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Formation of pyrazines from ascorbic acid and amino acids under dry-roasting conditions

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ABSTRACT

Although the participation of ascorbic acid in Maillard-type reactions has been described, the formation of flavour compounds resulting from the interaction of ascorbic acid with different amino acids has not been reported before. Therefore, the formation of flavour compounds from the model reactions of 20 amino acids with ascorbic acid was studied under dry-roasting conditions. Thirty-six different pyrazines were identified, mostly ethyl and methyl substituted pyrazines. The amounts of pyrazines detected were comparable to those formed from pentose sugars. Lysine was the most reactive amino acid and yielded the highest amounts of alkylpyrazines. The reducing activity of ascorbic acid influenced the reaction mechanism of pyrazine formation and thus the type of pyrazines produced. Addition of a base, such as potassium carbonate, significantly enhanced pyrazine formation from ascorbic acid for most amino acids. The formation of pyrazines from serine and threonine without a carbonyl compound was greatly enhanced by the addition of potassium carbonate as well. Furan was detected in all model systems in relatively low amounts and its formation was not enhanced by the addition of potassium carbonate.

1. Introduction

L-(+)-Ascorbic acid (vitamin C) is a common ingredient of the human diet, occurring especially in fruit and vegetables, herbs, and to a lesser extent in meat (liver). In addition, ascorbic acid is frequently used as a food additive, as an antioxidant and as a flour improver in bakeries. However, ascorbic acid is relatively unstable under common storage and processing conditions, such as heat, oxygen and exposure to heavy metal ions (Belitz, Grosch, & Schieberle, 2004). Understanding the mechanisms of ascorbic acid degradation in foods is of high importance.

The decomposition of ascorbic acid is typically classified in two pathways, a nonoxidative pathway and an oxidative pathway, initiated with the oxidation of L-ascorbic acid to dehydro-L-ascorbic acid. Under nonoxidative conditions, ascorbic acid undergoes a spontaneous decarboxylation and dehydration to form 3-deoxy-L-pentos-2-ulose, which cyclises to furfural (Kurata & Sakurai, 1967a). Under oxidative conditions as well, furfural was identified, together with L-*threo*-pentos-2-ulose, 3-deoxy-*erythro*-pentos-2-ulose-1,4-lactone and 1,2-butanedione (Kurata & Sakurai, 1967b). Velísek, Davídek, Kubelka, Zelinková, and Pokorný (1976) identified ten substituted furans upon thermal degradation of ascorbic acid and dehydroascorbic acid. Feather and co-workers studied the degradation of ascorbic acid in the absence and presence of *N*-acylated lysine. They showed that threose is formed via the oxidative pathway, reacts with amino groups and degrades further to 3-deoxytetros-2-ulose and glyceraldehyde (Lopez & Feather, 1992; Li & Feather, 1992). Upon dry thermal degradation of ascorbic acid at 300 °C, mainly substituted furans and α,β-unsaturated cyclic ketones with a five-membered ring were identified (Vernin, Chakib, Rogacheva, Obretenov, & Parkanyi, 1998).

Many of these carbonyl compounds, reactive intermediates and furan derivatives are the same as those obtained in the Maillard reaction or nonenzymatic browning. The Maillard reaction includes a very complex set of reactions, initiated with the condensation reaction between a reducing sugar and an amino compound. It is of high importance in food processing, since it results in a multitude of reaction products, ranging from volatile flavour compounds to brown-coloured polycondensation products, called melanoidins (Nursten, 2005). The participation of ascorbic acid and its degradation products in the Maillard reaction has been shown before. For instance, the development of browning in the reaction of ascorbic acid with 20 amino acids was studied in function of different parameters (temperature, time, additives) (Yu, Wu, Wang, & Salunkhe, 1974). The reaction of dehydro-L-ascorbic acid with amino acids is known to give browning as well. In this case, Strecker degradation of dehydro-L-ascorbic acid with an α-amino acid yields the amino reductone scorbamic acid, which upon condensation with dehydro-L-ascorbic acid gives a red pigment (Kurata, Fujimaki, & Sakurai, 1973). These reactions can initiate unwanted browning in citrus juices and dried fruits. The formation and properties of high molecular weight melanoidins prepared with ascorbic acid have been studied in model systems (Adams, Abbaspour Tehrani,

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Kersiene, Venskutonis, & De Kimpe, 2003; Davies & Wezicha, 1994; Obretenov et al., 2004; Rogacheva, Kuntcheva, Panchev, & Obretenov, 1999). In addition, it has been shown that ascorbic acid and its reactive oxidation products are involved in the glycation of proteins, which is especially important in the development of cataract by ageing of lens proteins (Fan et al., 2006).

