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Volatiles from the Maillard Reaction of L-Ascorbic Acid and L-Alanine at Different pHs

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Abstract The volatiles formed from the reactions of L-ascorbic acid with L-alanine at 5 different pH (5, 6, 7, 8, or 9) and 140±2°C for 2 hr was performed using solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) analysis were identified to be 25 different kinds. The reaction between L-ascorbic acid and L-alanine led mainly to the formation of pyrazines. Many of these were alkylpyrazines, such as 3-ethyl-2,5-dimethylpyrazine, 2,5-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 3,5-diethyl-2-methylpyrazine, methylpyrazine, 2-ethyl-6-methylpyrazine, and 2,3-diethyl-5-methylpyrazine, other compounds identified were furans, phenols, benzoquinones, 2,4,6-trimethylpyridine, and 2-methylbenzoxazole. The studies showed that furans, such as furfural and benzofuran were formed mainly at acidic pH. In contrast, higher pH values could promote the production of pyrazines.

Keywords: Maillard reaction, ascorbic acid, flavor, alanine, pyrazine

Introduction

The Maillard reaction is, together with lipid oxidation, without doubt the most important source for aroma compounds generated when food is cooked, baked, or roasted. The flavor industry, too, makes use of the Maillard reaction to produce meatlike, cocoalike, and other process flavors. After reducing carbohydrates, L-ascorbic acid (ASA) appears to be the most widely studied carbonyl component in the processes of non-enzymatic browning. This is due, on the one hand, to its significant presence in food products and, on the other, to the interesting chemical transformations it undergoes in the course of these processes. A series of researches on the behavior of ASA in the presence of amino acids via the Maillard reaction is reported in the literature (1-16).

But, the formation of volatiles is less of research findings. In the reaction of L-dehydroascorbic acid with ammonia and glycine, 5 alkylpyrazines were identified (2). Five derivatives of imidazole were found in the reaction mixture of ASA and ammonia/glycine (8). Rogacheva *et al.* (11) reported aroma compounds formation in the interaction of ASA with glycine/lysine/glutamic acid and Seck and Crouzet (13) reported formation of volatile compounds in ASA-phenylalanine model systems during heat treatment. Recently, Maillard reaction experiments of ASA with L-threonine/L-serine had been performed in our laboratory (16). Up to now, there was only one paper related to formation of aroma compounds in the model reactions of ASA with L-alanine (Ala) (1). Adams and De Kimpe (1) reported formation of 10 pyrazines produced by heating a model reactions of ASA with Ala under dry-roasting conditions in the presence of K_2CO_3.

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A number of factors influence the generation of flavors by the Maillard reaction. The pH at which the reaction is conducted, greatly influences the nature of the volatiles formed and, hence, the flavor of the final product and the reaction temperature and time mainly influence the kinetics of the reaction, while leaving the nature of the volatiles broadly unchanged (17). High temperature is a favorable condition for Maillard reaction (18). Solid-phase microextraction (SPME) is a suitable technique for the analysis of flavor release from Maillard reaction solutions (9). The present study is undertaken to study the effect of pH on the volatiles that are formed in the Maillard reaction between Ala and ASA. Experiments were performed 2 hr at 140±2°C in pH 5, 6, 7, 8, and 9 aqueous solutions. Volatiles were extracted by SPME.

Materials and Methods

Reagents L-Alanine (Ala) were from Shanghai Yuanju Biological Technology Co., Ltd. (Shanghai, China). L-Ascorbic acid (ASA), Na_2HPO_4, NaH_2PO_4, and NaOH were of analytical grade (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). Authentic samples for use as Co-GC were from J & K Chemical Ltd. (Beijing, China). C5-C22 *n*-alkanes were from Pure Chemical Analysis Co., Ltd. (Bornew, Belgium).

Model reaction of Ala with ASA Four mmol ASA was dissolved in 40 mL of phosphate buffer (0.2 mol/L), and the pH of the solution was adjusted to 5.00, 6.00, 7.00, 8.00, or 9.00 using NaOH. Four mmol Ala was added the solution. The mixtures was then sealed in a 48 mL Synthware® glass vials (Beijing Synthware Glass, Inc, Beijing, China) and heated while stirring at 140±2°C for 2 hr in an oil bath. The reactions were immediately stopped by cooling under a stream of cold water and then adjusted samples to pH 7 before SPME analysis.

