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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