Postprint of Food Chemistry Volume 304, 30 January 2020, 125284
DOI: https://doi.org/10.1016/j.foodchem.2019.125284

Food Chem. 304 (2020) 125284

Oligomerization of reactive carbonyls in the presence of ammonia-producing compounds: A route for the production of pyridines in foods

Rosario ZAMORA, Cristina M. LAVADO-TENA, and Francisco J. HIDALGO*

Instituto de la Grasa, Consejo Superior de Investigaciones Científicas, Carretera de Utrera km 1, Campus Universitario – Edificio 46, 41013-Seville, Spain

* Corresponding author. Tel.: +34 954 611 550; fax: +34 954 616 790. *E-mail address:* fhidalgo@ig.csic.es (F. J. Hidalgo)

Running title: Formation of pyridines from reactive carbonyls

ABSTRACT

The reactions of different lipid-derived reactive carbonyls with ammonia-producing compounds were studied to investigate the formation of pyridines in foods. 2-Alkyl, 3-alkyl-, and 2,5-dialkylpyiridines were produced by oligomerization of short-chain aldehydes in the presence of ammonia. Thus, acetaldehyde/crotonaldehyde mixtures and 2,4-alkadienals were the main responsible for the formation of 2-alkylpyridines; acrolein or 2,4-alkadienals were needed for the formation of 3-alkylpyridines; and 2-alkenals were responsible for the formation of 2,5-dialkylpyridines. On the contrary, 2,6-dialkylpyridines were produced by cyclization of unsaturated ketones. Reactions pathways for formation of these pyridines are proposed, and confirmed by isotopic labelling experiments. Aldehydes and ketones required for their formation are produced in the course of lipid oxidation. Therefore, pyridine formation seems to be an additional consequence of the lipid oxidation pathway. This new knowledge can employed for the optimization of reactions to achieve the desired targeted flavor generation during food processing.

Keywords: Carbonyl-amine reactions; Food flavors; Lipid Oxidation; Maillard reaction; Pyridines; Reactive carbonyls

Chemical compounds studied in this article: Acrolein (PubChem ID: 7847); Crotonaldehyde (PubChem ID: 447466); 2,5-Dimethylpyridine (PubChem ID: 11526); 2,6-Dimethylpyridine (PubChem ID: 7937); 2,4-Heptadienal (PubChem ID: 5283321); 2-Methylpyridine (PubChem ID: 7975); 3-Methylpyridine (PubChem ID: 7970); 2-Pentenal (PubChem ID: 5364752)

1. Introduction

Carbonyl-amine reactions are responsible for the formation of numerous food flavors during processing (Liu, Yang, Yang, Ayed, Linforth, & Fisk, 2019; Zhao, Wang, Xie, Xiao, Du, Wang, Cheng, & Wang, 2019). Among them, Maillard reaction is recognized as the most important route for the formation of these compounds (Adams & De Kimpe, 2006; Cui, Yu, Xia, Duhoranimana, Huang, & Zhang, 2019; Scalone, Lamichhane, Cucu, De Kimpe, & De Meulenaer, 2019). Nevertheless, reactive carbonyls in foods are not only produced from carbohydrates. Other major food components also produce carbonyl compounds and, therefore, contribute to flavor generation by carbonyl-amine reactions (Hidalgo & Zamora, 2019). Thus, for example, lipid-derived reactive carbonyls have been shown to convert amino acids into Strecker aldehydes, α-oxoacids, and amine compounds, among other flavor-contributing substances (Hidalgo & Zamora, 2016), and quinones have been shown to promote Strecker degradation of amino acids (Delgado, Zamora, & Hidalgo, 2015). On the other hand, polyphenols, from which quinones are formed, have been shown to trap carbonyl compounds and inhibit, in this way, carbonyl-amine reactions and flavor production (Ou, Wang, Zheng, & Ou, 2019; Zamora & Hidalgo, 2016).

Because of this variety of origins, it is frequently difficult to know the carbonyl compound responsible for the formation of a specific flavor. Therefore, it is difficult the optimization of reactions to achieve the desired flavors during food processing (Paravisini & Peterson, 2019). This is especially right in the case of pyridines. These compounds, which are produced to a lower extent than pyrazines in most foods (Maga, 1981), have been found in a wide range of processed foods and their concentration has been shown to increase with temperature. In particular, they have been shown to be the best markers for evaluation of the temperature exposure in heated fish powders (Mjøs &

61 Solvang, 2006). These compounds have different flavor properties, including green,
62 bitter, astringent, roasted, or burnt, although some have more pleasant and characteristic
63 sensations (Maga, 1981).

64 In spite of their contribution to overall food flavors, their formation pathways are
65 unclear, and the role of both carbohydrates (Paravisini & Peterson, 2019) and lipids
66 (Farmer & Mottram, 1990; Horiuchi, Umano, & Shibamoto, 1998) has been suggested.
67 The hypothesis of this study was both that pyridines are produced as a consequence of
68 oligomerization of reactive carbonyls in the presence of ammonia, and that the origin
69 (and the structure) of the involved reactive carbonyl will determine the kind of pyridine
70 produced. Thus, depending on food composition and reaction conditions, some
71 pyridines would be favored over others. Hence, the objective of this research was to
72 study comparatively the ability of different reactive carbonyls to produce some of the
73 pyridines most commonly found in foods.

74 The pyridines most frequently found in food products are collected in Table S-1 of
75 the Supporting Information. As can be observed, most of them have small alkyl groups
76 at positions 2 or 3, and, when two substituents are present, positions 2 and 5, or
77 positions 2 and 6 are the preferred. This should be a consequence of both the kind of
78 compound responsible for its formation and the reaction mechanism involved. The
79 identification of the carbonyl compounds responsible for the formation of 2-alkyl, 3-
80 alkyl, 2,5-alkyl-, and 2,6-dialkylpyridines, as well as the formation pathways involved,
81 has been carried out in this study.

82 **2. Materials and methods**

83 *2.1. Materials*

As reactive carbonyls, 2-alkenals (acrolein [2-propenal], crotonaldehyde [2-butenal], 2-pentenal, 2-hexenal, 2-heptenal, and 2-octenal) and 2,4-alkadienals (2,4-hexadienal, 2,4-heptadienal, 2,4-octadienal, 2,4-nonadienal, and 2,4-decadienal) were employed. In addition, other compounds were also tested as carbonyl compounds or precursors of carbonyl compounds, including menhaden oil, acetaldehyde (ethanal), propionaldehyde (propanal), butyraldehyde (butanal), 3,5-heptadien-2-one, 6-methyl-5-hepten-2-one, and alanine. Also, different compounds were tested as ammonia producers, including ammonia, ammonium chloride, glutamine, urea, and creatinine.

The following standard pyridines were purchased for identification and quantification purposes: 2-methylpyridine, 3-methylpyridine, 4-methylpyridine, 2-ethylpyridine, 3-ethylpyridine, 2-pentylpyridine, 2,5-dimethylpyridine, 2,6-dimethylpyridine, 5-ethyl-2-methylpyridine, 3-acetylpyridine, and 3-hydroxy-2-methylpyridine.

All these compounds, as well as other chemicals used in this research were of the highest available grade and were purchased from reliable commercial sources including: Sigma-Aldrich (St. Louis, MO), Merck (Darmstadt, Germany), Alfa Aesar (Haverhill, Massachutsetts), or TCI (Tokyo, Japan).

2.2. Oligomerization of reactive carbonyls in the presence of ammonia-producing compounds

Mixtures of the reactive carbonyl(s) (50 μmol in 50 μL of methanol) and the ammonia-producing compound (10 μmol in 45 μL of water) were singly homogenized with 0.063–0.20 mm silica gel (300 mg) (Macherey-Nagel, Düren, Germany), which was employed as a support (Hidalgo, Delgado, & Zamora, 2009), 30 μL of 0.3 mol/L sodium phosphate, pH 6.5, and 50 μL of water. Samples were heated at 180 ºC in closed

test tubes for 1 h. After cooling, 700 μL of methanol and 30 μL of the internal standard solution (19 μmol of methyl heptanoate per mL of methanol) were added. Suspensions were stirred for 1 min and centrifuged for 5 min at 2000 *g*. The supernatant was studied by gas chromatography coupled to mass spectrometry (GC-MS).