Headspace-SPME-gas chromatography-mass spectrometry (GC-MS) The assayed fibers were divinylbenzene/carboxen/--polydimethylsiloxane (DVB/CAR/PDMS) (50/30 μm thickness) (Supelco, Bellefonte, PA, USA). Before the SPME fiber was inserted into the vial, the sample was equilibrated for 15 min at 40°C. The extraction time is 50 min at 40°C.

Analyzes were performed using a Agilent 6890N gas chromatograph coupled to a Agilent 5975i mass selective detector (Agilent Technologies Inc., Santa Clara, CA, USA). Volatiles were separated using a DB-5 capillary column (30 m×0.25 mm i.d×0.25 μm). The SPME fiber was desorbed and maintained in the injection port at the oven temperature (250°C) and for the time (4.0 min) suggested by the manufacturer. The injection port was in split mode and split ratio was 1:30. The temperature program was isothermal for 5 min at 40°C, raised to 260°C at a rate of 5°C/min and then raised to 280°C at a rate of 15°C/min and held for 1 min. C5-C22 *n*-alkanes were run under the same chromatographic conditions as the samples to calculate the linear retention indices (LRI) of detected compounds. The transfer line to the mass spectrometer was maintained at 280°C. The mass spectra were obtained using a mass selective detector by electronic impact at 70 eV, a multiplier voltage of 1,753 V, and collecting data at a rate of 1 scan/sec over the *m/z* range of 30-400 u.m.a. Compounds were identified by comparing their mass spectra with those contained in the Nist05 and Wiley275 libraries and by comparison of their LRI with those reported in the literature, as well as, whenever possible, co-injection with authentic samples (Co-GC) available in our laboratories. Area counts of volatiles were provided by integrating in initial threshold 16.5 using Agilent chemstation. Analysis of each tested condition was repeated twice and expressed as mean value ±standard deviation (SD)

Results and Discussion

Volatile compounds of the Maillard reaction system Total ion chromatogram of volatiles produced by heating a model system containing ASA with Ala at pH 8 is shown in Fig. 1. The major headspace components at 5 different pHs (5, 6, 7, 8, and 9) are listed in Table 1, according to their elution order. The 25 compounds presented were those which gave significant peaks in GC-total ion chromatography (TIC). They can be classified as 15 pyrazines, 4 furans, 2 phenols, 2 benzoquinones, 2,4,6-trimethylpyridine, and 2-methylbenzoxazole. The identifications were achieved by comparing their mass spectra with those contained in the Nist05 and Wiley275 libraries and comparing their LRI with published data (much of these data are from http://webbook.nist.gov/chemistry/), compound No. 1, 2, 3, 4, 5, 14, 15, 18, 23, 24, and 25 (Table 1) also by means of Co-GC. The MS of compound No. 7 and 8; compound No. 10, 11, and 12; compound No. 15, 16, and 17; and compound No. 20 and 21 were very similar. The identifications of compound No. 7 and 8 were achieved by their elution order on DB-5 according to literature (19); compounds No. 10, 11, and 12 were by their elution order on HP-5MS according to literature (20); compound No. 15, 16, and 17 were by their elution order on HP-5 according to literature (21); and compound No. 20 and 21 were by their elution order on DB-5 according to literature (22). 2,5-Furandicarbaldehyde and triethylmethylpyrazine were tentatively identified. Identified compounds can corroborate with each other at different pHs.

As mentioned above, Adams and De Kimpe (1) reported 10 pyrazines produced by heating a model reaction of ASA with Ala under dry-roasting conditions in the presence of K_2CO_3. Among the identified 10 pyrazines in a model reaction of ASA with Ala under dry-roasting conditions in

