Flavour and Vinylogous Compounds Generated by Maillard-Type Reactions

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Abstract: The formation of vinylogous compounds and Strecker aldehydes from amino acids was studied in binary dry mixtures of fructose and valine, asparagine or phenylalanine at 180°C. Volatile compounds were monitored by GC-MS and proton transfer reaction mass spectrometry. Acrylamide was the major vinylogous compound followed by styrene and 2-methylpropene. On the contrary, methylpropanal was the most abundant odour-active Strecker aldehyde followed by phenylacetaldehyde, whereas 3-oxopropanamide could not unequivocally be identified. These data suggest that Strecker aldehydes and vinylogous compounds are generated through different pathways in the Maillard reaction.

Keywords: Maillard reaction; Strecker aldehydes; vinylogous compounds; acrylamide; styrene

INTRODUCTION

The recent discovery of relatively high amounts of acrylamide in carbohydrate-rich foods obtained by thermal processing [1, 2] has led to numerous studies indicating Maillard-type reactions as a major reaction pathway, in particular in the presence of asparagine, which directly provides the backbone of acrylamide [3–6]. Similarly, other vinylogous compounds have been identified as reaction products of specific amino acids, such as acrylic acid [7], but-3-enamide [7, 8], and styrene [9] generated from aspartic acid, glutamine, and phenylalanine, respectively. The mechanism explaining acrylamide formation from asparagine can basically be applied to other amino acids, as suggested in our recent paper [9]. It is based on a Strecker-type degradation of the Schiff base leading to azomethine ylide intermediates followed by a β-elimination reaction of the decarboxylated Amadori compound to afford the vinylogous compound [9]. Unfortunately, the large majority of studies dealing with acrylamide and other processing contaminants do not consider flavour or colour formation, despite the fact that they are also formed by Maillard-type reactions implying similar reaction pathways [10]. Therefore, the objective of this study was to simultaneously monitor the formation of flavour compounds and vinylogous-type processing contaminants.

EXPERIMENTAL

Materials. L-Valine (Val), L-asparagine (Asn), L-phenylalanine (Phe), D-fructose (Fru), 2-methylpropene, acrylamide, styrene, α,β,β-$^2H_3$-styrene (isotopic purity 98%), methylpropanal, and phenylacetaldehyde were from Fluka/Aldrich (Buchs, Switzerland).

Analytical methods. *Gas chromatography/Mass Spectrometry (GC/MS)* [9] – This was performed using a GC 6890A coupled to an MSD 5973N (both Agilent) equipped with a DB-Wax capillary column (J&W Scientific): 60 m × 0.25 mm, film thickness 0.25 μm. Helium was used as a carrier gas (2.4 ml/min). Samples (1 μl) were introduced *via* splitless injection at 250°C. The oven temperature program was: 35°C (2 min), 6°C/min to 240°C (10 min). The electron impact (EI) mass spectra were generated at 70 eV. The temperature of the ion source was 280°C. Quantification of styrene by isotope dilution assay was performed in the EI-MS mode by measuring the molecular ions

of analyte and labelled internal standard at *m/z* 104 and 107, respectively.

Proton Transfer Reaction Mass Spectrometry (PTR-MS) [11] – Samples for on-line measurement of headspace volatiles obtained in pyrolysis experiments were analysed by PTR-MS. The precursors (each 0.35 mol) were ground, mixed, and heated from room temperature to 190°C at a 5°C/min heating rate. Acrylamide (*m/z* 72), styrene (*m/z* 105), 2-methylpropene (*m/z* 57), and 3-oxopropanamide (*m/z* 88), phenylacetaldehyde (*m/z* 121), methylpropanal (*m/z* 73) were monitored in the scan mode (*m/z* 21–200, 0.2 s/mass).

Pyrolysis procedure. The chemicals of interest were heated in a temperature controlled heating module (Brouwer) at 180°C in tightly closed 6 ml Pyrex vacuum hydrolysis tubes (16 cm × 0.9 mm) that were immersed in silicone oil. After a defined heating period (e.g. 5 min), the tubes were cooled on ice. For quantification of styrene, fructose (0.2 mmol) and phenylalanine (0.2 mmol) were placed in 20 ml crimp cap vial (Chromacol) and heated in a silicone bath at 180°C for 15 min. After cooling down, the reaction sample was dissolved in water (2 ml), spiked with α,β,β-$^2H_3$-styrene (5.12 μg in MeOH) and extracted with diethylether (2 ml). The organic phase was dried over sodium sulphate and analysed by GC-MS. The experiments were performed in duplicate.