When mixtures of two reactive carbonyls were studied, 50 μmol of each one was dissolved in the 50 μL of methanol. When alanine was added in addition to the ammonia producing compound, this amino acid was dissolved in the 50 μL of water. Therefore, all studied samples had always the same amount of solvents.

Reactions involving menhaden oil were carried out similarly, although some modifications were required. Briefly, mixtures of the oil (1 g), glutamine (30 μmol in 150 μL of water), 25 μL of methanol, and 30 μL of 0.3 mol/L sodium phosphate, pH 6.5, were heated at 180 ºC in closed test tubes for 1 h. After cooling, 700 μL of acetonitrile and 30 μL of the internal standard solution (19 μmol of methyl heptanoate per mL of methanol) were added. Suspensions were stirred for 1 min and centrifuged for 5 min at 2000 *g*. The supernatant was studied by gas chromatography coupled to mass spectrometry (GC-MS).

2.3. Isotopic labelling

Deuteration experiments were carried out to obtain further insight into reaction pathways. In these experiments, deuterated water (D_2O) was employed in the place of water and produced pyridines were studied by GC-MS.

2.4. GC-MS analyses

The equipment employed for GC-MS analyses was an Agilent 7820A gas chromatograph coupled with an Agilent 5977 mass selective detector, quadrupole type

(Agilent Technologies, Santa Clara, CA). The pulsed splitless mode was used to inject one microliter of sample, which was fractionated on a fused-silica HP-5MS UI capillary column (30 m length, 0.25 mm inner diameter, 0.25 μm coating thickness). The conditions employed were: carrier gas, helium (1 mL/min at constant flow); injector, 250 ºC; transfer line to mass selective detector, 280 ºC; electron ionization (EI), 70 eV; ion source temperature, 230 ºC; and mass range, 28-550 amu. The oven was programmed from 40 ºC (3 min) to 200 ºC at 20 ºC/min, and then held at 200 ºC for 1 min.

2.5. Determination of pyridines

Pyridines were quantified by preparing standard curves of 2-methylpyridine, 3-methylpyridine, 2-ethylpyridine, 3-ethylpyridine, 2-pentylpyridine, 2,5-dimethylpyridine, 2,6-dimethylpyridine, 5-ethyl-2-methylpyridine, 3-acetylpyridine, and 3-hydroxy-2-methylpyridine, which were determined after being added to 300 mg of silica gel and following the same procedure described above (without heating). Seven amounts of pyridines (0–10 μmol) were used for each compound. Compound content was directly proportional to compound/internal standard area ratio ($r > 0.99$, $p < 0.001$). RSD was always < 10%.

In addition to the employed standards, other pyridines were also determined. Because standards were not available for these pyridines and calibration curves could not be prepared, their concentration was estimated by using the calibration curve of the compound with the closest chemical structure. Thus, for example, the calibration curve of 2-ethylpyridine was employed for determining 2-propylpyridine, and the calibration curve of 2-pentylpyridine was employed for determining 2-butylpyridine.

2.6. Statistical analysis

All data are mean ± SD values of, at least, three independent experiments. For comparison of the mean values obtained, analysis of variance was employed. When *F* values were significantly different, group differences were evaluated by the Tukey test (Snedecor & Cochran, 1980). Statistical comparisons were carried out using Origin® v. 7.0 (OriginLab Corporation, Northampton, MA). The significance level is $p < 0.05$ unless otherwise indicated.

3. Results and Discussion

3.1. Formation of pyridines by oligomerization of crotonaldehyde in the presence of ammonia-producing compounds

Preliminary experiments showed that the heating of lipid-derived reactive carbonyls in the presence of amine compounds produced pyridines (data not shown). Pyridines were formed to different extents depending on the amine and carbonyl compounds involved. This formation was hypothesized to be produced by oligomerization of the reactive carbonyl in the presence of amine compounds, analogously to the previously described formation of pyrrole derivatives (Hidalgo & Zamora, 2004).

The role of ammonia as the compound responsible for this oligomerization was firstly confirmed. Thus, crotonaldehyde (a lipid oxidation product) was heated in the presence of ammonia and different ammonia-producing compounds. As observed in Table 1, the same four pyridines (2-methyl, 3-methyl-, 2,5-dimethyl, and 5-ethyl-2-methylpyridine) were always produced for the different amine compounds assayed. These pyridines have been found in processed foods (Table S-1 of the Supplementary Material) and, to the best of our knowledge, their formation has not been clearly linked to the lipid oxidation pathway.

178 Obtained results suggested that ammonia was the responsible for crotonaldehyde
179 oligomerization. Thus, all assayed compounds produce ammonia upon heating (Chen,
180 Xing, Chin, & Ho, 2000; Riha, Izzo, Zhang, & Ho, 1996; Zamora, Alcon, & Hidalgo,
181 2014). In addition, crotonaldehyde oligomerization was not produced in the absence of
182 the amine compound. Moreover, with the exception of the nitrogen atom, the
183 incorporation of other parts of the amine compound into the structure of the produced
184 pyridine was not observed because the same pyridines were always produced in spite of
185 the structural differences among the different amino compounds assayed.

186 Although the same pyridines were always produced, their amounts and the
187 proportions among them were different for the different amino compounds. Thus, the
188 highest amount of pyridines was produced in the presence of ammonia (almost 50% of
189 the initial nitrogen was incorporated into the produced pyridines). A similar amount of
190 pyridines was produced in the presence of urea. In addition, pyridines were also
191 produced to a high extent in the presence of glutamine or ammonium chloride. On the
192 other hand, heating of crotonaldehyde in the presence of creatinine produced the lowest
193 amount of pyridines among the assayed amino compounds (Table 1). Because pyridines
194 were produced to a high extent in the presence of glutamine and this is a common food
195 component, this amino acid was selected for the rest of this study.

196 *3.2. Formation of 2-alkylpyridines by oligomerization of reactive carbonyls in the*
197 *presence of glutamine*

198 Different 2-alkylpyridines were produced when reactive carbonyls and their mixtures
199 were heated in the presence of glutamine (Table 2). The produced 2-alkylpyridine and
200 the reaction yield depended on the reactive carbonyl compound involved. However, all
201 2-alkylpyridines were produced similarly (see below).

Table 2 shows that 2-methylpyridine was produced by many reactive carbonyls (and their mixtures) in the presence of glutamine. However, the presence of any of three reactive carbonyls (hexadienal, acetaldehyde, or crotonaldehyde) was required for its formation, and the main reaction yield (50.21 ± 1.66 μmol/mmol of glutamine) was obtained when a mixture of acetaldehyde and crotonaldehyde was employed as reactive carbonyl compounds. The reason for this behavior is related to the formation pathways.

Figure 1 shows a possible reaction pathway for the formation of 2-methylpyridine. As can be observed, acetaldehyde, crotonaldehyde, and hexadienal are, all of them, precursors of 2-methylpyridine because these carbonyls are interconverted among them. Thus, aldol condensation of acetaldehyde produces crotonaldehyde in a first step and then hexadienal (this last compound was always observed in the chromatogram when acetaldehyde or crotonaldehyde was heated under the reaction conditions described in the Materials and Methods section). In addition, previous studies showed that 2,4-alkadienals are degraded into 2-alkenals and alkanals, and 2-alkenals into alkanals (Zamora, Navarro, Aguilar & Hidalgo, 2015). The higher reactivity of the mixture between crotonaldehyde and acetaldehyde is explained because ammonia is likely to be added to crotonaldehyde and the formed amine reacts with acetaldehyde. A cyclization of the formed adduct is responsible for the formation of 2-methylpyridine. A proof of this reaction pathway was obtained when water was replaced by deuterated water in the reaction mixture. In this case, a di-deuterated 2-methylpyridine was obtained. As shown in Figure 1, formation of this pyridine is explained by deuteration of interchangeable protons during ring formation.

