Formation pathways of 2,3-pentanedione in model systems and real foods

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Abstract

The formation of the buttery smelling 2,3-pentanedione was studied in glucose/glycine and glucose/proline reaction systems under different conditions and compared with the formation of 2,3-pentanedione upon extrusion cooking. The CAMOLA approach was applied to determine the relative importance of different reaction pathways. The results indicate a strong impact of moisture on the formation of 2,3-pentanedione. Under dry heating conditions, the majority of 2,3-pentanedione (70% to 82%) was formed from the intact glucose backbone, irrespectively of the pH and the type of amino acid. On the other hand, under aqueous conditions, both pH and the type of amino acid played an important role. At pH 5 the majority of 2,3-pentanedione was formed from the intact sugar backbone (60% in the presence of proline and 86% in the presence of glycine) while at pH 9 this diketone was almost exclusively formed by recombination of C₃/C₂ and C₄/C₁ sugar fragments. Upon extrusion cooking the major part of the 2,3-pentanedione (83%) was formed via the intact glucose backbone.

Introduction

Numerous studies were conducted up to date to better understand the generation of the buttery smelling 2,3-pentanedione from reducing sugars. The use of labelled precursors and the introduction of the so-called Carbon Module Labeling (CAMOLA) technique have allowed to propose several formation pathways, but also to determine their relative importance [1]. The formation mechanisms were shown to be strongly affected by reaction conditions such as moisture, temperature, pH and type of amino acid [1-4]. For example, under aqueous conditions at pH 7 and 135°C, the glucose/proline model system generated 2,3-pentanedione exclusively by recombination of sugar fragments, whereas at pH 5 the same precursor system generated 2,3-pentanedione both from the intact glucose skeleton (about 30%) and by recombination of sugar fragments (70%). Similar to 2,3-pentanodione, the generation of 2,3-pentanedione was shown to proceed via several mechanisms, e.g. from intact skeleton, recombination of sugar fragments (both of C₄/C₁ and C₃/C₂) or by alanine-mediated chain elongation of methylglyoxal [3-6]. Nevertheless, the impact of reaction conditions on the importance of the individual pathways is much less understood as compared to 2,3-pentanodione.

The aim of this study was to better understand the impact of reaction conditions on the formation of 2,3-pentanedione in model systems containing glucose and glycine or proline and to compare the results with those obtained for extruded cereals.

Experimental

Materials

The following chemicals were commercially available: D-glucose, glycine, L-proline, 2,3-butanedione, 2,3-pentanedione, monosodium dihydrogenphosphate anhydrous, disodium hydrogenphosphate dihydrate, trisodium phosphate, sodium
sulphate anhydrous (Sigma-Aldrich, Buchs, Switzerland); [U-\textsuperscript{13}C\textsubscript{6}]-glucose (Cambridge Isotope Laboratories, Inc., Andover, USA); [\textsuperscript{13}C\textsubscript{4}]2,3-butanedione, [\textsuperscript{13}C\textsubscript{2}]2,3-pentanediione, (Aroma Lab, Planegg, Germany).

**Aqueous systems**

Amino acid (either glycine or proline; 0.1 mmol each) and a 1:1 mixture of [\textsuperscript{12}C\textsubscript{6}]-glucose (0.15 mmol) and [U-\textsuperscript{13}C\textsubscript{6}]-glucose (0.15 mmol) were placed in a 20 mL headspace vial and dissolved in phosphate buffer (1 mL; 0.5 M; pH 5, 7 or 9). Vials were sealed with a crimp cap and heated in a silicon oil bath at 135 °C for 20 min. After cooling down with ice water, anhydrous sodium sulphate (2 g) was added, the vials were vortexed, and directly analysed by HS-SPME GCxGC-TOFMS.

**Dry systems**

Mixtures were prepared as described for aqueous systems, however the samples were freeze dried prior to heating (135 °C for 20 min). After cooling down with ice, the mixtures were dissolved in water (1g), anhydrous sodium sulphate (2 g) was added, the vials were vortexed, and directly analysed by HS-SPME GCxGC-TOFMS.

**Extrusion trials**

The extrusion trials were performed on a twin-screw extruder BC-21 (Clextral, France) using a model rice recipe. Rice flour was spiked with glycine (0.05 mol/kg) and a 1:1 mixture of [\textsuperscript{12}C\textsubscript{6}]-glucose (0.075 mol/kg) and [U-\textsuperscript{13}C\textsubscript{6}]-glucose (0.075 mol/kg) and extruded under moderate extrusion conditions (135 °C, 20% moisture, 400 rpm). The extruded products were dried in an Aerotherm oven (Wiesheu, Germany) at 120 °C for 6 min.

**Gas-Chromatography-Mass spectrometry**

The samples were analysed by HeadSpace Solid Phase Micro-Extraction in combination with 2D Gas Chromatography-Time-of-Flight-Mass Spectrometry (HS-SPME-GCxGC-TOFMS) as described previously [2]. The contribution of individual reaction pathways to the formation of 2,3-pentanediione was calculated from the relative distribution of the isotopologues. All results were corrected for the \textsuperscript{13}C content of the natural isotope. The obtained percentage after correction <0.5% was set to 0% by definition.

