

Study To Elucidate Formation Pathways of Selected Roast-Smelling Odorants upon Extrusion Cooking

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ABSTRACT: The formation pathways of the N-containing roast-smelling compounds 2-acetyl-1-pyrroline, 2-acetyl-1(or 3),4,5,6-tetrahydropyridine, and their structural analogues 2-propionyl-1-pyrroline and 2-propionyl-1(or 3),4,5,6-tetrahydropyridine were studied upon extrusion cooking using the CAMOLA approach. The samples were produced under moderate extrusion conditions (135 °C, 20% moisture, 400 rpm) employing a rice-based model recipe enriched with flavor precursors ([U-¹³C₆]-D-glucose, D-glucose, glycine, L-proline, and L-ornithine). The obtained data indicate that the formation of these compounds upon extrusion follows pathways similar to those reported for nonshared model systems containing D-glucose and L-proline. 2-Acetyl-1-pyrroline is formed (i) by acylation of 1-pyrroline via C₂ sugar fragments (major pathway) and (ii) via ring-opening of 1-pyrroline incorporating C₃ sugar fragments (minor pathway), whereas 2-propionyl-1-pyrroline incorporates exclusively C₃ sugar fragments. 2-Acetyl-1(or 3),4,5,6-tetrahydropyridine and the corresponding propionyl analogue incorporate C₃ and C₄ sugar fragments, respectively. In addition, it has been shown that the formation of 2-acetyl-1-pyrroline in low-moisture systems depends on the pH value of the reaction mixture.

KEYWORDS: roast-smelling odorants, pathways, extrusion, Maillard reaction, CAMOLA

INTRODUCTION

Extrusion cooking is gaining growing importance in the food industry as a versatile process combining in one step several unit operations such as conveying, mixing, cooking, texturizing, and shaping. Although extrusion of cereals offers numerous technological and operational advantages, some challenges remain to use extrusion cooking more efficiently. Flavor generation is one of them, as the extruded products are generally inferior in flavor compared to conventionally cooked cereals. The short residence time and aroma stripping at the die are often pointed out as the main causes for the less intense flavor of extruded products.^{1–5}

Several studies have been published dealing either with the identification of odorants in extrudates produced from different raw materials or focusing on possibilities to improve the flavor by the addition of precursor molecules.^{6,7} Unfortunately, only a few studies were published using the aroma extract dilution analysis approach to judge the contribution of the individual odorants to the overall flavor of extrudates.⁸ The N-containing heterocycles 2-acetyl-1-pyrroline (2-AP, **1**) and the tautomers 2-acetyl-1(or 3),4,5,6-tetrahydropyridine (2-ATHP, **2a/b**) are frequently mentioned as important flavor molecules found in numerous thermally processed cereal products including bread^{9,10} or extrudates enriched with L-proline and D-glucose.^{11,12} These compounds exhibit a roasty, popcorn-like aroma at very low threshold concentrations (0.02 ng/L for 2-AP (**1**) and 0.06 ng/L for 2-ATHP (**2a/b**); both in air).¹³

The mechanisms underlying the formation of 2-AP (**1**) and 2-ATHP (**2a/b**) as well as their structural analogues 2-propionyl-1-pyrroline (2-PP, **3**) and 2-propionyl-1(or 3),4,5,6-tetrahydropyridine (2-PTHP, **4a/b**) were studied in detail in model systems^{14–21} and were recently reviewed in detail.²² According to these studies all of these compounds are formed through the common intermediate 1-pyrroline, which is

generated either by Strecker degradation of the secondary amino acid L-proline or by cyclization of 4-aminobutanal, the Strecker aldehyde of L-ornithine.^{17,21} 1-Pyrroline is further converted into 2-AP (**1**) by reacting with either acetylformoin¹⁹ or 2-oxopropanal.¹⁷ The first proposal on the formation of 2-ATHP (**2a/b**) described N-2-oxopropyl-4-aminobutanal as the key intermediate,¹⁵ but was disproved in a later study.¹⁴ Other mechanisms published in the literature comprise the reaction of L-proline with 1-desoxyosone²⁰ or the interaction of 1-pyrroline with 1-hydroxy-2-propanone, a well-known sugar degradation product.¹⁷ The formation of 2-PTHP (**4a/b**) was suggested to proceed by an analogous mechanism starting from 1-pyrroline and 1-hydroxy-2-butanone instead of 1-hydroxy-2-propanone. Similarly, the formation of 2-PP (**3**) was proposed to proceed by the interaction of 1-pyrroline with hydrated 2-oxobutanal instead of 2-oxopropanal.^{20,23} Currently, no data are available concerning the formation mechanism of these N-containing, roast-smelling compounds upon extrusion.