To the best of our knowledge, however, no information is available on the formation of low molecular weight flavour compounds from the reaction of ascorbic acid with amino acids. Therefore, the formation of flavour compounds from ascorbic acid and 20 amino acids was studied under dry-roasting conditions, since it was found that particularly high amounts of alkylpyrazines were formed under specific reaction conditions. Alkylated pyrazines are an important group of flavour compounds, which contribute substantially to the unique roasted aroma of various food products (Maga, 1992), and their formation from the interaction of amino acids with ascorbic acid reveals an additional precursor system in foods.

Since ascorbic acid has been shown to be an important source of furan in food (Limacher, Kerler, Condé-Petit, & Blank, 2007), the formation of this compound in the model reactions was also evaluated. The formation of furan received particular attention in recent years because of its classification as "possibly carcinogenic to humans" (IARC, 1995) and because of the relatively high amounts of furan detected in heat-treated canned and jarred food products (US Food and Drug Administration, 2004).

2. Materials and methods

2.1. Chemicals

1,3-Dihydroxyacetone (DHA, dimer 98%), L-(+)-ascorbic acid, arabinose, alanine, proline, arginine monohydrate, lysine monohydrate, D_4-furan (99%), *o*-phenylenediamine (98%) and potassium carbonate were from Acros Organics (Geel, Belgium). Glycine, valine, asparagine, aspartic acid, glutamine, glutamic acid, threonine, histidine, methionine, and tryptophan were from Sigma–Aldrich (Bornem, Belgium). Serine, phenylalanine, cysteine, leucine, and isoleucine were from Janssen Chimica (Geel, Belgium). Tyrosine was from Difco Laboratories (BD, Erembodegem, Belgium).

2.2. Model reactions

For the model reactions, 5 mmol of amino acid were mixed and ground with 5 mmol of ascorbic acid in a 20-mL headspace vial closed with a magnetic crimp cap with septum (Gerstel, Mülheim a/d Ruhr, Germany). Where indicated, 0.5 mmol of potassium carbonate was added. The reaction mixtures (without solvent) were heated in an oil bath at 160 °C for 20 min and rapidly cooled in an ice bath afterwards. When the reactivity of lysine with different carbohydrates was compared, lower amounts of reagents were used because of the high reactivity of lysine. For these model reactions (as indicated in the tables), 1 mmol of amino acid was mixed and ground with 1 mmol of ascorbic acid in a 20-mL vial and heated for 20 min in a preheated oven at 160 °C. Model reactions in aqueous conditions were performed in pressure resistant glass test tubes (10 mL) and heated in an oil bath at 130 °C for 1 h. For this purpose, 15 mmol of the reagents were dissolved in a minimal amount of water (22.5 mL). For each sample, 8 mL was taken and the pH was adjusted to 3, 7 or 9 with aqueous NaOH (2 N) or HCl (2 N).

2.3. Determination of α-dicarbonyl compounds

For the in situ trapping of the α-dicarbonyl compounds resulting from ascorbic acid degradation, ascorbic acid (5 mmol) was heated with 0.5 mmol of potassium carbonate and 5 mmol of *o*-phenylenediamine at 160 °C in an oil bath (20 min). Afterwards, two replicate samples were analysed by means of SPME-GC-MS as described below. Two additional replicate samples were dissolved in 10 mL of water, extracted with dichloromethane (3×15 mL) and dried ($MgSO_4$). The resulting extract was directly analysed by means of GC-MS.

2.4. Furan determination

Stock solutions of furan and D_4-furan were prepared by adding 10 μL of (D_4-)furan via a gastight syringe through the septum of a 20-mL headspace vial (Gerstel, Mülheim a/d Ruhr, Germany) containing 20 mL methanol. The weight increase was measured to determine the exact concentration of furan. The working solutions were prepared by adding 50 μL of stock solution to a 20-mL headspace vial containing 20 mL of water. After heating, reaction mixtures were spiked with 50 μL of D_4-furan working solution by means of a gastight syringe. All samples were kept in ice during spiking and were closed as fast as possible after spiking to minimise losses of furan. For calibration, exact amounts of furan working solutions were added to 1 g of sand, which was spiked with 50 μL of D_4-furan working solution. All samples were analysed in duplicate.

2.5. Analysis of flavour compounds

The volatiles formed from model reactions were directly sampled by means of headspace solid phase microextraction (SPME) during 30 min at 30 °C with a 50/30 μm DVB/Car/PDMS fibre (divinylbenzene/Carboxen/polydimethylsiloxane, Supelco, Bornem, Belgium). Desorption was done during 2 min at 250 °C in the gas chromatograph-mass spectrometer (GC-MS) inlet. The SPME extraction and desorption were performed automatically by means of a Multipurpose Sampler (MPS-2, Gerstel). For the determination of furan, a 85 μm Carboxen/PDMS fibre (Supelco, Bornem, Belgium) was used. The fibre was exposed to the headspace of the samples during 25 min at 35 °C. Desorption was carried out at the temperature of 300 °C for 5 min.