In addition to this main mechanism, an alternative mechanism can be suggested for 2,4-hexadienal (Figure 1). In this case, the formation of the corresponding imine is

226 followed by the formation of the pyridine ring. This last reaction pathway was already
227 previously proposed (Kim & Ho, 1998).

228 Similar reaction mechanisms can be suggested for the formation of other 2-
229 alkylpyridines. Thus, 2-ethylpyridine (30.95 ± 0.34 μmol/mmol Gln) was produced
230 from 2,4-heptadienal, 2-propylpyridine (27.15 ± 1.82 μmol/mmol Gln) was produced
231 from 2,4-octadienal, 2-butylpyridine (22.42 ± 1.03 μmol/mmol Gln) was produced from
232 2,4-nonadienal, and 2-pentylpyridine (14.68 ± 0.58 μmol/mmol Gln) was produced
233 from 2,4-decadienal. As observed, there was a decrease of the amount of pyridine
234 produced when the chain length of the reactive carbonyl increased.

235 Analogously, 2-alkylpridines were also produced when mixtures of 2-alkenals and
236 acetaldehyde were heated in the presence of glutamine. Thus, the mixture of 2-pentenal
237 and acetaldehyde produced 2-ethylpyridine (8.81 ± 1.54 μmol/mmol Gln), and the
238 mixture of 2-hexenal and acetaldehyde produced 2-propylpyridine (15.61 ± 1.04
239 μmol/mmol Gln).

240 Finally, acetaldehyde precursors, such as alanine, also produced 2-alkylpyridines, but
241 usually to a much lower extent (Table 2).

242 *3.3. Formation of 3-alkylpyridines by oligomerization of reactive carbonyls in the*
243 *presence of glutamine*

244 Analogously to the formation of 2-alkylpyridines, different 3-alkylpyridines were
245 also produced in the reaction of reactive carbonyls and glutamine, and the kind of 3-
246 alkylamine produced and the reaction yield also depended on the reactive carbonyl
247 involved.

248 Differently to the formation of 2-methylpyridine, the main carbonyl compound
249 responsible for the formation of 3-methylpyridine was acrolein. In fact, the mixture of

acrolein and propanal produced 67.02 ± 4.18 μmol/mmol Gln, followed by the mixture of acrolein and acetaldehyde (45.05 ± 0.82 μmol/mmol Gln) and the mixture of acrolein and alanine (30.06 ± 0.65 μmol/mmol Gln). Acrolein alone also produced 3-methylpyridine, but to a lower extent (17.46 ± 1.47 μmol/mmol Gln). Finally, 2,4-hexadienal also produced 3-methylpyridine to a significant extent (12.06 ± 1.40 μmol/mmol Gln), although to a lower extent than 2-methylpyridine. This ability of 2,4-hexadienal to produce 3-methylpyridine might be related to the described decomposition of the 2,4-alkadienal into acetaldehyde (Zamora, Navarro, Aguilar, & Hidalgo, 2015) and acrolein (Ewert, Granvolg, & Schieberle, 2014).

A reaction pathway that explains the formation of 3-methylpyridine by heating acrolein and propanal in the presence of glutamine is shown in Figure 2. The reaction would be initiated by the addition of ammonia to acrolein and the later formation of an imine with propanal. The cyclization of the produced adduct would be the origin of the 3-methylpyridine. Analogously to the above described for 2-methylpyridine, this pathway was confirmed by carrying out the reaction in the presence of deuterated water. In this case, the mono-deuterated pyridine was mainly produced.

Differently to the mixture acrolein/propanal, the formation of 3-methylpyridine in mixtures of acrolein/acetaldehyde or acrolein/alanine (alanine is a precursor of acetaldehyde, Hofmann, Münch, & Schieberle, 2000) is not so clear because one additional carbon is required. One possibility is the dimerization of acetaldehyde followed by the loss of one carbon (perhaps as formaldehyde or formic acid), and this product would then react with acrolein or the adduct acrolein/ammonia. Further studies are required to explain this result, which is also likely related to the formation of 3-alkylpyridines from 2,4-alkadienals. Thus, in addition to the formation of 3-methylpyridine from 2,4-hexadienal, 3-ethylpyridine was produced from 2,4-

heptadienal, 3-propylpyridine was produced from 2,4-octadienal, 3-butylpyridine was produced from 2,4-nonadienal, and 3-pentylpyridine was produced from 2,4-decadienal. As described above, this formation is likely a consequence of being the alkadienal the origin of both alkanals and acrolein.

3.4. Formation of 2,5-dialkylpyridines by oligomerization of reactive carbonyls in the presence of glutamine

Although 2,5-dimethylpyridine was produced in the presence of only one reactive carbonyl (acetaldehyde or crotonaldehyde), much higher contents of this pyridine were found when a couple of reactive carbonyls were present, in particular the mixture of propanal and crotonaldehyde. The formation of 2,5-dimethylpyridine in the presence of propanal and crotonaldehyde is suggested to take place as shown in Figure 3. The reaction would be initiated by addition of ammonia to crotonaldehyde to produce 3-aminopropanal, which would then react with propanal. The later cyclization of the produced adduct would be the origin of 2,5-dimethylpyridine, analogously to that described for other pyridines. A proof of this pathway was the formation of the mono-deuterated pyridine when the reaction was carried out in the presence of deuterated water.

This pathway is also valid for the formation of 2,5-dimethylpyridine from other reactive carbonyls. Thus, for example, thermal decomposition of 2,4-heptadienal produces propanal (Zamora, Navarro, Aguilar, & Hidalgo, 2015). Therefore, the mixture of 2,4-heptadienal and crotonaldehyde should be a source of 2,5-dimethylpyridine (as observed). Acetaldehyde produced 2,5-dimethylpyridine because of its conversion into crotonaldehyde. In addition, as described in the previous section, acetaldehyde should be able to be converted into a three-carbon aldehyde to some extent and 2,5-dimethylpyridine should be produced in that way.

300 This pathway is also valid for the formation of other 2,5-dialkylpyridines. Thus,
301 when propanal was substituted by 2-pentenal, the mixture 2-pentenal/crotonaldehyde
302 produced 2,5-diethylpyridine to a significant extent (19.09 ± 2.13 μmol/mmol Gln). In
303 this case, the ammonia is firstly added to 2-pentenal and the amine produced reacts then
304 with crotonaldehyde producing the corresponding imine in a first step and, after
305 cyclization, the 2,5-diethylpyridine.

306 Finally, this pathway is also valid for the formation of 2,5-dialkylpyridines with
307 different substituents. These compounds are produced by oligomerization of 2-alkenals
308 in the absence of other reactive carbonyls. This kind of pyridines were produced to a
309 high extent. Thus, 5-ethyl-2-methylpyridine (321.6 ± 11.9 μmol/mmol Gln) was
310 produced by oligomerization of crotonaldehyde, 2-ethyl-5-propylpyridine (101.9 ± 12.5
311 μmol/mmol Gln) was produced by oligomerization of 2-pentenal, 5-butyl-2-
312 propylpyridine (156.9 ± 18.0 μmol/mmol Gln) was produced by oligomerization of 2-
313 hexenal, 2-butyl-5-pentylpyridine (257.8 ± 9.3 μmol/mmol Gln) was produced by
314 oligomerization of 2-heptenal, and 5-hexyl-2-pentylpyridine (336.6 ± 43.9 μmol/mmol
315 Gln) was produced by oligomerization of 2-octenal.

316 The oligomerization pathway of 2-alkenals to produce 2,5-dialkylpyridines is shown
317 in Figure S-1 (Supplementary Material). The mechanism is analogous to that collected
318 in Figure 3, but there are significant differences that can explain the much higher yield
319 obtained for these pyridines. The first one is the existence of an additional double bond
320 that facilitates the formation of the extended conjugated system before the cyclization of
321 the molecule. The second one is that the oxidation step is not required. The consequence
322 is that the observed yields for this kind of pyridines were 10–34%, and the best yield
323 obtained for 2,5-dimethylpyridine was 8%.