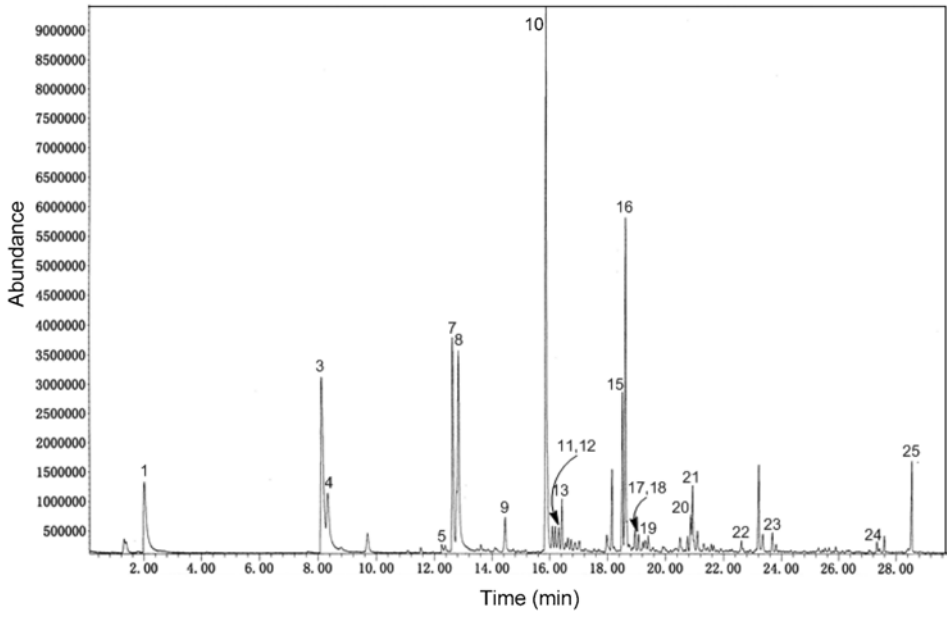

Fig. 1. Total ion chromatogram of volatiles in the Maillard reaction system at pH 8 and 140±2°C for 2 hr. Peak numbers correspond to Table 1.

Table 1. Effect of pH on the formation of volatiles in the Maillard reaction system (GC-TIC peak areas×10^6)

No.[1)]	Compound	Mw	LRI[2)]	Identification	pH 5	pH 6	pH 7	pH 8	pH 9
1	Methylpyrazine	94	811±4.8	MS, LRI, Co-GC[3)]	2.1±0.2[4)]	21.6±2.4	76.4±7.6	83.3±7.5	77.7±10.1
2	Furfural	96	821±1.4	MS, LRI, Co-GC	61.9±8.7	5.6±0.8	ND	ND	ND
3	2,5-Dimethylpyrazine	108	902±4.9	MS, LRI, Co-GC	42.8±6.8	83.8±16.7	137.7±22.0	174.5±26.2	170.9±17.1
4	Ethylpyrazine	108	905±5.1	MS, LRI, Co-GC	ND	21.8±4.6	57.4±10.9	69.2±6.2	54.2±6.5
5	2,4,6-Trimethylpyridine	121	981±2.8	MS, LRI, Co-GC	ND	ND	ND	4.4±0.8	3.0±0.5
6	Benzofuran	118	986±0.7	MS, LRI	11.5±0.7	4.9±0.63	ND	ND	ND
7	2-Ethyl-6-methylpyrazine	122	988±2.3	MS, LRI	4.4±0.4	11.6±0.4	69.8±9.1	121.0±13.3	135.3±10.8
8	2-Ethyl-5-methylpyrazine	122	992±2.2	MS, LRI	45.5±5.9	89.0±11.6	146.8±11.7	131.7±4.1	144.4±7.2
9	2,5-Furandicarbaldehyde	124	1,031±1.8	MS	21.2±3.4	19.2±2.7	20.1±2.2	23.9±5.0	22.3±2.0
10	3-Ethyl-2,5-dimethylpyrazine	136	1,069±1.1	MS, LRI	113.4±6.8	184.9±12.9	269.9±16.2	305.2±21.4	300.5±24.0
11	2-Ethyl-3,5-dimethylpyrazine	136	1,075±1.9	MS, LRI	2.9±0.2	5.0±0.6	10.3±1.2	15.1±1.2	14.1±2.1
12	5-Ethyl-2,3-dimethylpyrazine	136	1,077±1.2	MS, LRI	ND	4.9±0.9	10.1±1.6	14.1±1.3	13.4±1.5
13	2,5-Diethylpyrazine	136	1,083±1.1	MS, LRI	5.7±1.1	13.9±2.2	24.1±2.2	26.6±3.9	23.8±1.4
14	2-Methylbenzoxazole	133	1,105±1.4	MS, Co-GC	ND	2.2±0.2	1.6±0.2	ND	ND
15	2,3-Diethyl-5-methylpyrazine	150	1,144±0.5	MS, LRI, Co-GC	22.7±2.0	42.5±6.8	63.9±1.9	67.5±8.1	56.0±7.3
16	3,5-Diethyl-2-methylpyrazine	150	1,147±0.5	MS, LRI	45.0±2.7	86.5±12.1	138.5±8.3	143.8±5.8	118.0±16.5
17	3,5-Dimethyl-2-propylpyrazine	150	1,151±0.6	MS, LRI	ND	2.0±0.3	4.9±0.7	3.0±0.2	5.3±1.0
18	3,4-Dihydroxybenzaldehyde	138	1,165±0.6	MS, Co-GC	ND	ND	3.1±0.3	4.4±0.4	4.3±0.6
19	1-Furfurylpyrrole	147	1,171±0.8	MS, LRI	6.0±0.8	12.5±1.1	10.2±1.1	6.7±1.2	6.4±0.6
20	2,6-Diethyl-3,5-dimethylpyrazine	164	1,214±0.6	MS, LRI	ND	ND	4.9±0.7	7.4±0.3	5.6±0.8
21	2,5-Diethyl-3,6-dimethylpyrazine	164	1,217±0.6	MS, LRI	ND	ND	8.0±1.2	15.6±1.7	11.5±0.5
22	Triethylmethylpyrazine	178	1,279±0.7	MS	ND	ND	ND	5.6±0.7	3.7±0.6
23	2,3,5,6-Tetramethyl-*p*-benzoquinone	164	1,316±0	MS, Co-GC	ND	ND	6.2±1.1	9.9±0.9	7.3±1.1
24	2,6-di-*tert*-Butyl-*p*-benzoquinone	220	1,458±0	MS, LRI, Co-GC	ND	1.9±0.3	3.1±0.5	2.4±0.3	1.8±0.2
25	2,6-di-*tert*-butyl-*p*-cresol (BHT)	220	1,502±0	MS, LRI, Co-GC	8.3±0.8	15.6±1.4	17.9±1.6	36.5±5.8	22.4±1.1
Total volatiles (mean values)					393.4	629.4	1,084.9	1,271.8	1,201.9