**Results and discussion**

The formation of 2,3-pentanediione from hexoses has been shown to proceed via several pathways including recombination of fragments as well as formation from the intact sugar skeleton [3-6]. The impact of reaction conditions was studied in model systems containing equimolar mixtures of unlabelled and \textsuperscript{13}C\textsubscript{6}-labelled glucose (CAMOLA approach) in the presence of glycine or proline. The relative importance of the individual pathways generating 2,3-pentanediione in glucose/proline systems under different reaction conditions is shown on Figure 1. Under aqueous conditions, the importance of individual pathways depended on the pH of the reaction mixture. While the formation from the intact glucose skeleton was the major pathway contributing to 2,3-pentanediione at pH 5 (60%), this pathway was not active at pH 7 and pH 9. Under neutral and alkaline aqueous conditions, 2,3-pentanediione was exclusively formed by recombination of glucose fragments. The recombination of C\textsubscript{3}/C\textsubscript{2} fragments (e.g. 1-hydroxypropanone and acetaldehyde as proposed by Hofmann [5]) was the major pathway (72% to 74%) while the recombination of C\textsubscript{4}/C\textsubscript{1} fragments (e.g. 2,3-butanediione and formaldehyde as proposed by Weenen [4]) contributed to about
one quarter of the 2,3-pentanedione formed (24% to 28%). Contrary to aqueous conditions, under dry heating the formation of 2,3-pentanedione was almost independent of the pH value. The majority of the compound was formed from the intact sugar skeleton (72% to 82%) followed by recombination of C\textsubscript{3}/C\textsubscript{2} fragments (12% to 18%). The recombination of the C\textsubscript{4}/C\textsubscript{1} fragments contributed only marginally under dry heating conditions (6% to 8%).

![Figure 1](image1.png)

**Figure 1:** Relative contribution of different pathways generating 2,3-pentanedione in glucose/proline model systems under aqueous (A) and dry (B) heating conditions as calculated from the isotopologue distribution of CAMOLA experiments

In the presence of glycine, the contribution of the individual pathways to 2,3-pentanedione was different as compared to the system containing proline (Figure 2). In general, the contribution of the intact skeleton was more pronounced in the system containing glycine. The formation from the intact sugar skeleton was the major pathway generating 2,3-pentanedione at pH 5 (86%). The formation through recombination of sugar fragments was very limited at pH 5, however the importance of these pathways strongly increased with pH. At pH 9 the majority of 2,3-pentanedione was formed by recombination of C\textsubscript{3}/C\textsubscript{2} fragments (62%), followed by recombination of C\textsubscript{4}/C\textsubscript{1} fragments (24%). The presence of glycine, as compared to proline, triggered also limited formation of 2,3-pentanedione by recombination of C\textsubscript{4} sugar fragment and C\textsubscript{1} glycine fragment (most probably formaldehyde, the Strecker aldehyde of glycine). The importance of the latter pathway slightly increased with the pH of the aqueous system, but remained marginal.

![Figure 2](image2.png)

**Figure 2:** Relative contribution of different pathways generating 2,3-pentanedione in glucose/glycine model systems under aqueous (A) and dry (B) heating conditions as calculated from the isotopologue distribution of CAMOLA experiments

Under dry heating condition, the results obtained in the glucose/glycine system were quite similar to that obtained in the glucose/proline system. Irrespectively of the pH, the
The majority of the 2,3-pentanedione has been formed from intact glucose backbone. The generation of 2,3-pentanedione through recombination of C₃/C₂ and C₄/C₁ sugar fragments was slightly less important in the systems containing glycine as compared to those containing proline, in favour of generation via recombination of the C₄ sugar fragment and C₁ glycine fragment. Indeed, at pH 5 and pH 7, the dry heating system seems to produce more easily the C₁ fragment (formaldehyde) from glycine than from glucose, which is not the case for pH 9 where generation of the C₁ fragment from both precursors was comparable. In contrast, under aqueous conditions the generation of the C₁ fragment from glucose was favoured over the generation from glycine, irrespectively to pH value.

The relative contribution of different pathways generating 2,3-pentanedione from glucose in the presence of glycine under extrusion cooking is shown in Figure 3. The results indicate that upon extrusion cooking, the major part of the 2,3-pentanedione originates from the added precursors and only a small part (about 7%) is formed from the inherent precursors of rice flour. The majority of 2,3-pentanedione (83%) that originated from added precursors was generated from the intact glucose backbone. The recombination of C₃/C₂ sugar fragments contributed to only about 11% and recombination of C₄ sugar fragment and C₁ glycine fragment to the remaining 6% of 2,3-pentanedione. Under extrusion conditions, the generation of the C₁ fragment from glycine is favoured over the generation from glucose, indicating that extruded systems seem to behave more like dry systems than aqueous systems.

Figure 3: Relative contribution of different pathways generating 2,3-pentanedione upon extrusion cooking from added precursors (glucose/glycine) and inherent precursors of rice as calculated from the isotopologue distribution of CAMOLA experiments

In conclusion, the generation of 2,3-pentanedione from glucose strongly depends on the reaction conditions as well as on the type of co-reacting amino acids. Therefore, extrapolation of the results from models systems to food systems must be done with caution and should be validated by experiments using authentic food systems.

References