3.5. Formation of 2,6-dialkylpyridines by oligomerization of reactive carbonyls in the presence of glutamine

Differently to the other determined pyridines, 2,6-dialkylpyridines were not produced by oligomerization reactions of short-chain aldehydes but by cyclization of ketones. As collected in Table 2, 2,6-dimethylpyridine was mainly produced from 3,5-heptadien-2-one in the presence of glutamine, although it was also produced by the heating of 6-methyl-5-hepten-2-one in the presence of glutamine. A reaction pathway for the formation of 2,6-dimethylpyridine from 2,4-heptadien-6-one is shown in Figure 4. The reaction can be initiated either by formation of the corresponding imine with ammonia and then cyclization, or by addition of ammonia to the γ,δ carbon-carbon double bond and then the formation of the imine. In any case, a later oxidation of the cyclic intermediate would produce the corresponding 2,6-dimethylpyridine.

Analogously to other pyridines, the proposed reaction pathway was tested by carrying out the reaction in the presence of deuterated water. Although Figure 4 only shows one mono-deuterated pyridine, the existence of other poly-deuterated derivatives is also possible because the methyl group can interchange until three protons. In addition, deuteration of aromatic protons at position 3 and 5 can also occur. Thus, when the reaction was carried out in the presence of deuterated water, mono- and di-deuterated 2,6-dimethylpyridines were detected to a high extent. Also, small amounts of tri-deuterated 2,6-dimethylpyridine were also produced.

This reaction pathway can also be valid for the formation of 2,6-dimethylpyridine from 6-methyl-5-hepten-2-one, but in this case the formation of the pyridine ring implied the exit of the methyl group at position 6 of the alkyl chain of the ketone. The fate of this methyl group was not investigated.

3.6. Lipid oxidation as an origin of 2-alkyl-, 3-alkyl-, 2,5-dialkyl-, and 2,6-dialkylpyridines in foods

As described previously, the reactive carbonyls that play a major role in the formation of 2-alkyl-, 3-alkyl-, 2,5-dialkyl-, and 2,6-dialkylpyridines are reactive carbonyls commonly produced in the course of the lipid oxidation pathway. Thus, 2-alkylpyridines are mostly produced by acetaldehyde/crotonaldehyde mixtures or alkadienals; 3-alkylpyridines are mostly produced by mixtures involving acrolein; 2,5-dialkylpyridines are mostly produced by 2-alkenals or mixtures between 2-alkenals and alkanals; and 2,6-dialkylpyridines are produced by cyclization of unsaturated ketones. All of these reactive carbonyls are produced by oxidative degradation of fatty acyl chains. Thus, for example, acrolein is a major oxidation product formed during oil frying (Sordini, Veneziani, Servili, Esposto, Selvaggini, Lorefice, & Taticchi, 2019); crotonaldehyde is present in heat-processed edible fats and oils as well as food (Granvogl, 2014); 2,4-alkadienals play a major role in the overall aroma of chicken broth (Feng, Cai, Fu, Zheng, Xiao, & Zhao, 2018); and 6-methyl-5-hepten-2-one has been related to the light-induced off-flavor development in cloudy apple juice (Hashizume, Gordon, & Mottram, 2007). Therefore, lipid oxidation should have been produced in foods where alkylpyridines have been detected. As shown in Table S-1 of the Supporting Information, most of foods where alkylpyridines appeared are susceptible to oxidation. Furthermore, most of these foods are rich in polyunsaturated fatty acids prone to be oxidized. Moreover, pyridines have been suggested as chemical markers for evaluation of the temperature exposure of heated fish powders where ω3 fatty acyl chains can be easily oxidized (Mjøs & Solvang, 2006)

To confirm the involvement of lipid oxidation in the formation of alkylpyridines, menhaden oil was heated in the presence of glutamine. The appearance of hexanal in the

oil was parallel to the formation of pyridines. Among them, 3-methylpyridine (34.13 ± 6.61 μmol/mmol of amine compound) was the main pyridine detected, most likely because of the high amount of acrolein produced as a consequence of lipid oxidation (Shibata, Uemura, Hosokawa, & Miyashita, 2018). 2-Ethylpyridine (4.34 ± 0.45 μmol/mmol of amine compound) was also detected to a significant extent, most likely because of the formation of 2,4-heptadienal as a consequence of ω3 fatty acyl chain breakage (Nogueira, Scolaro, Milne, & Castro, 2019).

4. Conclusions

Obtained results have confirmed that pyridines are produced as a consequence of oligomerization of reactive carbonyls in the presence of ammonia. Therefore, the origin (and the structure) of the involved reactive carbonyl will determine the kind of pyridine produced. Thus, acetaldehyde/crotonaldehyde mixtures and alkadienals are mainly responsible for the formation of 2-alkylpyridines; acrolein or alkadienals are needed for the formation of 3-alkylpyridines; 2-alkenals are mostly responsible for the formation of 2,5-dialkylpyridines; and unsaturated ketones are responsible for the formation of 2,6-dialkylpyridines. All these carbonyl compounds are produced in the course of lipid oxidation. Therefore, formation of 2-alkyl, 3-alkyl, 2,5-dialkyl, and 2,6-dialkylpyridines seems to be an additional consequence of the lipid oxidation pathway. Because phenolic compounds have been shown to have lipid-derived reactive carbonyl-trapping abilities (Zamora & Hidalgo, 2016), these compounds might be employed to modulate the formation of pyridines in foods.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

We are indebted to José L. Navarro for technical assistance. This study was supported in part by the European Union (FEDER funds), the Plan Nacional de I + D of the Ministerio de Economía y Competitividad of Spain (project AGL2015-68186-R), and the Programa Estatal de I+D+I of the Ministerio de Ciencia, Innovación y Universidades of Spain (project RTI2018-096632-B-100).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at

405 **References**

406 Adams, A., & De Kimpe, N. (2006). Chemistry of 2-acetyl-1-pyrroline, 6-acetyl-
407 1,2,3,4-tetrahydropyridine, 2-acetyl-2-thiazoline, and 5-acetyl-2,3-dihydro-4*H*-
408 thiazine: Extraordinary Maillard flavor compounds. *Chemical Reviews, 106*, 2299–
409 2319.

410 Chen, Y., Xing, J. S., Chin, C. K., & Ho, C. T. (2000). Effect of urea on volatile
411 generation from Maillard reaction of cysteine and ribose. *Journal of Agricultural and*
412 *Food Chemistry, 48*, 3512–3516.

413 Cui, H. P., Yu, J. Y., Xia, S. Q., Duhoranimana, E., Huang, Q. R., & Zhang, X. M.
414 (2019). Improved controlled flavor formation during heat-treatment with a stable
415 Maillard reaction intermediate derived from xylose-phenylalanine. *Food Chemistry,*
416 *271*, 47–53.

417 Delgado, R. M., Zamora, R., & Hidalgo, F. J. (2015). Contribution of phenolic
418 compounds to food flavors: Strecker-type degradation of amines and amino acids
419 produced by *o*- and *p*-diphenols. *Journal of Agricultural and Food Chemistry, 63*,
420 312–318.

421 Ewert, A., Granvolg, M., & Schieberle, P. (2014). Isotope-labeling studies on the
422 formation pathway of acrolein during heat processing of oils. *Journal of Agricultural*
423 *and Food Chemistry, 62*, 8524–8529.

424 Farmer, L. J., & Mottram, D. S. (1990). Interaction of lipid in the Maillard reaction
425 between cysteine and ribose: the effect of a triglyceride and three phospholipids on
426 the volatile products. *Journal of the Science of Food and Agriculture, 53*, 505–525.

427 Feng, Y., Cai, Y., Fu, X., Zheng, K., Xiao, Z., & Zhao, M. (2018). Comparison of
428 aroma-active compounds in broiler broth and native chicken broth by aroma extract

dilution analysis (AEDA), odor activity value (OAV) and omission experiment. *Food Chemistry*, *265*, 274–280.

Granvogl, M. (2014). Development of three stable isotope dilution assays for the quantitation of (*E*)-2-butenal (crotonaldehyde) in heat-processed edible fats and oils as well as in food. *Journal of Agricultural and Food Chemistry*, *62*, 1272–1282.

Hashizume, M., Gordon, M. H., & Mottram, D. S. (2007). Light-induced off-flavor development in cloudy apple juice. *Journal of Agricultural and Food Chemistry, 55*, 9177–9182.