RESULTS AND DISCUSSION

Vinylogous compounds such as acrylamide have recently been claimed as food processing contaminants and associated with safety risks [1]. This is due to the fact that they can lead to highly reactive epoxides (i.e. glycidamide), which may for example react with nucleophiles forming haemoglobin adducts [2]. In analogy to acrylamide **1**, valine and phenylalanine may lead to 2-methylpropene **3** and styrene **5**, respectively (Figure 1). They are formed *via* the Maillard reaction under low-moisture conditions by a Strecker-type degradation of the intermediary Schiff base leading to a decarboxylated Amadori compound that upon β-elimination may release the vinylogous compounds [5, 6, 9]. Similar reactions can also lead to Strecker aldehydes, i.e. 3-oxopropanamide **2**, methylpropanal **4**, and phenylacetaldehyde **6** (Figure 1), some of which are odour-active contributing to the overall flavour of food products [10, 12].

PTR-MS was used as a suitable tool to monitor the formation of the compounds **1–6** due to their volatility. As shown in Figure 2A, acrylamide **1** (*m/z* 72) was the major vinylogous compound formed, followed by 2-methylpropene **3** (*m/z* 57) and styrene **5** (*m/z* 105). However, these data are only indicative because the release of compounds into the headspace depends on their volatility. For example, acrylamide **1** is very polar having a high boiling point of 125°C (25 mm), whereas 2-methylpropene **3** is very apolar and volatile (b.p. –6.9°C). Styrene **5** is also apolar having a higher boiling point (145°C) due to π-stacking interactions. Interestingly, **1** and **3** show one major peak corresponding to 150–160°C while styrene **5** is basically formed in two portions, i.e. at 150°C and under harsher pyrolytic conditions at 190°C. As the trace at *m/z* 57 is not well representative for **3**, it may correspond to several compounds. On the

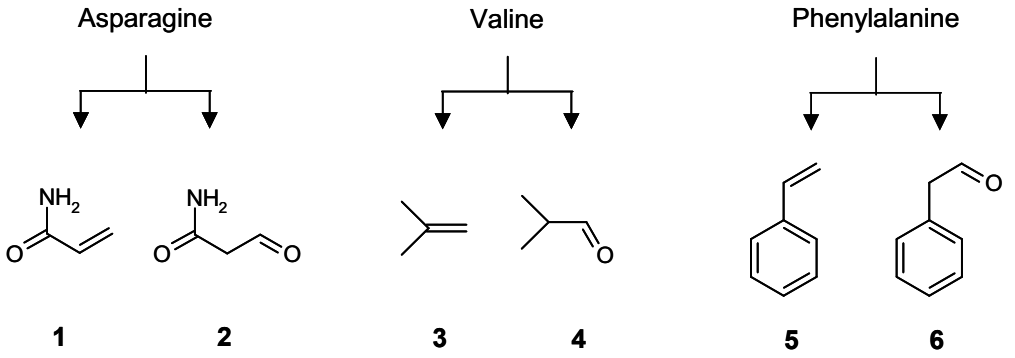

Figure 1. Formation of vinylogous compounds (**1**, **3**, **5**) and Strecker aldehydes (**2**, **4**, **6**) by Maillard-type reactions (see text for explanation)

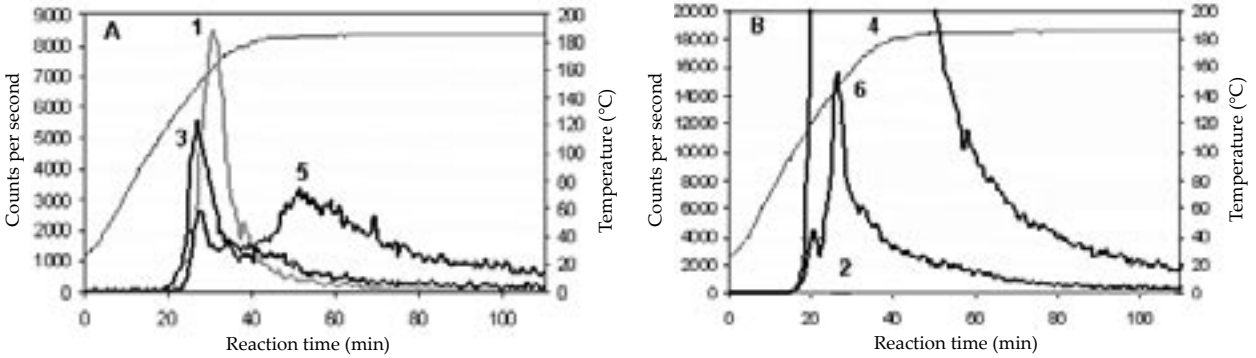

Figure 2. Formation of (A) vinylogous compounds: acrylamide **1**, 2-methylpropene **3**, styrene **5** and (B) Strecker aldehydes: 3-oxopropanamide **2**, methylpropanal **4**, phenylacetaldehyde **6**

contrary, *m*/*z* 72 and *m*/*z* 105 are characteristic for acrylamide **1** and styrene **5**, respectively. Styrene **5** has previously been reported as reaction product of dry and aqueous sugar/Phe systems [13, 14].