2.6. Gas chromatography analysis

For the analysis of the flavour compounds a Hewlett–Packard 6890 GC Plus coupled with a HP 5973 MSD (Mass Selective Detector-Quadrupole type), equipped with a CIS-4 PTV (Programmed Temperature Vaporisation) Injector (Gerstel), and a DB5-MS capillary column (30 m × 0.25 mm i.d.; coating thickness 0.25 μm) was used. Working conditions were: injector 250 °C; transfer line to MSD 250 °C; oven temperature programmed from 35 to 180 °C at 8 °C min^{-1} and from 180 to 260 °C at 30 °C min^{-1}, hold 10 min; carrier gas (He) 1.2 mL min^{-1}; split 1/10; ionisation EI 70 eV. For the determination of furan, a Varian CP-PoraBOND Q capillary column was used (25 m × 0.32 mm i.d.; coating thickness 5 μm). In this case, the oven temperature was programmed from 50 to 260 °C at 8 °C min^{-1}, hold 7 min. All other GC-MS parameters were kept constant. Substances were identified by comparison of their mass spectra and retention times with those of reference substances and by comparison with the Wiley (6th) and the NIST Mass Spectral Library (Version 1.6d, 1998). Linear retention indices were calculated and compared with literature values (Adams, 2007; Wagner, Czerny, Bielohradsky, & Grosch, 1999). When only MS data were available, identities were considered to be tentative.

3. Results and discussion

Water activity has an important influence on the course of the Maillard reaction. It has been reported repeatedly that the Maillard

reaction occurs faster in the absence of water (Adams, Abbaspour Tehrani, Kersiene, & De Kimpe, 2004; Eichner & Karel, 1972). Therefore, the degradation of ascorbic acid was studied under dry-roasting conditions (160 °C) in the absence and presence of amino acids.

In the absence of an amino compound very little browning and flavour formation occurred from ascorbic acid under dry-roasting conditions. Only low amounts of furfural, 2-acetylfuran and 2-furylmethanol were detected (Table 1). It is known that nonenzymatic browning of dry sugar mixtures can be greatly enhanced by the addition of a base, such as potassium carbonate, which initiates the retroaldol reactions required for the degradation of carbohydrates (Severin, Hiebl, & Popp-Ginsbach, 1984). Ascorbic acid caramelisation indeed increased substantially after addition of 10% potassium carbonate. Large amounts of the following furan derivatives were detected, in decreasing order of GC peak area: furfural, 2-(2-furylmethyl)furan, 2-(2-furylmethyl)-5-methylfuran, 2-furylmethanol, 2-acetylfuran, 2-(5-methyl-2-furyl)furan and 2-(2-furyl)furan (Table 1).

In the reaction of ascorbic acid with amino acids, 36 different pyrazines were detected (Table 2). Mostly methyl and ethyl substituted pyrazines were identified. High amounts of pyrazines were formed in particular from the reaction of ascorbic acid with lysine. Since α-dicarbonyl compounds are crucial reagents in pyrazine formation, ascorbic acid was heated in the presence of the trapping reagent *o*-phenylenediamine to determine the major α-dicarbonyl compounds produced. Solid phase microextraction as well as liquid extraction followed by GC-MS analysis showed the predominant formation of 2-oxopropanal, followed by ethanedial, 2,3-butanedione, 1,2-butanedione and 2,3-pentanedione (data not shown). In a previous study, ethanedial, 2-oxopropanal, 2,3-butanedione, tetros-2-ulose, 3-deoxytetros-2-ulose and 3-deoxypentos-2-ulose were identified by means of non-chromatographic tandem mass spectrometry after thermal treatment of ascorbic acid in aqueous solution (120 °C, 120 min) (Schulz, Trage, Schwarz, & Kroh, 2007). The predominant mechanism of pyrazine formation from α-dicarbonyl compounds and α-amino acids is shown in Scheme 1. In complex systems, however, additional mechanisms of pyrazine formation can occur (Adams, Polizzi & De Kimpe, 2008). A first step in pyrazine formation is the Strecker degradation between α-dicarbonyl compound **1** or **5** and α-amino acid **2** with the formation of α-aminoketone **4** or **6** and Strecker aldehyde **3**. Condensation of two α-aminoketones **4** and **6** yields dihydropyrazine **7**. This dihydropyrazine **7** can either oxidise spontaneously to the corresponding alkylpyrazine **8** (pathway A) or perform a nucleophilic attack from an unsubstituted position towards a carbonyl compound, e.g. Strecker aldehyde **3**, leading to alkylpyrazine **9** (pathway B). Following pathway A, the presence of 2-oxopropanal and 2,3-butanedione account for the formation of methyl substituted pyrazines while ethyl substituted pyrazines result from the reaction of 1,2-butanedione or 2,3-pentanedione. Incorporation of ethanedial leads to the formation of pyrazine and methylpyrazine. Depending on the amino acid side chain and the resulting Strecker aldehyde, additional alkyl substituents onto pyrazines are formed, following pathway B (Table 2). In this case, the oxidation step is not necessary since rearrangement of the double bonds with the side chain yields the aromatic pyrazine. As such, the formation of 2-methylpropyl substituted pyrazines from valine can be explained by the addition of intermediate dihydropyrazines to the Strecker aldehyde 2-methylpropanal. For instance, the addition of 3,6-dimethyl-2,5-dihydropyrazine to 2-methylpropanal followed by elimination of water yields 2,5-dimethyl-3-(2-methylpropyl)pyrazine (Table 2). A similar mechanism can be constructed for the formation of propylpyrazines from the Strecker aldehyde 2-hydroxypropanal from threonine, although in this case, an additional reduction step is required, which can be catalysed by ascorbic acid. Trace amounts of acetyl and *E*-1-propenyl substituted pyrazines from asparagine, and of ethenyl substituted pyrazines from lysine were detected. These substituents cannot be directly explained based on the reaction mechanism shown in Scheme 1 and probably originate from the recombination of degradation products.

