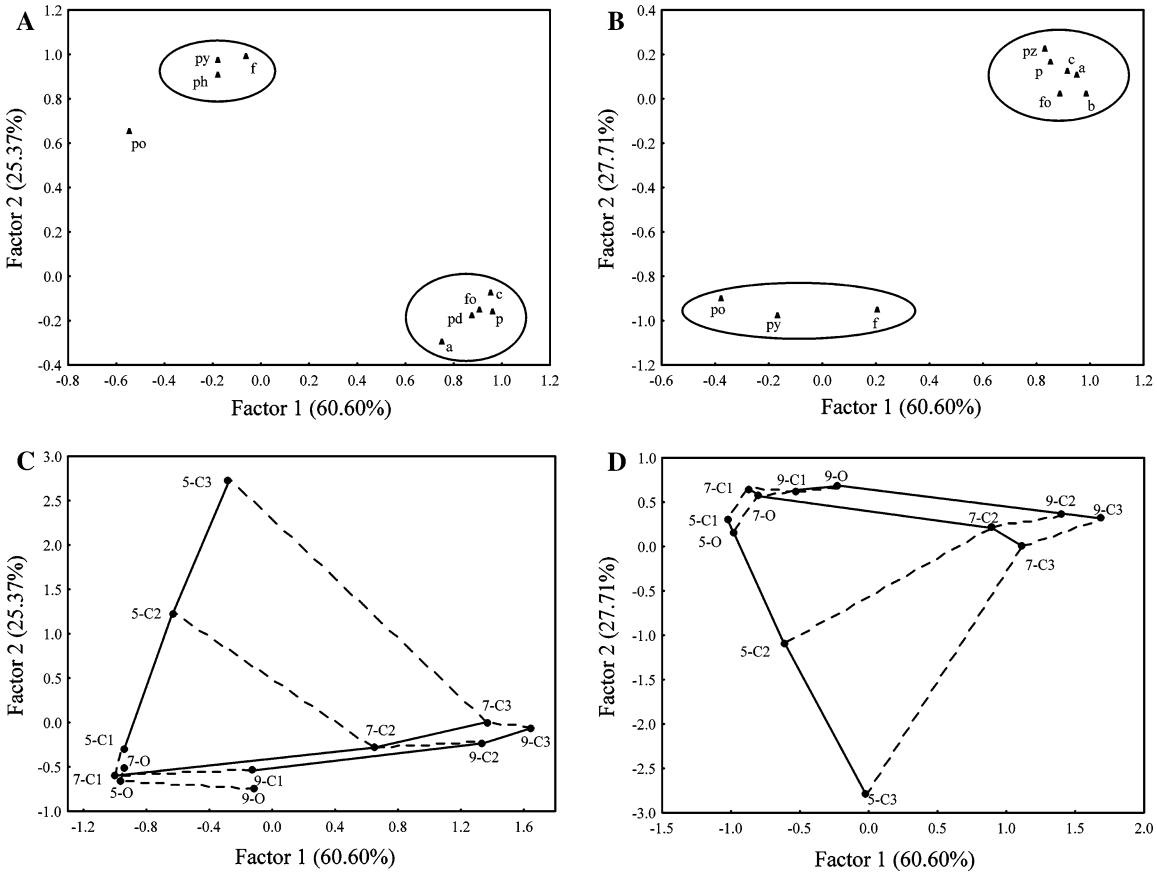

Fig. 1 Plots of factor loadings (**a**, **b**) and factor scores (**c**, **d**) in factor analysis for the volatile MRPs prepared under different conditions in Glc–Tyr (**a**, **c**) and Glc–His (**b**, **d**) systems (*a* aliphatic compounds, *c* cyclopentenones, *p* pyrazines, *f* furans, *po* pyrones, *fo* furanones, *py* pyrroles, *pd* pyridines, *ph* phenols, *b* 1H-benzimidazol-2-ylmethanamine, *pz* 2-acetylpyrido[3,4-d]imidazole; 5-O, 7-O, 9-O, representing 5-h open reaction at pH = 5, 7 and 9; 5-C1(C2/C3), 7-C1(C2/C3), 9-C1(C2/C3), representing 0.5 (1.5/2.5) h-close reaction at pH = 5, 7 and 9)

representing 1.5-h and 2.5-h close reaction showed increasing tendency along first dimension and decreasing tendency along second dimension, illustrating that under 1.5-h or 2.5-h close reaction, pyrazines, cyclopentenones, pyridines, furanones and aliphatics were favored under alkaline condition, while phenols, furans and 2-acetylpyrrole were favored under acidic condition. Solid lines demonstrated that volatiles increased over reaction time regardless of pH level; however, larger increase was observed in acidic condition for phenols, furans and 2-acetylpyrrole, while pyrazines, cyclopentenones, pyridines, furanones and aliphatics showed larger increase in neutral and alkaline conditions. It was noteworthy that not all volatiles increased over reaction time. Pyrones were of negative loadings at factor 1 and decreased with increasing time under neutral and alkaline conditions, in consistent with the previous discussion.

In Glc–His system, factor 1 could be used to interpret 1H-benzimidazole-2-ylmethanamine, 2-acetylpyrrole, aliphatic compounds, cyclopentenones, furanones, pyrazines and 2-acetylpyrido[3,4-d] imidazole, which showed higher loadings at factor 1, while factor 2 could be applied in the explanation of pyrones, furans and 2-acetylpyrrole, which showed substantial negative loadings at factor 2 (Fig. 1b). In view of the interpretation about factors, it was found from Fig. 1d that: (1) influence of pH on the volatile generation was negligible in the early stage of Maillard reaction as points representing 0.5-h close and 5-h open reaction under different pH levels were close to each other; (2) in close system, volatiles increased over reaction time regardless of pH level; however, 1H-benzimidazole-2-ylmethanamine, 2-acetylpyrrole, aliphatic compounds, cyclopentenones, furanones, pyrazines and 2-acetylpyrido[3,4-d] imidazole, which were favored under alkaline condition as illustrated through dash lines, showed larger increase over reaction time in alkaline condition, while larger increase was observed in acidic condition for pyrones, 2-acetylpyrrole and furans, which were favored under acidic condition as illustrated from dash lines.