The formation of the Strecker aldehydes shows a different behaviour (Figure 2B). The malt-like smelling methylpropanal **4** was the most abundant compound followed by the honey-like smelling phenylacetaldehyde **6**, both generated at about 150°C. Surprisingly, the Strecker aldehyde of asparagine (**2**) was hardly detectable, indicating that this amino acid preferably forms the vinylogous compound. On the contrary, valine favours the generation the Strecker aldehyde. Phenylalanine

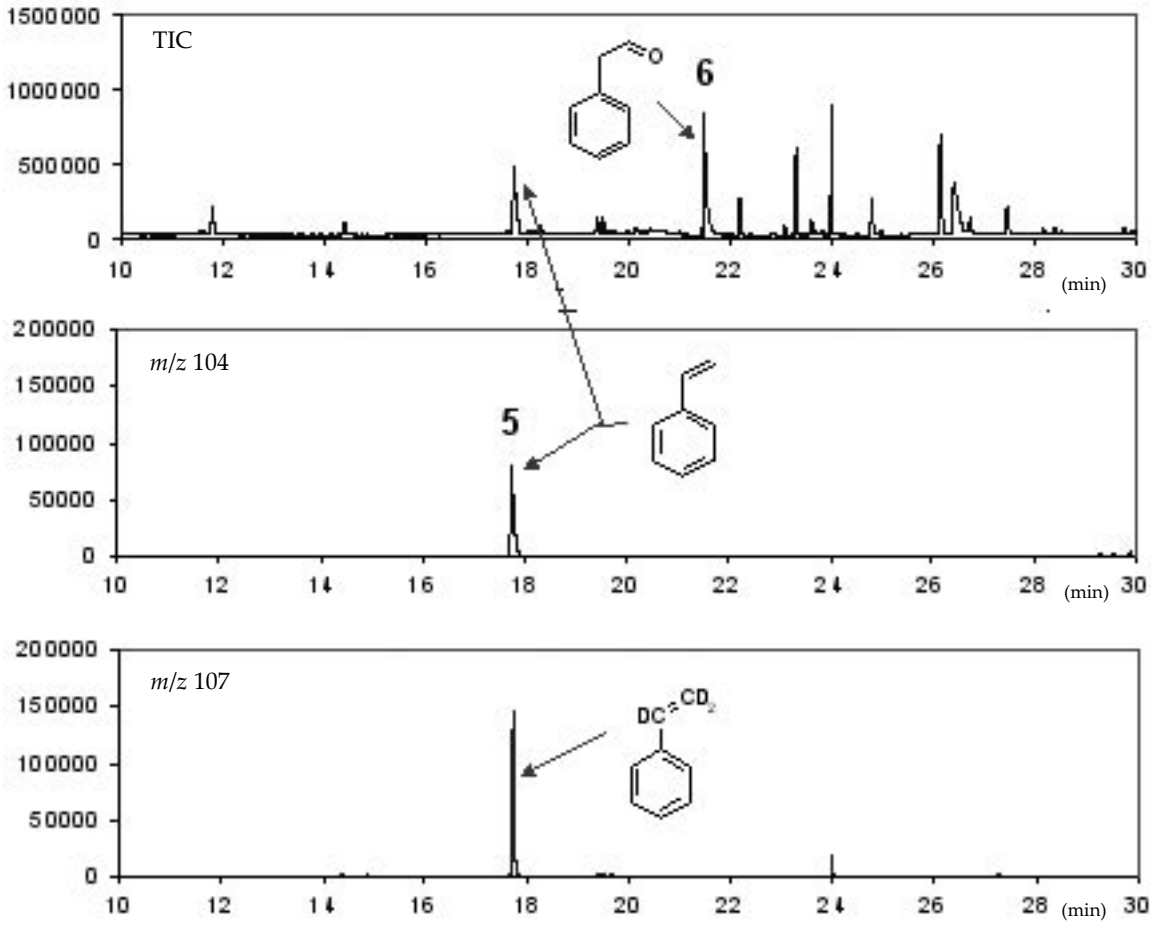

Figure 3. Formation of styrene **5** and phenylacetaldehyde **6** from Fru/Phe (TIC: total ion current)

seems to form both chemical entities under the same conditions. Again, the PTR-MS traces depend on the boiling point of the Strecker aldehydes, i.e. 63°C and 195°C for **4** and **6**, respectively.

The formation of styrene **5** and phenylacetaldehyde **6** from Fru/Phe was studied by GC-MS. Styrene **5** was quantified using the deuterated analogue (d_3-styrene): about 200–300 μmol/mol Phe was found after heating at 180°C for 15 min. As indicated in Figure 3, the ratio of Strecker aldehyde (**6**) to vinylogous compound (**5**) was about 2.5:1 based on the peak area.

CONCLUSIONS

Strecker aldehydes and vinylogous compounds show different formation patterns, suggesting that these molecules are generated by different reaction pathways. Therefore, it should be possible to favour the formation of flavour-active components while controlling the amounts of vinylogous compounds. However, more work is required including strong collaboration between academia and industry to develop food products with desirable flavour notes and reduced amounts of processing contaminants.

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