For the other amino acids tested (cysteine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophane, tyrosine), no pyrazines were detected after reaction with ascorbic acid. Other flavour compounds were identified, being mainly furan derivatives and, for instance, thiophenes from cysteine (data not shown).

In a previous study, similar model reactions were performed of amino acids with 1,3-dihydroxyacetone as a precursor of 2-oxopropanal (90 °C, 30 min) (Adams et al., 2008). In these model mixtures, a high excess of 2,5-dimethylpyrazine was found for most amino acids, illustrating the importance of pathway A (Scheme 1) in this case. Although about 85% (as observed from GC peak areas) of the α-dicarbonyl compounds resulting from ascorbic acid degradation consisted of 2-oxopropanal, ascorbic acid/amino acid model reactions formed much less 2,5-dimethylpyrazine (pathway A) and relatively more 3-alkyl-2,5-dimethylpyrazines (pathway B) as compared to 1,3-dihydroxyacetone/amino acid model reactions. Therefore, the reducing activity of ascorbic acid probably influences the course of the reaction, inhibiting the spontaneous oxidation of intermediate dihydropyrazines, and thereby promoting pathway B.

The amounts of pyrazines formed from ascorbic acid and lysine were compared with the amounts generated from a hexose (glucose), from a pentose (arabinose), and from a sugar degradation product (1,3-dihydroxyacetone) (Table 3). Except for methylpyrazine and trimethylpyrazine, the amounts of pyrazines detected from ascorbic acid were of the same order of magnitude as from arabinose. This can be expected, since the first decomposition products of ascorbic acid, 3-deoxypentosulose and 3,4-dideoxypentosulos-3-ene, are the same as the intermediates from pentoses in Maillard browning (Davies & Wezicha, 1994). Higher amounts of 2,5-dimethylpyrazine and trimethylpyrazine were formed from the corresponding model reactions with glucose and especially with the fragmentation product 1,3-dihydroxyacetone.

As it was shown that the addition of potassium carbonate increased ascorbic acid degradation substantially (Table 1), the influence of potassium carbonate on the ascorbic acid/amino acid model reactions was studied. In Table 4, the GC peak areas of some

Table 1
Substituted furans (GC-MS peak area $\times 10^{-7}$) as detected by SPME in the headspace of heated ascorbic acid (5 mmol, with or without 0.5 mmol K_2CO_3, heated for 20 min at 160 °C in an oil bath).

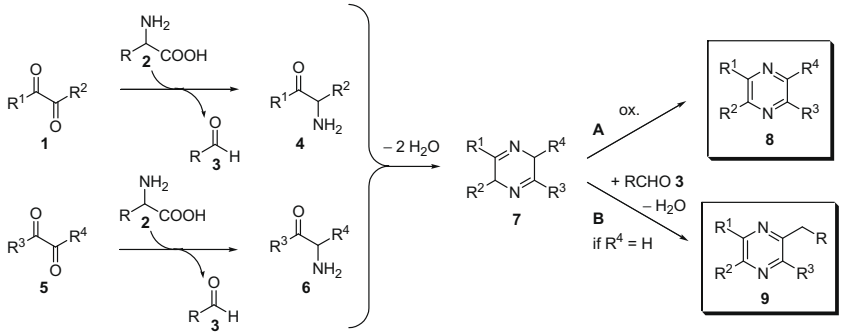
Compound	LRI[b]	Blank AsA	AsA + K_2CO_3
Furfural	833	87.69	135.77
2-Furylmethanol	862	3.84	28.33
2-Furylmethyl formate[a]	911	–[c]	1.11
2-Acetylfuran	912	1.84	27.23
Benzofuran[a]	991	–	1.10
1-(2-Furyl)-1-propanone[a]	1010	–	2.44
2-(2-Furyl)furan[a]	1036	–	13.30
2,3-Dihydrobenzofuran[a]	1075	–	4.65
2-(2-Furylmethyl)furan[a]	1085	–	46.60
2-Methylbenzofuran[a]	1102	–	1.78
2-(5-Methyl-2-furyl)furan[a]	1146	–	20.78
2-(2-Furylmethyl)-5-methylfuran[a]	1188	–	31.90

[a] Tentatively identified. Compound identifications correspond with our previous findings, except for 2-furylmethyl formate (which was not identified then) (Adams, Borrelli, Fogliano, & De Kimpe, 2005).
[b] Linear retention index (DB-5 stationary phase).
[c] Not detected.