Hidalgo, F. J., & Zamora, R. (2004). Strecker-type degradation produced by the lipid oxidation products 4,5-epoxy-2-alkenals. *Journal of Agricultural and Food Chemistry, 52*, 7126–7131.

Hidalgo, F. J., & Zamora, R. (2016). Amino acid degradations produced by lipid oxidation products. *Critical Reviews in Food Science and Nutrition, 56*, 1242–1252.

Hidalgo, F. J., & Zamora, R. (2019). Formation of phenylacetic acid and benzaldehyde by degradation of phenylalanine in the presence of lipid hydroperoxides: new routes in the amino acid degradation pathways initiated by lipid oxidation products. *Food Chemistry X, 2*, 100037.

Hidalgo, F. J., Delgado, R. M., & Zamora, R. (2009). Degradation of asparagine to acrylamide by carbonyl-amine reactions initiated by alkadienals. *Food Chemistry, 116*, 779–784.

Hofmann, T., Münch, P., & Schieberle, P. (2000). Quantitative model studies on the formation of aroma-active aldehydes and acids by Strecker-type reactions. *Journal of Agricultural and Food Chemistry, 48*, 434–440.

452 Horiuchi, M., Umano, K., & Shibamoto, T. (1998). Analysis of volatile compounds
453 formed from fish oil heated with cysteine and trimethylamine oxide. *Journal of*
454 *Agricultural and Food Chemistry, 46*, 5232–5237.

455 Kim, Y.-S., & Ho, C.-T. (1998). Formation of pyridines from thermal interaction of
456 glutamine or glutamic acid with a mixture of alkadienals in aqueous and oil media.
457 *Journal of Food Lipids, 5*, 173–182.

458 Liu, C., Yang, N., Yang, Q., Ayed, C., Linforth, R., & Fisk, I. D. (2019). Enhancing
459 Robusta coffee aroma by modifying flavor precursors in the green coffee bean. *Food*
460 *Chemistry, 281*, 8–17.

461 Maga, J. A. (1981). Pyridines in foods. *Journal of Agricultural and Food Chemistry, 29*,
462 895–898.

463 Mjøs, S. A., & Solvang, M. (2006). Patterns in volatile components over heated fish
464 powders. *Food Research International, 39*, 190–202.

465 Nogueira, M. S., Scolaro, B., Milne, G. L., & Castro, I. A. (2019). Oxidation products
466 from omega-3 and omega-6 fatty acids during a simulated shelf life of edible oils.
467 *LWT-Food Science and Technology, 101*, 113–122.

468 Ou, J., Wang, M., Zheng, J., & Ou, S. (2019). Positive and negative effects of
469 polyphenol incorporation in baked foods. *Food Chemistry, 284*, 90–99.

470 Paravisini, L., & Peterson, D. G. (2019). Reactive carbonyl species as key control point
471 for optimization of reaction flavors. *Food Chemistry, 274*, 71–78.

472 Riha, W. E., Izzo, H. V., Zhang, J., & Ho, C. T. Nonenzymatic deamidation of food
473 proteins. *Critical Reviews in Food Science and Nutrition, 36*, 225–255.

474 Scalone, G. L. L., Lamichhane, P., Cucu, T., De Kimpe, N., & De Meulenaer, B.
475 (2019). Impact of different enzymatic hydrolysates of whey protein on the formation
476 of pyrazines in Maillard model systems. *Food Chemistry, 278*, 533–544.

477 Shibata, A., Uemura, M., Hosokawa, M., & Miyashita, K. (2018). Acrolein as a major
478 volatile in the early stages of fish oil TAG oxidation. *Journal of Oleo Science, 67*,
479 515–524.

480 Snedecor, G. W., & Cochran, W. G. (1980). *Statistical methods* (7th ed.). Ames: IA:
481 Iowa State University Press.

482 Sordini, B., Veneziani, G., Servili, M., Esposto, S., Selvaggini, R., Lorefice, A., &
483 Taticchi, A. (2019). A quanti-qualitative study of a phenolic extract as a natural
484 antioxidant in the frying processes. *Food Chemistry, 279*, 426–434.

485 Zamora, R., & Hidalgo, F. J. (2016). The triple defensive barrier of phenolic
486 compounds against the lipid oxidation-induced damage in food products. *Trends in*
487 *Food Science & Technology, 54*, 165–174.

488 Zamora, R., Alcon, E., & Hidalgo, F. J. (2014). Ammonia and formaldehyde participate
489 in the formation of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in
490 addition to creati(ni)ne and phenylacetaldehyde. *Food Chemistry, 155*, 74–80.

491 Zamora, R., Navarro, J. L., Aguilar, I, & Hidalgo, F. J. (2015). Lipid-derived aldehyde
492 degradation under thermal conditions. *Food Chemistry, 174*, 89–96.

493 Zhao, J., Wang, T. Z., Xie, J. C., Xiao, Q. F., Du, W. B., Wang, Y. X., Cheng, J., &
494 Wang, S. (2019). Meat flavor generation from different composition patterns of
495 initial Maillard stage intermediates formed in heated cysteine-xylose-glycine reaction
496 systems. *Food Chemistry, 274*, 79–88.

497

Table 1

Pyridines produced by crotonaldehyde oligomerization in the presence of ammonia and ammonia-producing compounds

ammonia-producing compound	pyridine (μmol/mmol of amine compound)			
	2-methyl pyridine	3-methyl pyridine	2,5-dimethyl pyridine	5-ethyl-2-methyl pyridine
ammonia	22.89 ± 7.01 a	1.10 ± 0.13 a	4.44 ± 0.96 a,b	463.3 ± 81.1 a
ammonium chloride	9.56 ± 1.52 b,c	4.41 ± 0.98 b	2.04 ± 0.66 c,d	258.1 ± 37.4 b
glutamine	14.96 ± 0.33 c	3.34 ± 0.66 b	3.66 ± 0.70 b,d	321.6 ± 11.9 b,d
creatinine	7.59 ± 2.34 b,c	1.11 ± 0.31 a	2.09 ± 0.39 c,d	119.8 ± 6.9 c
urea	27.51 ± 4.10 a	1.82 ± 0.28 a	5.70 ± 1.10 a,b	388.9 ± 59.0 a,d

Values are mean ± standard deviation (SD) for, at least, three independent experiments.

Means in the same column with a different letter are significantly different ($p < 0.05$).

503 **Table 2**

504 Pyridines produced by reactive carbonyl oligomerization in the presence of glutamine

Pyridine	RI	Identification	Amount (μmol/mmol glutamine)	Carbonyl compound or precursor
2-methyl	814	RI, MS, ST	9.93 ± 1.35 a	CROT
			34.07 ± 1.33 b	HxD
			13.90 ± 2.79 a,c	ACET
			16.58 ± 1.04 c	CROT/HxD
			1.55 ± 0.26 d	ACET/ACR
			50.21 ± 1.66 e	ACET/CROT
			2.46 ± 0.59 d	PROP/CROT
			1.59 ± 0.51 d	ACR/Ala
3-methyl	863	RI, MS, ST	17.46 ± 1.47 a	ACR
			12.06 ± 1.40 a,b	HxD
			2.37 ± 0.82 c,d	ACET
			3.56 ± 0.46 c,d	PROP
			0.71 ± 0.08 c,d	Ala
			11.04 ± 3.81 a,b	ACR/CROT
			4.92 ± 1.03 b,d	ACR/PENT
			7.08 ± 1.05 b,d	ACR/HxD
			5.49 ± 1.44 b,d	CROT/HxD
			45.05 ± 0.82 e	ACET/ACR
			1.40 ± 0.04 c,d	ACET/CROT
			67.02 ± 4.18 f	PROP/ACR
			1.70 ± 0.36 c,d	PROP/CROT
			30.06 ± 0.65 g	ACR/Ala
			5.23 ± 1.79 b,d	BUT/ACR
2,6-dimethyl	889	RI, MS, ST	26.93 ± 1.38 a	HDO
			3.48 ± 0.47 b	MHDO
2-ethyl	909	RI, MS, ST	30.95 ± 0.34 a	HpD
			1.85 ± 0.33 b	ACR/HpD
			19.01 ± 0.50 c	CROT/HpD
			8.81 ± 1.54 d	ACE/PENT
2,5-dimethyl	938	RI, MS, ST	8.61 ± 1.43 a	ACET
			0.75 ± 0.04 b	PROP
			5.13 ± 0.56 c	ACR/CROT
			3.66 ± 0.39 d	CROT/HpD
			1.35 ± 0.47 b	ACET/ACR