[1)]No. correspond to Fig. 1.
[2)]Calculated for a DB-5 capillary column; Mean±SD.
[3)]Co-GC, co-injection with authentic sample.
[4)]Mean±SD; ND, not detected.

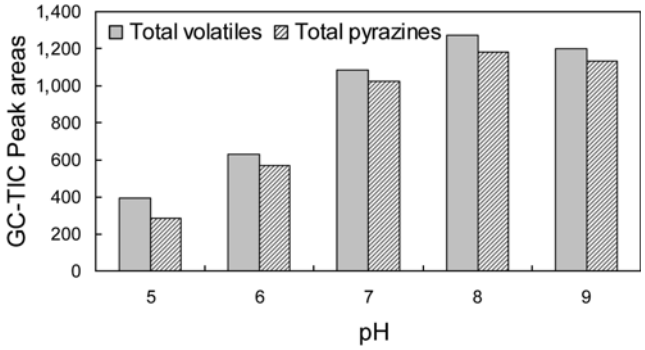

Fig. 2. Total volatiles and total pyrazines generation in pH 5, 6, 7, 8, and 9 aqueous solutions in the Maillard reaction system. Total GC-TIC peak areas×10^6.

the presence of K_2CO_3, there were 8 pyrazines in accordance with our studies. But they did not report 2,5-dimethylpyrazine, 2,6-diethyl-3,5-dimethylpyrazine, ethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 5-ethyl-2,3-dimethylpyrazine, 2,5-diethylpyrazine, and 3,5-dimethyl-2-propylpyrazine, and 4 furans, 2 phenols, 2 benzoquinones, 2,4,6-trimethylpyridine, and 2-methylbenzoxazole. Whereas, trimethylpyrazine and 2,3,5-triethyl-6-methylpyrazine were not identified in our studies. This could be attributed to the different reaction conditions, as for instance, with or without solvent, or pH, reaction temperature, and reaction time.