Table 2
Pyrazines (GC-MS peak area $\times 10^{-7}$) as detected by SPME in the headspace of model reactions of ascorbic acid with various amino acids (5 mmol, 0.5 mmol K_2CO_3, heated for 20 min at 160 °C in an oil bath).

Compounds	LRI [b]	Ala	Arg	Asn	Asp	Gln	Glu	Gly	Lys	Ser	Thr	Val
Pyrazine	760	–[c]	–	–	–	–	–	–	–	0.66	–	–
Methylpyrazine	831	0.14	0.37	4.17	0.06	2.78	0.29	0.10	2.36	1.56	0.25	0.01
2,5-Dimethylpyrazine	911	–	1.57	7.29	–	2.93	1.32	0.73	24.68	–	6.41	–
2,6-Dimethylpyrazine	912	–	–	2.49	–	2.40	–	–	5.18	–	6.96	–
Ethylpyrazine	915	–	1.51	2.70	–	4.01	–	–	–	7.63	–	–
2,3-Dimethylpyrazine	919	–	–	–	–	–	–	–	–	2.56	–	–
2-Ethyl-6-methylpyrazine[a]	997	0.68	0.66	3.51	–	3.72	0.27	0.25	0.84	4.84	1.32	–
2-Ethyl-5-methylpyrazine[a]	1000	0.72	4.81	4.89	0.57	4.87	1.60	0.60	15.59	0.99	1.22	–
Trimethylpyrazine	1001	0.44	2.91	2.31	–	1.90	0.69	10.84	14.19	–	2.07	–
2-Ethyl-3-methylpyrazine	1003	–	–	0.62	0.63	–	–	–	1.93	1.51	–	–
3-Ethyl-2,5-dimethylpyrazine	1079	4.53	1.75	6.81	2.01	0.73	0.10	0.46	35.11	7.26	8.88	–
2,6-Diethylpyrazine[a]	1081	–	–	–	–	–	–	–	–	2.24	–	–
2-Ethyl-3,5-dimethylpyrazine	1085	–	0.31	0.31	–	0.27	0.03	0.88	3.29	0.78	3.88	–
2-Methyl-6-propylpyrazine[a]	1087	–	–	–	–	–	–	–	–	–	2.23	–
5-Ethyl-2,3-dimethylpyrazine	1089	–	0.37	0.55	–	0.21	0.05	2.16	3.81	1.57	–	–
2,5-Diethylpyrazine[a]	1090	–	1.48	0.61	–	0.91	0.07	-	2.11	0.94	–	–
3-Ethenyl-2,5-dimethylpyrazine	1099	–	–	–	–	–	–	–	0.30	–	–	–
2-Acetyl-5-methylpyrazine[a]	1129	–	–	0.05	–	–	–	–	–	–	–	–
2-Acetyl-6-methylpyrazine[a]	1134	–	–	0.32	–	–	–	–	–	–	–	–
2,3-Diethyl-5-methylpyrazine	1153	0.63	0.13	0.67	0.27	0.03	–	–	1.74	0.18	1.69	–
3,5-Diethyl-2-methylpyrazine[a]	1156	1.60	0.32	1.60	0.53	0.06	–	–	5.16	0.80	0.85	–
2,3,5-Trimethyl-6-ethylpyrazine[a]	1159	–	–	0.65	–	–	–	–	–	–	–	–
2,5-Dimethyl-3-propylpyrazine	1166	–	–	–	–	–	–	–	–	–	20.69	–
3,5-Dimethyl-2-propylpyrazine	1176	–	–	–	–	–	–	–	3.04	–	12.42	–
2-Methyl-5*H*-6,7-dihydrocyclopentapyrazine[a]	1196	–	–	0.66	0.12	–	0.22	–	–	–	–	–
2,5-Dimethyl-3-(2-methylpropyl)pyrazine	1204	–	–	–	–	–	–	–	–	–	–	1.76
3,5-Dimethyl-2-(2-methylpropyl)pyrazine	1216	–	–	–	–	–	–	–	–	–	–	0.15
2,5-Dimethyl-3-(*E*-1-propenyl)pyrazine[a]	1226	–	–	0.19	-	–	–	–	–	–	–	–
2,6-Diethyl-3,5-dimethylpyrazine[a]	1235	–	–	0.08	0.03	–	–	–	–	–	–	–
2,5-Diethyl-3,6-dimethylpyrazine[a]	1240	1.00	–	0.23	0.05	–	–	–	–	0.33	1.81	–
2,3,5-Triethyl-6-methylpyrazine[a]	1249	0.69	–	–	–	–	–	–	–	–	–	–
2,3,5-Trimethyl-6-propylpyrazine[a]	1249	–	–	–	–	–	–	–	–	–	2.51	–
6-(2-Methylpropyl)-2,3,5-trimethylpyrazine[a]	1279	–	–	–	–	–	–	–	–	–	–	0.44
2-(2-Methylpropyl)-3,5,6-trimethylpyrazine[a]	1285	–	–	–	–	–	–	–	–	–	–	0.88
Triethylmethylpyrazine[a]	1298	0.69	–	–	–	–	–	–	–	–	0.28	–
2,5-Dimethyl-3,6-dipropylpyrazine[a]	1394	–	–	–	–	–	–	–	–	–	6.63	–