To study the formation of the target molecules, the carbon module labeling (CAMOLA) approach was proven as a versatile and convenient tool. It is based on using an equimolar mixture of unlabeled and stable isotope labeled reaction precursors and studying their incorporation into the target molecule. The CAMOLA approach has been successfully applied to clarify formation pathways of numerous odorants in model systems and food matrices.²⁴

The formation mechanisms of key odorants upon extrusion cooking, including N-containing heterocycles, might differ from

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those described in nonsheared systems, as during extrusion the raw materials are transformed by a combination of thermal and mechanical energy. Therefore, the aim of the present study was to investigate in-depth the formation pathways of 2-AP (1), 2-PP (3), 2-ATHP (2a/b), and 2-PTHP (4a/b) upon extrusion in a model rice recipe using the CAMOLA approach. Furthermore, the impact of the pH value on the formation pathways was investigated in a low-moisture model system.

EXPERIMENTAL PROCEDURES

Chemicals. The following chemicals were commercially available: D-glucose, glycine, L-proline, L-ornithine monohydrochloride, disodium hydrogenphosphate dihydrate, sodium sulfate anhydrous (Sigma-Aldrich, Buchs, Switzerland); 2-acetyl-1-pyrroline, 2-acetyl-3,4,5,6-tetrahydropyridine (Toronto Research Chemicals Inc., ON, Canada); [$U\text{-}^{13}\text{C}_6$]-D-glucose (Cambridge Isotope Laboratories, Inc., Andover, MA, USA); 2-propionyl-1-pyrroline (Aroma Lab, Freising, Germany).

Materials. Rice flour Remyflo R7 250T was obtained from Beneo-Remy NV (Leuven, Belgium).

Extrusion Trials (CAMOLA Experiments). The extrusion trials were performed on a BC-21 twin-screw extruder (Cletral, France) using a rice-based model recipe. Rice flour was enriched with glycine (0.05 mol/kg), L-proline (0.02 mol/kg), L-ornithine monohydrochloride (0.02 mol/kg), and a mixture of D-glucose and [$U\text{-}^{13}\text{C}_6$]-D-glucose (1:1; 0.05 mol/kg each) 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. After milling, an aliquot of the sample (1 g) was mixed with anhydrous sodium sulfate (4 g) and water (6 g), the vials were vortexed, and the samples were directly analyzed by SPME-GC×GC-TOFMS.

Similar trials were performed using solely unlabeled D-glucose (0.1 mol/kg) instead of a mixture of D-glucose and [$U\text{-}^{13}\text{C}_6$]-D-glucose (1:1) to facilitate identification of the compounds of interest.

Low-Moisture Model Rice Systems. L-Proline (0.1 mmol) and [$U\text{-}^{13}\text{C}_6$]-D-glucose (0.3 mmol) were placed in a 20 mL headspace vial containing phosphate buffer (1 mL; 0.5 M; pH 5.5 or 8.2) and water (0.5 mL). After complete dissolution, rice flour (1 g) was added, and the mixtures were homogenized by vortexing and then freeze-dried in the vials. The moisture of the freeze-dried mixtures was afterward adjusted to 20% (corresponding to the moisture of extruded systems) by the addition of water (270 μL). The vials were sealed with a crimp cap and heated in a silicon oil bath at 135 °C for 20 min. After cooling in an ice bath, the mixtures were dispersed in water (6 g), anhydrous sodium sulfate (2 g) was added, the vials were vortexed, and the samples were directly analyzed by SPME-GC×GC-TOFMS.

pH Measurements. The pH of nonheated low-moisture rice systems was measured prior to freeze-drying after the addition of water (5.5 mL), whereas the pH of the heated systems was measured after dispersion in water (6 mL). In the case of extrusion trials, dry mix (nonextruded) or milled product (5 g) was suspended in cold water (30 mL) and extracted during 1 h under constant stirring prior to pH measurement.