			7.22 ± 0.31 a	ACET/CROT
			82.82 ± 0.75 e	PROP/CROT
			0.85 ± 0.08 b	ACR/Ala
3-ethyl	965	RI, MS, ST	17.85 ± 0.59 a	HpD
			15.93 ± 4.43 a,b	ACET
			4.89 ± 1.69 c,d	ACR/CROT
			8.60 ± 0.80 c,e	CROT/HpD
			5.91 ± 0.66 c,d	ACET/ACR
			1.92 ± 0.08 d	ACET/CROT
			2.63 ± 0.31 d	ACR/Ala
			10.99 ± 2.05 b,e	BUT/ACR
2-propyl	1003	MS	27.15 ± 1.82 a	OD
			15.61 ± 1.04 b	ACE/HEX
5-ethyl-2-methyl	1034	RI, MS, ST	321.6 ± 11.9 a	CROT
			32.63 ± 1.51 b,c	ACET
			2.55 ± 0.96 d	ACR/CROT
			24.33 ± 2.14 b	CROT/PENT
			63.77 ± 3.05 e	CROT/HxD
			43.58 ± 3.79 c	CROT/HpD
			171.5 ± 12.5 f	ACET/CROT
			33.32 ± 8.76 b,c	PROP/CROT
3-propyl	1060	MS	12.12 ± 1.38	OD
2-butyl	1102	MS	22.42 ± 1.03	ND
2,5-diethyl	1123	MS	19.09 ± 2.13	CROT/PENT
3-butyl	1163	MS	12.18 ± 0.33	ND
2-pentyl	1205	RI, MS, ST	14.68 ± 0.58	DD
2-ethyl-5-propyl	1211	MS	101.9 ± 12.5 a	PENT
			1.83 ± 0.25 b	ACR/PENT
			29.95 ± 8.44 c	CROT/PENT
			1.34 ± 0.06 b	ACR/CROT/ PENT
3-pentyl	1263	MS	8.42 ± 0.53	DD
5-butyl-2-propyl	1398	MS	156.9 ± 18.0 a	HEX
			82.24 ± 10.94 b	ACE/HEX
2-butyl-5-pentyl	1599	MS	257.8 ± 9.3	HEP
5-hexyl-2-pentyl	1806	MS	336.6 ± 43.9	OCT

505 Values are mean ± standard deviation (SD) for, at least, three independent experiments.
506 For each pyridine, means in the same column with a different letter are significantly
507 different ($p < 0.05$). Methods employed for identification: RI, retention index; MS, mass
508 spectrum; ST, co-elution with a reference compound. Identifications carried out

509 exclusively on the basis of MS should be considered only tentative. Abbreviations:
510 ACET, acetaldehyde; ACR, acrolein; Ala, alanine; BUT, butyraldehyde; CROT,
511 crotonaldehyde; DD, 2,4-decadienal; HDO, 3,5-heptadien-2-one; HEP, 2-heptenal;
512 HEX, 2-hexenal; HpD, 2,4-heptadienal; HxD, 2,4-hexadienal; MHDO, 6-methyl-3,5-
513 heptadien-2-one; ND, 2,4-nonadienal; OCT, 2-octenal; OD, 2,4-octadienal; PENT, 2-
514 pentenal; PROP, propanal.

515

Figure legends

Fig. 1. Proposed pathways for the formation of 2-methylpyridine from crotonaldehyde and its precursors (alanine and acetaldehyde) or from 2,4-hexadienal in the presence of ammonia (or ammonia-producing compounds). When the reaction is carried out in the presence of D_2O, the corresponding di-deuterated pyridine is produced. The stereochemistry of aldimines should be *E*. They have been drawn in the *Z* form to better understand the reaction mechanism.

Fig. 2. Proposed pathway for the formation of 3-methylpyridine from acrolein and propanal. When the reaction is carried out in the presence of D_2O, the corresponding mono-deuterated pyridine is produced. The stereochemistry of aldimines should be *E*. They have been drawn in the *Z* form to better understand the reaction mechanism.

Fig. 3. Proposed pathway for the formation of 2,5-dimethylpyridine from crotonaldehyde and propanal. When the reaction is carried out in the presence of D_2O, the corresponding mono-deuterated pyridine is produced. The stereochemistry of aldimines should be *E*. They have been drawn in the *Z* form to better understand the reaction mechanism.

Fig. 4. Proposed pathway for the formation of 2,6-methylpyridine from 3,5-heptadien-2-one. When the reaction is carried out in the presence of D_2O, several pyridines with different degree of deuteration were produced. In the figure, the mono-deuterated pyridine in one of the methyl groups is shown. Deuteration of positions 3 and 5 of the pyridine ring is also possible as shown in Figures 1–3.

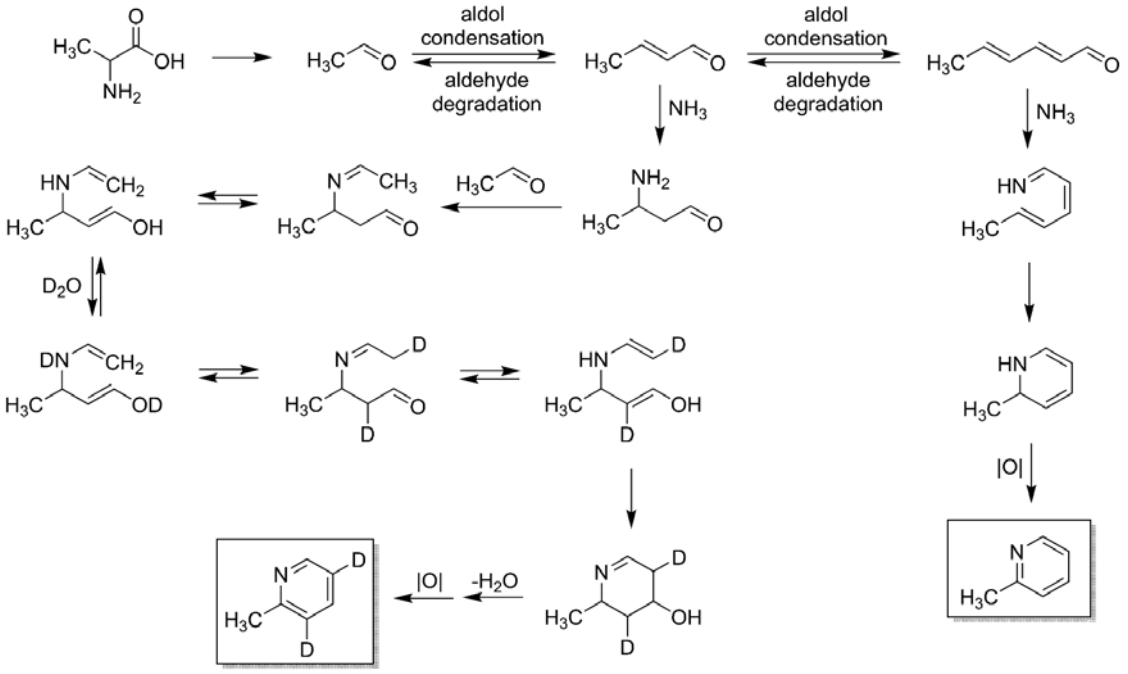
aldol condensation
aldehyde degradation
aldol condensation
aldehyde degradation
NH3
NH3
D2O
|O|
-H2O
|O|