[a] Tentatively identified.
[b] Linear retention index (DB-5 stationary phase).
[c] Not detected.



Scheme 1. Mechanism of formation of alkylpyrazines **8** and **9** from α-dicarbonyl compounds **1** and **5** and α-amino acid **2**.

selected pyrazines and furans from the ascorbic acid/amino acid model reactions are compared in the absence and presence of K_2CO_3. In most cases, addition of K_2CO_3 enhanced the formation of pyrazines. For instance, in the absence of K_2CO_3, no pyrazines were detected in the headspace of model mixtures of ascorbic acid with alanine or glycine, while in the presence of K_2CO_3 pyrazines were formed and a pleasant odour was generated. For most amino acids, such as asparagine and threonine, the addition of K_2CO_3 enhanced the formation of the pyrazines. In the case of valine, the formation of 2-methylpropyl substituted pyrazines also increased in the presence of K_2CO_3 (data not included in Table 4). Without K_2CO_3 only 2,5-dimethyl-3-(2-methylpropyl)-pyrazine (0.06×10^7) was formed, while with K_2CO_3 2,5-dimethyl-3-(2-methylpropyl)-pyrazine (1.76×10^7), 3,5-dimethyl-2-(2-methylpropyl)-pyrazine (0.15×10^7), 6-(2-methylpropyl)-2,3,5-trimethylpyrazine (0.44×10^7) and 2-(2-methylpropyl)-3,5,6-trimethylpyrazine (0.88×10^7) were detected (Table 2). However, in the reaction of ascorbic acid with lysine or glutamine, the addition of K_2CO_3 did not increase pyrazine formation. These amino acids showed a very high reactivity with ascorbic acid, even without K_2CO_3. Probably, the amino acids themselves are able to catalyse the degradation of ascorbic acid. In the case of lysine, this can easily be explained by the high pK_a of the side chain, but, in analogy, a similar reactivity would be expected from arginine, which did not seem to be the

Table 3
Pyrazines (GC-MS peak area $\times 10^{-7}$) as detected by SPME in the headspace of model reactions of lysine with various carbonyl compounds (1 mmol, heated for 20 min at 160 °C in an oven).

Compounds	Ascorbic acid	Glucose	Arabinose	1,3-Dihydroxyacetone
Methylpyrazine	3.61 ± 0.43[b]	9.78 ± 0.68	11.05 ± 0.61	4.90 ± 0.84
2,5-Dimethylpyrazine	48.73 ± 3.65	96.44 ± 8.32	47.35 ± 3.13	187.51 ± 11.11
2-Ethyl-5-methylpyrazine[a]	7.17 ± 1.34	16.02 ± 0.58	8.14 ± 0.32	–[c]
Trimethylpyrazine	19.57 ± 1.36	60.61 ± 3.16	54.70 ± 5.46	224.04 ± 26.61
3-Ethyl-2,5-dimethylpyrazine	20.84 ± 5.02	19.46 ± 2.12	20.27 ± 0.98	33.26 ± 4.05
2,3-Diethyl-5-methylpyrazine	0.51 ± 0.16	0.54 ± 0.13	0.54 ± 0.04	–
3,5-Diethyl-2-methylpyrazine[a]	1.71 ± 0.39	1.01 ± 0.14	1.40 ± 0.08	–

[a] Tentatively identified.
[b] Mean ± standard deviation ($n = 2$).
[c] Not detected.

Table 4
Selected flavour compounds (GC-MS peak area $\times 10^{-7}$) as detected by SPME in the headspace of model reactions of ascorbic acid with various amino acids (5 mmol, with or without 0.5 mmol K_2CO_3, heated for 20 min at 160 °C in an oil bath).