Analysis of N-Containing Roast-Smelling Compounds. The samples were analyzed by solid phase microextraction (SPME) in combination with 2D gas chromatography–time-of-flight-mass spectrometry (SPME-GC×GC-TOFMS). After an equilibration (70 °C, 15 min), the aroma compounds were extracted from the headspace during 30 min at 70 °C under agitation (250 rpm) using a divinylbenzene–carboxen–polydimethylsiloxane fiber (StableFlex DVB/CAR/PDMS; 2 cm; film thickness, 50/30 μm ; Supelco, Buchs, Switzerland). The extracted compounds were thermally desorbed for 2 min into a split/splitless injector maintained at 250 °C and operated in split mode (1:20). GC×GC-TOFMS analyses were performed using an Agilent 7890A gas chromatograph (Palo Alto, CA, USA) coupled to a Pegasus 4D TOFMS (LECO Corp., St. Joseph, MI, USA). The system was equipped with a CombiPAL autosampler (CTC Analytics AG, Zwingen, Switzerland). LECO ChromaTOF software (version 4.24) was used to operate the GC×GC-TOFMS system. Two column

sets were used. The first column set consisted of a DB-1701 capillary column (30 m × 0.25 mm; film thickness = 0.25 μm ; J&W Scientific, Folsom, CA, USA) coupled to a DB-WAX capillary column (2 m × 0.1 mm; film thickness = 0.1 μm ; J&W Scientific), whereas the second column set comprised a DB-SMS UI capillary column (30 m × 0.25 mm; film thickness = 0.25 μm ; J&W Scientific) coupled to a DB-WAX capillary column (2 m × 0.1 mm; film thickness = 0.1 μm ; J&W Scientific). The GC oven was temperature programmed from 30 °C (2 min) to 230 °C (10 min) at 5 °C/min. Carrier gas (He) was held at constant flow of 1.2 mL/min. The modulation period was set to 6 s. The modulator temperature and secondary oven temperature were kept at 35 and 30 °C above the primary oven temperature, respectively. The MS transfer line temperature was 250 °C and the MS source temperature was set to 200 °C. The MS detector voltage was –1850 V. Electron-induced ionization was performed at 70 eV. The mass spectral data acquisition rate was 200 Hz, and data were collected over a mass range of 29–350 amu. The analyses were performed in duplicate. The identification of 2-acetyl-1-pyrroline (2-AP, 1), 2-propionyl-1-pyrroline (2-PP, 3), and 2-acetyl-1(or 3),4,5,6-tetrahydropyridine (2-ATHP, 2a/b) was accomplished by comparing their mass spectra and RI values on two capillary columns of different polarities with those of authentic standards. The identification of 2-propionyl-1(or 3),4,5,6-tetrahydropyridine (2-PTHP, 4a/b) was performed by comparing the mass spectra and RI obtained on an apolar capillary column with literature data and by examination of spectra obtained from labeling experiments.²³

RESULTS AND DISCUSSION

The analysis of the extrudate produced on the pilot scale extruder BC-21 under moderate conditions (135 °C, 20% moisture, 400 rpm) employing a rice recipe enriched with unlabeled precursors revealed the presence of four roast-smelling N-containing heterocycles: 2-AP (1), 2-PP (3), 2-ATHP (2a/b), and 2-PTHP (4a/b) (see Table 1 for the

Table 1. Retention Indices of the Identified Roast-Smelling Heterocyclic Odorants Obtained on Two Capillary Columns of Different Polarities

odorant	RI on DB-SMS UI	RI on DB-1701
2-acetyl-1-pyrroline (1)	921	1009
2-propionyl-1-pyrroline (3)	1025	1113
2-acetyl-3,4,5,6-tetrahydropyridine (2b)	1047	1141
2-acetyl-1,4,5,6-tetrahydropyridine (2a)	1150	1265
2-propionyl-3,4,5,6-tetrahydropyridine (4b)	1163	1240
2-propionyl-1,4,5,6-tetrahydropyridine (4a)	1221	1360

corresponding RIs on apolar and medium polar capillary columns). The first three compounds are well-known constituents of thermally treated foods including cereals,²² whereas the generation of 2-PTHP (4a/b) has been reported in dry-heated and aqueous model systems containing D-glucose and L-proline.²³