Figure 1

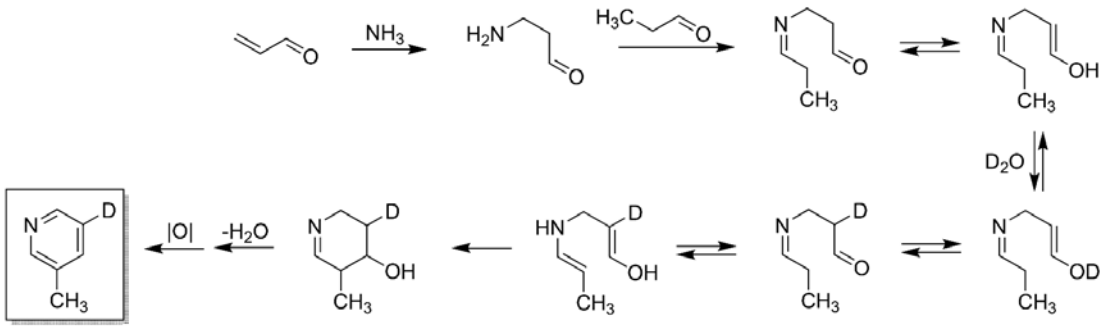

Figure 2

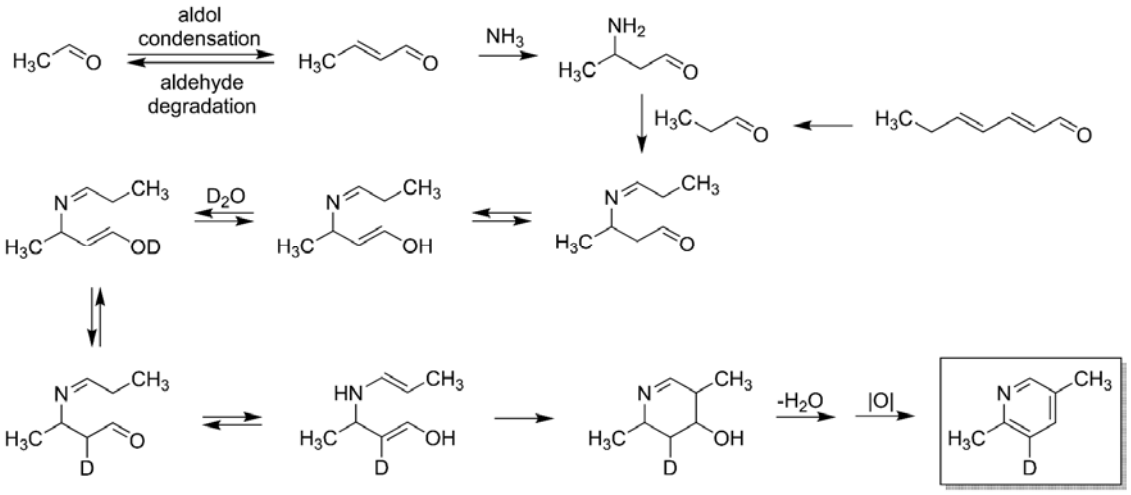
aldol
condensation
aldehyde
degradation
NH3
NH2
H3C
O
D2O
CH3
N
OD
OH
HN
D
-H2O
|O|

Figure 3

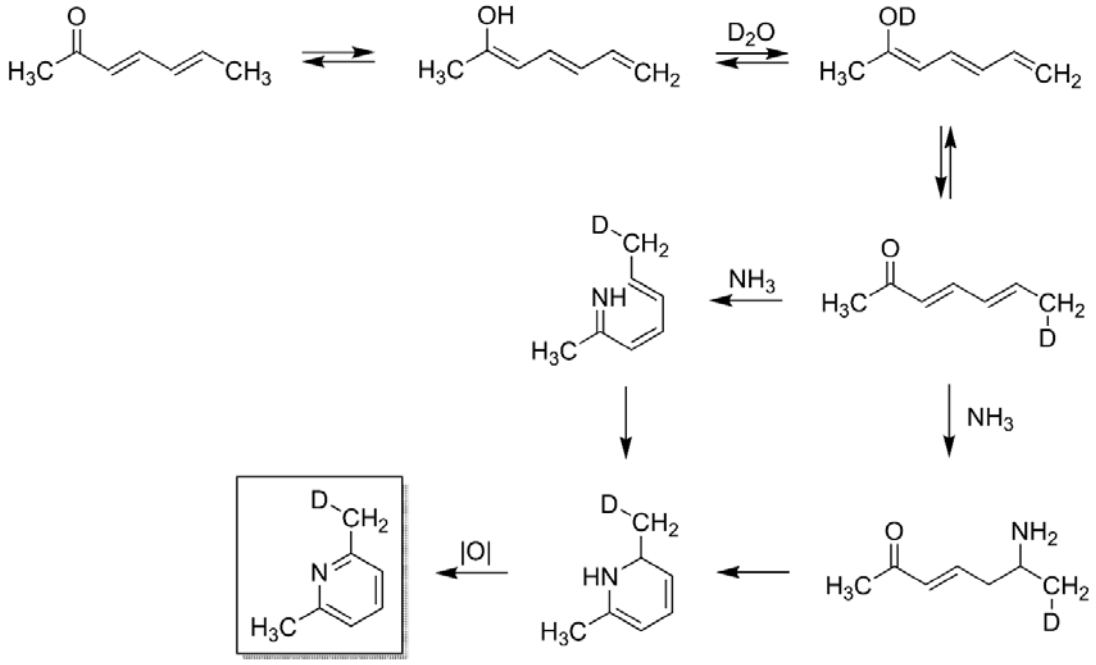
O
H3C
CH3
OH
H3C
CH2
D2O
OD
H3C
CH2
D
CH2
NH
H3C
NH3
O
H3C
CH2
D
NH3
D
CH2
HN
H3C
O
NH2
H3C
CH2
D
|O|
D
CH2
N
H3C

Figure 4

Supporting Information

Oligomerization of reactive carbonyls in the presence of ammonia-producing compounds: A route for the production of pyridines in foods

Rosario ZAMORA, Cristina M. LAVADO-TENA, and Francisco J. HIDALGO*

Instituto de la Grasa, Consejo Superior de Investigaciones Científicas, Carretera de Utrera km 1, Campus Universitario – Edificio 46, 41013-Seville, Spain

Table 1

Pyridines found in foods

Pyridine	Food	References
2-methyl	broiled pork	Biller, Boselli, Obiedzinski, Karpinski, & Wasxkiewicz-Robak, 2016
	coffee	Amanpour, & Selli, 2016; Sanz, Maeztu, Zapelena, Bello, & Cid, 2002; Moon & Shibamoto, 2009.
	chocolate	Afoakwa, Paterson, Fowler, & Ryan, 2009.
	goat meat	Madruga, Elmore, Dobson, & Mottram, 2009.
	fish powder	Mjøs & Solvang, 2006.
	scallops	Chung, Yung, & Kim, 2001; Chung, Yung, Ma, & Kim, 2002.
	rice	Cho, Nuijten, Shewfelt, & Kays, 2014.
2-ethyl	fish powder	Mjøs & Solvang, 2006.
	black pepper oil	Clery, Hammond, & Wright, 2006.
2-butyl	black pepper oil	Clery, Hammond, & Wright, 2006.
2-pentyl	roasted peanuts	Wang, Adhikari, & Hung, 2017.
	roasted *Pistacia t.*	Gogus, Ozel, Kocak, Hamilton, & Lewis, 2011
2-heptyl	black pepper oil	Clery, Hammond, & Wright, 2006.
2-isopropyl	black pepper oil	Clery, Hammond, & Wright, 2006.
2-acetyl	coffee	Amanpour, & Selli, 2016; Sanz, Maeztu, Zapelena, Bello, & Cid, 2002.
	black pepper oil	Clery, Hammond, & Wright, 2006.
3-methyl	coffee	Sanz, Maeztu, Zapelena, Bello, & Cid, 2002.
	fish powder	Mjøs & Solvang, 2006.
	scallops	Chung, Yung, & Kim, 2001; Chung, Yung, Ma, & Kim, 2002.
	roasted hazelnuts	Nicolotti, Cordero, Bicchi, Rubiolo, Sgorbini, & Liberto, 2013.
3-ethyl	coffee	Amanpour, & Selli, 2016
	scallops	Chung, Yung, & Kim, 2001; Chung, Yung, Ma, & Kim, 2002.
	table olives	Sansone-Land, Takeoka, & Shoemaker, 2014.
	fried bacon	Timon, Carrapiso, Jurado, & van de Lagemaat, 2004.
3-ethenyl	table olives	Sansone-Land, Takeoka, & Shoemaker, 2014.
3-hydroxy	Coffee	Amanpour, & Selli, 2016; Moon & Shibamoto, 2009
4-methyl	fried bacon	Timon, Carrapiso, Jurado, & van de Lagemaat, 2004.
	smoked salmon	Varlet, Serot, Cardinal, Knockaert, & Prost, 2007.
2,5-dimethyl	fish powder	Mjøs & Solvang, 2006.
2,5-diethyl	Coffee	Rocha, Maeztu, Barros, Cid, & Coimbra, 2003.
5-ethyl- 2-methyl	fish powder	Mjøs & Solvang, 2006.
	black pepper oil	Clery, Hammond, & Wright, 2006.
	cheese	Qian, & Reineccius, 2002.
2,6-dimethyl	rice	Cho, Nuijten, Shewfelt, & Kays, 2014.
	black pepper oil	Clery, Hammond, & Wright, 2006.
	smoked salmon	Varlet, Serot, Cardinal, Knockaert, & Prost, 2007.
2-ethyl-6-methyl	fish powder	Mjøs & Solvang, 2006.
3,4-dimethyl	roasted bacon	Timon, Carrapiso, Jurado, & van de Lagemaat, 2004.
3-ethyl-4-methyl	table olives	Sansone-Land, Takeoka, & Shoemaker, 2014.
6-methyl-3-hydroxy	Coffee	Moon & Shibamoto, 2009