Reagents: ascorbic acid +	Methyl-pyrazine	Furfural	2,5-Dimethyl-pyrazine	2-Acetyl-furan	2-Ethyl-6-methylpyrazine[a]	2-Ethyl-5-methylpyrazine[a]	Trimethyl-pyrazine	2,3-Diethyl-5-methylpyrazine	3,5-Diethyl-2-methylpyrazine[a]
Ala	–[b]	13.77	–	11.41	–	–	–	–	–
Ala + K_2CO_3	0.30	1.48	tr[c]	8.48	0.68	0.72	0.44	0.63	1.60
Arg	0.19	–	0.67	1.09	0.24	1.87	0.97	–	–
Arg + K_2CO_3	0.37	–	1.57	0.83	0.66	4.81	2.91	0.13	0.32
Asn	0.10	1.58	–	6.32	0.19	0.28	0.05	–	–
Asn + K_2CO_3	4.17	–	7.29	8.27	3.51	4.89	2.31	0.67	1.60
Asp	–	9.36	–	0.32	–	–	–	–	–
Asp + K_2CO_3	0.06	15.02	–	5.97	–	–	0.57	0.27	0.53
Gln	1.59	–	2.34	9.04	3.54	5.21	1.75	0.02	0.06
Gln + K_2CO_3	2.78	–	2.93	7.49	3.72	4.87	1.90	0.03	0.06
Glu	0.35	5.24	1.04	11.26	0.11	1.12	–	–	–
Glu + K_2CO_3	0.29	0.67	1.32	7.57	0.27	1.59	–	–	–
Gly	–	1.96	–	24.93	–	–	–	–	–
Gly + K_2CO_3	0.26	0.44	0.73	20.31	0.25	0.60	10.84	–	–
Lys	4.41	–	39.22	–	1.10	26.56	21.17	3.39	9.44
Lys + K_2CO_3	2.36	–	24.68	-	0.84	15.59	14.19	1.74	5.16
Ser	0.78	4.26	–	7.80	2.50	–	0.41	0.06	0.27
Ser + K_2CO_3	1.56	0.76	–	9.17	4.84	–	0.99	0.18	0.80
Thr	–	1.09	1.23	8.42	0.55	0.25	1.09	0.12	–
Thr + K_2CO_3	0.25	0.55	6.41	6.25	1.32	1.22	2.07	1.69	0.85

[a] Tentatively identified.
[b] Not detected.
[c] Trace amount.

case. Whereas the addition of K_2CO_3 increased the formation of furans from ascorbic acid caramelisation (Table 1), these furans did not increase (and mostly decreased) in the presence of amino acids. This phenomenon can be ascribed to competition effects. On one hand, reactive carbonyl compounds will preferentially react with the amino acid instead of cyclising to furans. On the other hand, there is an increased competition for adsorption sites on the SPME fibre by the increased amount of volatiles.

In order to evaluate whether the catalytic effect of a base could also be established in aqueous solution, ascorbic acid was reacted in a concentrated aqueous solution at various pH values, alone and in the presence of glycine, alanine or lysine. Whereas furfural was formed in ascorbic acid solutions at pH 3, the amounts of furfural decreased exponentially with increasing pH and from pH 6 upwards, no furfural was detected (data not shown). No pyrazines were detected. Addition of 10% K_2CO_3 to aqueous model mixtures of ascorbic acid (0.7 M) with alanine or glycine (0.7 M) increased the pH from a value of 1 to 3. Slightly lower amounts of furfural were detected but the difference was small (data not shown). Thus, it seems that only in dry-roasting conditions ascorbic acid degradation could be enhanced by the addition of potassium carbonate (as demonstrated in Table 1).

Because it has been shown that pyrazines can be formed from serine and threonine without a carbohydrate source (Shu, 1999), all amino acids were also heated without ascorbic acid in the absence and presence of K_2CO_3. When lysine was heated, traces of 2,5-dimethylpyrazine were detected, independently of the presence of K_2CO_3. Heating of serine and threonine, on the contrary, yielded higher amounts of pyrazines which increased greatly in the presence of K_2CO_3 (Table 5). In case of serine, the type of pyrazines formed with and without ascorbic acid were more or less the same. The amounts were slightly higher in the presence of ascorbic acid (Table 2). In case of threonine, a wide range of pyrazines was formed in high amounts in the presence of K_2CO_3 and without ascorbic acid. In addition to the pyrazines shown in Table 5, various higher substituted pyrazines, difficult to identify correctly, were also detected in the threonine model systems. In the presence of K_2CO_3 and ascorbic acid, different pyrazines and, in general, lower amounts were formed from threonine (Table 2). However, with the exception of serine and threonine, no pyrazines were detected from heated amino acids and thus, the pyrazines detected in the model systems described are formed from the interaction of the amino acids with ascorbic acid.

In a study on the influence of natural antioxidants on Maillard browning, the inhibition of pyrazine formation was described as a disadvantage of several antioxidants. Ascorbic acid as well was reported to decrease the formation of pyrazines in a microwaved

Table 5
Pyrazines (GC-MS peak area $\times 10^{-7}$) as detected by SPME in the headspace of heated amino acids serine and threonine (5 mmol, with or without 0.5 mmol K_2CO_3, heated for 20 min at 160 °C in an oil bath).