CAMOLA Experiments. To gain first insight into the formation of these roast-smelling compounds upon extrusion cooking, combining a thermal and mechanical energy input, the extrusion experiment was repeated with the unlabeled D-glucose replaced with an equimolar mixture of unlabeled and [$U\text{-}^{13}\text{C}_6$]-labeled D-glucose (1:1).

Formation of 2-AP (1). The mass spectrum of 1 obtained from CAMOLA experiments is shown in Figure 1. The presence of the ions m/z 113 and 114 clearly indicates the formation of doubly and triply labeled isotopomers, respec-

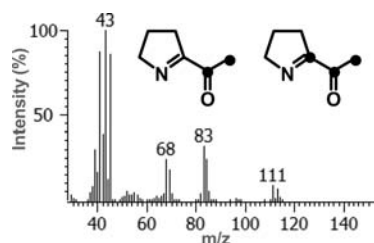


Figure 1. Mass spectrum of 2-AP (1) obtained from CAMOLA experiments upon extrusion: (●) carbon-13 label from $[U-^{13}C_6]$ -D-glucose.

tively, on top of the unlabeled isotopomer (m/z 111). The double-labeled isotopomer incorporated both labeled carbon atoms in the acetyl group, as confirmed by the presence of ions m/z 45 and 84 for the labeled compound versus m/z 43 and 83 for the unlabeled compound. The triply labeled isotopomer integrated two labeled carbon atoms in the acetyl group and one labeled carbon atom in the 1-pyrroline ring (as carbon-2 of the ring system) as confirmed by the ions m/z 45, 85, and 69/70. These findings are well in agreement with the data published for model systems.¹⁷ With the literature data taken into consideration, the incorporation of a C_2 sugar fragment into 2-AP (1) upon extrusion may be explained by the acetylation of 1-pyrroline either via acetylformoin as proposed by Rewicki¹⁹ or via hydrated 2-oxopropanal as proposed by Hofmann and Schieberle.¹⁷ The incorporation of a C_3 sugar fragment can be explained by the mechanism starting with a nucleophilic attack of tautomeric 2-pyrroline at carbon-1 of 2-oxopropanal, which includes ring-opening and elimination of one carbon atom of the pyrroline ring.¹⁷

The relative distribution of different isotopomers determined from the cluster of their molecular ions (Table 2) pointed out

Table 2. Relative Distribution of Isotopomers of Roast-Smelling Heterocyclic Compounds Generated during CAMOLA Experiments upon Extrusion

compound	relative distribution of isotopomers (%)				
	M^+	$(M + 1)^+$	$(M + 2)^+$	$(M + 3)^+$	$(M + 4)^+$
2-AP (1)	53	3	36	8	0
2-PP (3)	59	0	5	36	0
2-ATHP (2a/b)	51	0	8	41	0
2-PTHP (4a/b)	55	0	0	3	42

the incorporation of a C_2 sugar fragment as the major pathway and integration of the C_3 sugar fragment as a minor pathway leading to 1 (ratio about 4:1). Interestingly, a similar ratio was observed in the literature for a dry-heated D-glucose/L-proline model system, although the reaction conditions were quite different (dry heating at 160 °C for 10 min).¹⁷ However, by coincidence the extruded system and dry-heated system had similar pH values (about pH 7). To verify if there is a direct influence of pH on the importance of individual pathways, two low-moisture rice model systems (20% moisture) containing $[U-^{13}C_6]$ -D-glucose and L-proline, buffered at different pH values (pH 5.5 and pH 8.2), were prepared and heated at 135 °C for 20 min. These reaction conditions were close to extrusion conditions used in this study (longer reaction time was applied to compensate for the lack of mechanical energy obtained from the shearing conditions in the extruder). Under slightly acidic conditions, the ratio of doubly labeled to triply

labeled isotopomer was comparable to that obtained at pH 7 (about 3:1 at pH 5.5 vs 4:1 at pH 7). However, under alkaline conditions the concentration of the triply labeled isotopomer significantly increased and both isotopomers were formed at comparable levels (Table 3). Thus, alkaline conditions favor the generation of 1 from a C_3 sugar fragment, implying that the pH value is a key parameter affecting the different formation pathways.