DPPH radical scavenging activity of volatile MRPs

Generally, the antioxidant activity of volatile MRPs was measured by the oxidation of heptanal to heptanoic acid

[38–40], and there was no report on the radical scavenging activity of volatile MRPs to the best of our knowledge. Considering that DPPH radical is a free radical and acquired directly without preparation as well as the dissolvability in organic solvent, DPPH radical in ethanol solution was employed in this study to estimate the antioxidant activity of volatile MRPs. That is, the dichloromethane extract of model solution which was concentrated prior to GC–MS analysis was allowed to react with DPPH radical. Preliminary experiments showed internal standard (phenyl ethyl acetate), and dichloromethane had no DPPH radical scavenging activity, suggesting that there was no interruption of internal standard and dichloromethane on the measurement of DPPH radical scavenging activity of volatile MRPs when using the extract solution mentioned above as testing samples.

The DPPH radical scavenging activity of volatile MRPs prepared under different conditions was shown in Fig. 2, from which it was found that volatile MRPs showed increasing radical scavenging activity over reaction time in close system regardless of amino acid and pH level. Besides, in consistent with volatile composition, volatile MRPs prepared from 5-h open reaction showed equivalent radical scavenging activity of those from 0.5-h close reaction. Based on the comparison of Fig. 2a–c, it was demonstrated that the influence of pH on the antioxidant activity of volatile MRPs was associated with amino acid. In Glc–Tyr system, volatile MRPs generated in alkaline condition showed the highest radical scavenging activity that was barely higher than those in neutral condition especially under close reaction for 1.5 or 2.5 h. In Glc–His system, volatile MRPs showed decreasing radical scavenging activity with increasing pH, which was more significant in 5-h open or 0.5-h close reaction. Considering the discrepancy between volatile composition and their DPPH radical scavenging activity as affected by pH and reaction time, it could be deduced that not all volatiles showed radical scavenging activity or not all volatiles showed the equivalent radical scavenging activity. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one and 2-acetylpyrrole were reported to be an effective antioxidant in literatures [35, 39, 40], and such compounds need further investigation on the contribution to the antioxidant activity of total volatile MRPs.

Conclusions

The data obtained in our experiment as well as the factor analysis showed clearly that in both Glc–Tyr and Glc–His systems, volatiles generated in 5-h open system were of similar composition to those in 0.5-h close system regardless of pH level, implying higher pressure in close system could accelerate the volatile formation in Maillard reaction. Besides, with the extension of reaction time, volatiles

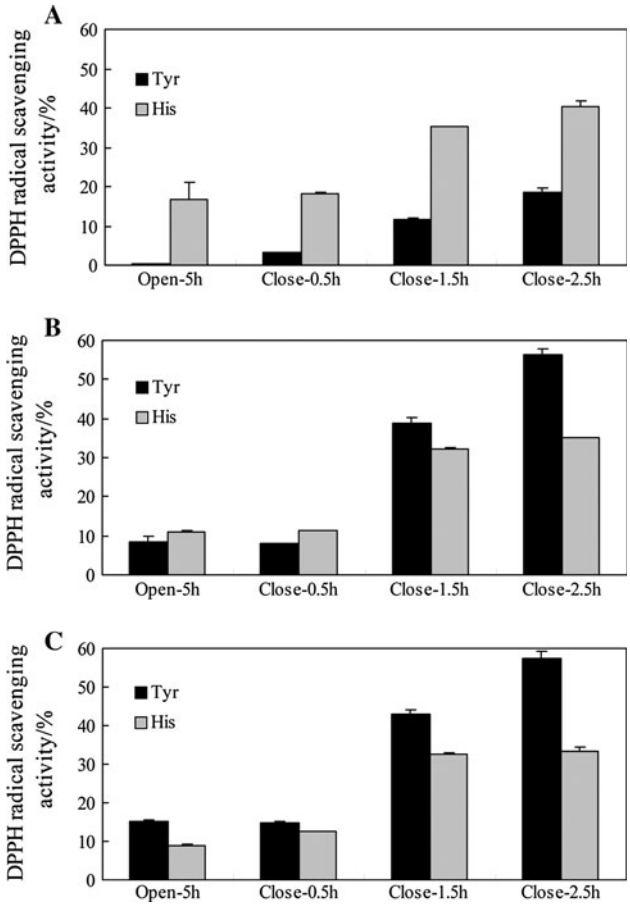

Fig. 2 Influences of pH and reaction time on the DPPH radical scavenging activity of volatile compounds prepared from Glc–Tyr/His model systems (**a**: pH = 5; **b**: pH = 7; **c**: pH = 9)

generated in neutral and alkaline conditions showed similar change pattern that was quite different from that observed in volatiles derived from acidic condition, especially for pyrones that showed decreasing tendency over reaction time under neutral and alkaline conditions. Additionally, most volatiles were favored under alkaline condition with the exception of pyrones, furans, phenols and 2-acetylpyrrole, which were favored under acidic condition. As for the measurement on the antioxidant activity, volatile MRPs prepared from Glc–Tyr and Glc–His systems possessed appreciable DPPH radical scavenging activity.

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Conflict of interest The authors declare that there are no conflicts of interest.

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