Compound	LRI[b]	Ser	Ser + K_2CO_3	Thr	Thr + K_2CO_3
Pyrazine	760	0.60	0.89	–	–
Methylpyrazine	831	0.24	0.62	-	–
2,5-Dimethylpyrazine	911	0.03	0.53	2.27	4.66
Ethylpyrazine	915	0.11	1.23	–	–
2,3-Dimethylpyrazine	919	0.02	0.14	–	–
2-Ethyl-6-methylpyrazine[a]	997	–[c]	0.42	–	–
2-ethyl-5-methylpyrazine[a]	1000	–	1.26	0.03	–
Trimethylpyrazine	1001	–	–	0.75	4.04
3-Ethyl-2,5-dimethylpyrazine	1079	–	2.89	0.28	51.83
2-Ethyl-3,5-dimethylpyrazine	1085	–	0.14	0.02	3.84
Tetramethylpyrazine	1087	–	–	0.16	0.83
2-Methyl-6-propylpyrazine[a]	1087	–	–	–	2.29
5-Ethyl-2,3-dimethylpyrazine	1089	–	0.42	–	–
2,5-Diethylpyrazine[a]	1090	–	0.26	–	–
2,3-Diethyl-5-methylpyrazine	1153	–	0.83	–	0.32
3,5-Diethyl-2-methylpyrazine[a]	1156	–	1.65	–	0.93
2,3,5-Trimethyl-6-ethylpyrazine[a]	1159	–	–	–	13.57
2,5-Dimethyl-3-propylpyrazine	1166	–	–	–	3.19
2,3-Dimethyl-5-propylpyrazine	1182	–	–	–	1.02
2,6-Diethyl-3,5-dimethylpyrazine[a]	1237	–	–	–	14.42
2,5-Diethyl-3,6-dimethylpyrazine[a]	1242	–	0.76	–	17.16
Ethyl-methyl-propylpyrazine[a]	1245	–	–	–	1.83
Ethyl-methyl-propylpyrazine[a]	1249	–	–	–	5.32
2,3,5-Trimethyl-6-propylpyrazine[a]	1256	–	–	–	6.99

[a] Tentatively identified.
[b] Linear Retention Index (DB-5 stationary phase).
[c] Not detected.

glucose/glycine model system (Porter et al., 2006). However, only 2,5-dimethylpyrazine and trimethylpyrazine were discussed and these are much less important in ascorbic acid model systems as compared to glucose model systems (Table 3). This is probably due to the inhibition of pyrazine formation following pathway A (Scheme 1), leading to the preferential formation of other types of pyrazines following pathway B. According to our results, pyrazine formation can be induced by the addition of ascorbic acid, which might thus contribute to its use as an antioxidant in certain food products.

In recent years, particular attention has been given to the formation of furan, because of its classification as "possibly carcinogenic to humans" (International Agency for Research on Cancer, 1995) and because of the relatively high amounts of furan detected in heat-treated canned and jarred food products (US Food and Drug Administration, 2004). Since ascorbic acid has been shown to be an important source of furan (Limacher et al., 2007), the formation of this compound in the model reactions was also evaluated. For this purpose, a Carboxen/PDMS SPME fibre was selected and a PLOT (Porous Layer Open Tubular) capillary column was used for the GC-MS analyses. Furan was detected in all model reactions tested (of ascorbic acid, with or without K_2CO_3, in the presence or absence of the amino acids alanine, asparagine, lysine, serine, and threonine) in relatively low amounts as compared to substituted furans. For the same model system, the peak area of furan was on average 29, 39 and 46 times lower than the peak areas of 2-methylfuran, furfural and 2-acetylfuran, respectively (data not shown). The highest amounts of furan were formed in the presence of threonine and the lowest amounts in the presence of lysine. The addition of a base, such as K_2CO_3, to the model systems did not have a promoting effect on furan formation. Attempts were undertaken to quantify furan in the model systems by SPME-GC-MS using D_4-furan as the internal standard (data not shown). However, the headspace peak areas of furan and D_4-furan showed a very high variation, although all samples were spiked with an equal amount of the deuterated standard. During heating, a cake is formed in the reaction mixtures, in which furan and D_4-furan may be unevenly distributed. Grinding and homogenisation of the samples before spiking, however, was not possible since this would lead to high losses of the volatile furan. Therefore, absolute quantification was not considered meaningful in this case. Investigations on the interaction of furan with food matrix constituents are currently in progress.

Ascorbic acid is widely used as an antioxidant in food products, but its contribution to flavour formation from the interaction with amino acids in heat-treated foods has not been described yet, although its degradation to various carbonyl compounds and furan derivatives has been reported. In this study, it was shown that the reaction of ascorbic acid with amino acids gives rise to the formation of a wide range of pyrazines, in amounts comparable to pentose sugars. The addition of potassium carbonate to the model mixtures enhanced ascorbic acid degradation and pyrazine formation considerably in most cases. The pyrazines described generally have pleasant roasted, nutty flavour characteristics and low odour thresholds (Wagner et al., 1999). Therefore, the potential formation of pyrazines in heat-treated foods containing ascorbic acid must be taken into account.

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