Table 3. Relative Distribution of 2-AP (1) Isotopomers Generated from L-Proline and $[U-^{13}C_6]$ -D-Glucose in a Low-Moisture Rice Model System under Different pH Conditions

2-AP (1) (m/z)	relative distribution of 2-AP (1) isotopomers (%)	
	pH 5.5	pH 8.2
111 (M^+)	9	4
112 ($M + 1$) ⁺	4	3
113 ($M + 2$) ⁺	67	50
114 ($M + 3$) ⁺	20	43

Formation of 2-PP (3). The mass spectrum of 3 obtained from the CAMOLA experiments is shown in Figure 2. Apart

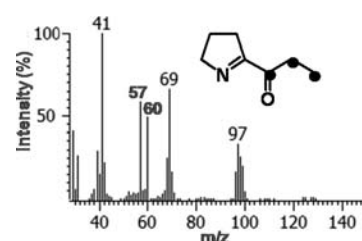


Figure 2. Mass spectrum of 2-PP (3) obtained from CAMOLA experiments upon extrusion: (●) carbon-13 label from $[U-^{13}C_6]$ -D-glucose.

from the molecular ion of the unlabeled isotopomer (m/z 125), the triply labeled isotopomer (m/z 128) was detected. The three labeled carbon atoms were all incorporated in the propionyl residue of the flavor compound as indicated by the fragment m/z 60 corresponding to the fragment m/z 57 of the unlabeled isotopomer.

The relative distribution of the different isotopomers determined from the cluster of their molecular ions (Table 2) pointed out the incorporation of the C_3 sugar fragment as a major pathway leading to 3. These data are in good agreement with the reaction mechanism proposed in the literature suggesting the formation of 2-PP by the interaction of hydrated 2-oxobutanal with 1-pyrroline.²³

Formation of 2-ATHP (2a/b). Gas chromatography led to the separation of two tautomers of this compound. The mass spectra of both tautomers (Figure 3) pointed out the presence of the unlabeled isotopomers (m/z 125) and triply labeled isotopomers (m/z 128). On the basis of the spectral data the position of the labeled carbon atoms was successfully attributed. Two labeled carbons were present in the acetyl group (m/z 45) and one in the piperidine ring (m/z 83/84). These data are well in line with the spectra of triply labeled 2a/b reported in the literature.²³

The unlabeled and triply labeled isotopomers represented >90% of all isotopomers formed (see Table 2). Therefore, the incorporation of C_3 sugar fragments can be considered as the

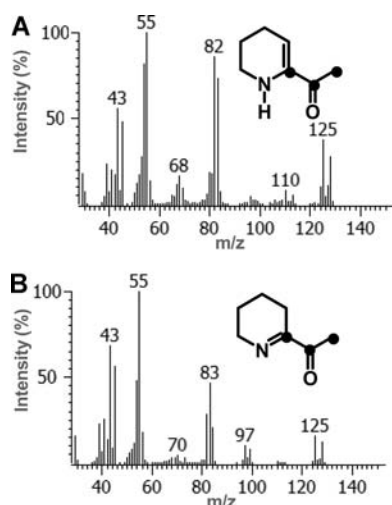


Figure 3. Mass spectra of 2-acetyl-1,4,5,6-tetrahydropyridine (**2a**, A) and 2-acetyl-3,4,5,6-tetrahydropyridine (**2b**, B) obtained from CAMOLA experiments upon extrusion: (●) carbon-13 label from [$U\text{-}^{13}\text{C}_6$]-D-glucose.

major pathway leading to **2a/b** by extrusion. On the basis of the results obtained from the model systems, several mechanisms were proposed to explain this incorporation. For example, formation via Strecker degradation of L-proline by acetylformoin and 1-deoxy-2,3-hexulose was proposed by Rewicki et al.¹⁹ and Schieberle,²⁰ respectively. In addition, the generation from 1-pyrroline and sugar fragments (1-hydroxy-2-propanone) has been proposed.¹⁷