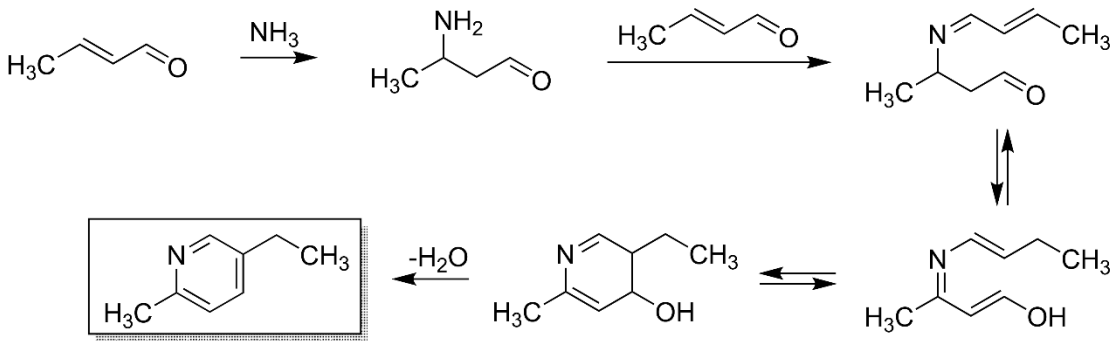

Figure S-1. Proposed pathway for the formation of 5-ethyl-2-methylpyridine by oligomerization of crotonaldehyde in the presence of glutamine. This pathway is also valid for the formation of 2,5-dialkylpyridines from other 2-alkenals, including 2-ethyl-5-propylpyridine, 5-butyl-2-propylpyridine, 2-butyl-5-pentylpyridine, and 5-hexyl-2-pentylpyridine.

References

Afoakwa, E. O., Paterson, A., Fowler, M., & Ryan, A. (2009). Matrix effects on flavour volatiles release in dark chocolates varying in particle size distribution and fat content using GC–mass spectrometry and GC–olfactometry. *Food Chemistry, 113*, 208–215.

Amanpour, A., & Selli, S. (2016). Differentiation of volatile profiles and odor activity values of Turkish coffee and French press coffee. *Journal of Food Processing and Preservation, 40*, 1116–1112.

Biller, E., Boselli, E., Obiedzinski, M., Karpinski, P., & Wasxkiewicz-Robak, B. (2016). The profile of volatile compounds in the outer and inner parts of broiled pork neck is strongly influenced by the acetic-acid marination conditions. *Meat Science, 121*, 292–301.

Cho, S., Nuijten, E., Shewfelt, R. L., & Kays, S. J. (2014). Aroma chemistry of African *Oryza glaberrima* and *Oryza sativarice* and their interspecific hybrids. *Journal of the Science of Food and Agriculture, 94*, 727–735.

Chung, H. Y., Yung, I. K. S., & Kim, J.-S. (2001). Comparison of volatile components in dried scallops (*Chlamys farreri* and *Patinopecten yessoensis*) prepared by boiling and steaming methods. *Journal of Agricultural and Food Chemistry, 49*, 192–202.

Chung, H. Y., Yung, I. K. S., Ma, W. C. J., & Kim, J.-S. (2002). Analysis of volatile components in frozen and dried scallops (*Patinopecten yessoensis*) by gas chromatography/mass spectrometry. *Food Research International, 35*, 43–53.

Clery, R. A., Hammond, C. J., & Wright, A. C. (2006). Nitrogen-containing compounds in black pepper oil (*Piper nigrum* L.). *Journal of Essential Oils, 18*, 1–3.

Gogus, F., Ozel, M. Z., Kocak, D., Hamilton, J. F., & Lewis, A. C. (2011). Analysis of roasted and unroasted *Pistacia terebinthus* volatiles using direct thermal desorption-GCxGC–TOF/MS. *Food Chemistry, 129*, 1258–1264.

Madruga, M. S., Elmore, J. S., Dobson, A. T., & Mottram, D. S. (2009). Volatile flavour profile of goat meat extracted by three widely used techniques. *Food Chemistry, 115*, 1081–1087.

Mjøs, S. A., & Solvang, M. (2006). Patterns in volatile components over heated fish powders. *Food Research International, 39*, 190–202.

Moon, J.-K., & Shibamoto, T. (2009). Role of roasting conditions in the profile of volatile flavor chemicals formed from coffee beans. *Journal of Agricultural and Food Chemistry, 57*, 5823–5831.

Nicolotti, L., Cordero, C., Bicchi, C., Rubiolo, P., Sgorbini, B., & Liberto, E. (2013). Volatile profiling of high quality hazelnuts (*Corylus avellana* L.): Chemical indices of roasting. *Food Chemistry, 138*, 1723–1733.

Qian, M., & Reineccius, G. (2002). Identification of aroma compounds in Parmigiano-Reggiano cheese by gas chromatography/olfactometry. *Journal of Dairy Science, 85*, 1362–1369.

Rocha, S., Maeztu, L., Barros, A., Cid, C., & Coimbra, M. A. (2003). Screening and distinction of coffee brews based on headspace solid phase microextraction/gas chromatography/principal component analysis. *Journal of the Science of Food and Agriculture, 84*, 43–51.

Sansone-Land, A., Takeoka, G. R., & Shoemaker, C. F. (2014). Volatile constituents of commercial imported and domestic black-ripe table olives (*Olea europaea*). *Food Chemistry, 149*, 285–295.

Sanz, C., Maeztu, L., Zapelena, M. J., Bello, J., & Cid, C. (2002). Profiles of volatile compounds and sensory analysis of three blends of coffee: influence of different proportions of Arabica and Robusta and influence of roasting coffee with sugar. *Journal of the Science of Food and Agriculture, 82*, 840–847.

Timon, M. L., Carrapiso, A. I., Jurado, A., & van de Lagemaat, J. (2004). A study of the aroma of fried bacon and fried pork loin. *Journal of the Science of Food and Agriculture, 84*, 825–831.

Varlet, V., Serot, T., Cardinal, M., Knockaert, C., & Prost, C. (2007). Olfactometric determination of the most potent odor-active compounds in salmon muscle (*Salmo salar*) smoked by using four smoke generation techniques. *Journal of Agricultural and Food Chemistry, 55*, 4518–4525.

Wang, S., Adhikari, K., & Hung, Y.-C. (2017). Acceptability and preference drivers of freshly roasted peanuts. *Journal of Food Science, 82*, 174–184.