Effect of pH on the volatiles of the Maillard reaction system A clear tendency was observed for some classes of compounds to be formed more at higher or lower pH. In Fig. 2, the formation of total volatiles and total pyrazines was compared; the total volatiles and total pyrazines increased significantly from pH 5 to 8. The results indicated that high pH conditions favored the formation of total volatiles. Compounds 5, 18, 20, 21, 22, and 23 (Table 1) were detected only when the reaction was carried out at pH 7, 8, and 9, and not under more acidic conditions. The quantities of compounds 1, 3, 4, 7, 8, 10, 11, 12, 13, 15, 16, and 25 (Table 1) increased with increasing pH value. On the other hand, furfural and benzofuran were formed only at acidic pH, and no traceable amounts were detected at pH 7.0. The pH had no obvious influence on the formation of 2,5-furandicarbaldehyde. Among all detected compounds in the model reaction system of Ala with ASA, we should pay most attention to the 15 pyrazines, which were main volatiles. The most predominant pyrazine compound in the model reaction system of Ala with ASA was 3-ethyl-2,5-dimethylpyrazine, which was also the most predominant in a model reactions of ASA with Ala under dry-roasting conditions in the presence of K_2CO_3 (1). High pH values could promote the production of pyrazines. While the pH of reaction solution was higher than 6, the total pyrazines increased obviously in the model system involving Ala with ASA. The main pyrazines are methylpyrazine, 2,5-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, 3-ethyl-2,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, and 3,5-diethyl-2-methylpyrazine generation in pH 5, 6, 7, 8, and 9 aqueous solutions by heating a model system containing Ala with ASA (Table 1). Many pyrazines were identified from the reaction of Ala with ASA from pH 7 to 9. The thermal degradation of ASA can produce many carbonyl compounds (23). The base catalysis was probably due both to the increased reactivity of the amino group of the amino acid toward the carbonyl and to the increased rearrangement and fragmentation of ASA (24). These findings support an earlier observation that pH has a great influence on volatile compounds formed in Maillard type reactions.

Possible formation pathways of main volatiles Pyrazine is one of the most important groups among the identified volatiles in the studied systems. They are widely distributed in food systems, especially foods processed at high temperatures and low water environment. Pyrazines may contribute to the toasted, roasted, nutty, and burnt notes. The review on pyrazines in food had been published (25). There is a precursor or pathway for pyrazine compounds generation. The α-amino carbonyls, which can be formed from the reactions between dicarbonyl compounds and amino acids during Strecker degradation, are generally considered to be the precursors of pyrazines. The dicarbonyl compounds such as ethylglyoxal, butanedione, glyoxal, and pyruvaldehyde can be produced by thermal degradation of ASA (23). These α-amino carbonyls may react with each other to generate pyrazines during thermal processing (26). The concentration of 3-ethyl-2,5-dimethylpyrazine is the highest in the model reaction system, and 2,5-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 3,5-diethyl-2-methylpyrazine, 2-ethyl-6-methylpyrazine, methylpyrazine, and 2,3-diethyl-5-methylpyrazine are higher than other pyrazines. Dihydropyrazines are postulated as intermediate products of pyrazine formation. According to Shibamoto *et al.* (27), 2,5-dimethyldihydropyrazine combines with acetaldehyde, which finally results in 3-ethyl-2,5-dimethylpyrazine. Acetaldehyde for the reaction with dihydropyrazines results from a Strecker degradation of Ala (28). The dihydropyrazines can be formed through condensation of 2 α-aminocarbonyl compounds, which result from the Strecker reaction of amino acids and α-dicarbonyl compounds such as pyruvaldehyde. The reaction route to 2-ethyl-5-methylpyrazine and 2-ethyl-6-methylpyrazine is in the first step methyldihydropyrazine is formed from aminoacetone and aminoacetaldehyde, which result from the Strecker reaction of pyruvaldehyde and glyoxal, respectively. Acetaldehyde reacts with the methyldihydropyrazine, finally resulting in 2 ethyldimethylpyrazine isomers (28). 2,5-Dimethylpyrazine, 3,5-diethyl-2-methylpyrazine, methylpyrazine, and 2,3-diethyl-5-methylpyrazine have similar formation pathways. Furfural (23), 2,6-di-*tert*-butyl-*p*-cresol (23), and benzofuran (29) are products from thermal degradation of ASA. Furfural and benzofuran were formed only at acidic pH and 2,6-di-*tert*-butyl-*p*-cresol increased with increasing pH, reached a maximum at pH 8. This could be attributed to the different thermal degradation mode of ASA at different pHs. However, these hypotheses need further studies.

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