Formation of 2-PTHP (4a/b). Similarly to **2a/b**, the GC analysis led to separation of two tautomers of this compound. Apart from the unlabeled isotopomers (m/z 139), the isotopomers containing four labeled carbon atoms (m/z 143) were clearly visible in the spectra of both tautomers (Figure 4). A comparison with the mass spectra of unlabeled and 4-fold labeled tautomers of **4a/b**, reported in the literature,²³ revealed that in both tautomers one labeled carbon atom is incorporated as carbon-2 of the piperidine ring (m/z 83/84 vs 82/83),

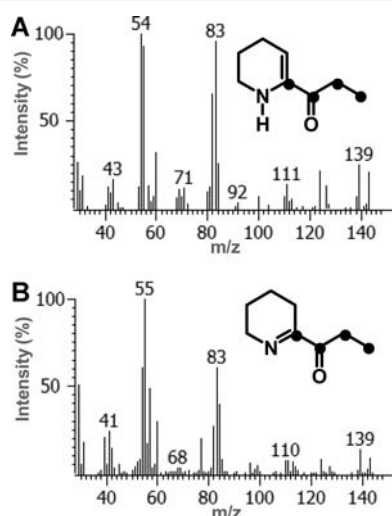


Figure 4. Mass spectra of 2-propionyl-1,4,5,6-tetrahydropyridine (**4a**, A) and 2-propionyl-3,4,5,6-tetrahydropyridine (**4b**, B) obtained from CAMOLA experiments upon extrusion: (●) carbon-13 label from [$U\text{-}^{13}\text{C}_6$]-D-glucose.

whereas the remaining three labeled carbons form the propionyl residue (m/z 60 vs 57).

The relative distribution of the different isotopomers determined from the cluster of their molecular ions (Table 2) indicated that upon extrusion 2-PTHP is almost exclusively generated from 1-pyrroline through incorporation of C_4 sugar fragments because unlabeled and 4-fold labeled isotopomers represent 97% of all isotopomers formed. To explain the formation of 2-PTHP, a mechanism analogous to the formation of 2-ATHP from 1-pyrroline and 1-hydroxy-2-propanone has been proposed, involving 1-hydroxy-2-butanone as the key intermediate.²³ Our data indicate that this mechanism could apply not only to nonsheared systems but also to sheared extruded systems.

In summary, the CAMOLA experiments permit a first insight into the formation pathways of roast-smelling pyrrolines and tetrahydropyridines upon extrusion. The obtained data indicate that the formation of these compounds upon extrusion follows pathways similar to those reported for nonsheared model systems containing D-glucose and L-proline. 2-AP (**1**) is principally formed by (i) acylation of 1-pyrroline via C_2 sugar fragments (major pathway) and (ii) via ring-opening of 1-pyrroline incorporating C_3 sugar fragments (minor pathway). 2-PP (**3**) integrates C_3 sugar fragments, whereas 2-ATHP (**2a/b**) and 2-PTHP (**4a/b**) incorporate C_3 and C_4 sugar fragments, respectively. In addition, it has been proven that the formation of **1** in low-moisture systems largely depends on the pH value. Under acidic and neutral conditions **1** mainly incorporates C_2 sugar fragments, whereas under alkaline conditions C_3 fragments were shown to contribute to the formation of the target molecule to the same extent as C_2 fragments.

The pH dependency of the different formation pathways might be a result of different ring-opening kinetics under alkaline and neutral conditions. Further understanding of this phenomenon is the subject of following studies.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

2-AP, 2-acetyl-1-pyrroline; 2-ATHP, 2-acetyl-1(or 3),4,5,6-tetrahydropyridine; 2-PP, 2-propionyl-1-pyrroline; 2-PTHP, 2-propionyl-1(or 3),4,5,6-tetrahydropyridine; CAMOLA, carbone module labeling; RI, retention index; SPME, solid phase microextraction; GC×GC, comprehensive two-dimensional gas chromatography; TOFMS, time-of-flight mass spectrometry

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