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Conversion of 5-Hydroxymethylfurfural into 6-(Hydroxymethyl)pyridin-3-ol: A Pathway for the Formation of Pyridin-3-ols in Honey and Model Systems

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ABSTRACT

The formation of 6-(hydroxymethyl)pyridin-3-ol by ring expansion of 5-(hydroxymethy)furfural (HMF) in the presence of ammonia-producing compounds was studied to determine routes of formation of pyridin-3-ols in foods. 6-(Hydroxymethyl)pyridin-3-ol was produced from HMF in model systems, mostly at neutral pH values, as a function of reaction times and temperature, and with an activation energy (E_a) of 74 ± 3 kJ/mol, which was higher than that of HMF disappearance (43 ± 4 kJ/mol). A reaction pathway is proposed, which is general for the formation of pyridin-3-ols from 2-oxofurans. Thus, it explains the conversions of furfural into pyridin-3-ol and of 2-acetylfuran into 2-methylpyridin-3-ol, which were also studied. When honey and sugarcane honey were heated, they produced different pyridin-3-ols, although 6-(hydroxymethyl)pyridin-3-ol was the pyridine-3-ol produced to the highest extent. Obtained results suggest that formation of pyridin-3-ols in foods is unavoidable when 2-oxofurans are submitted to thermal heating and ammonia (or ammonia-producing compounds) is present.

KEYWORDS: *2-Acetylfuran; Carbonyl-amine reactions; Furfural; Honey; 5-Hydroxymethylfurfural; Maillard reaction; Pyridin-3-ols; Reactive carbonyls*

INTRODUCTION

Maillard reaction is a major route for the formation of aromas as a consequence of food processing.[1–3] The compounds responsible for these aromas have a great diversity of structures. Thus, the formation of aldehydes, alcohols, thiols, sulfides, acids, lactones, and a large variety of heterocyclic compounds, has been described.[4] Among these last compounds, the stale flavors of furan-2-carbaldehydes,[5] or the roasted flavors of pyrazines,[6] for example, have been widely studied. However, some of the compounds responsible for the produced aromas are not always stable and aroma changes are usually observed with further processing or storage. Many of these changes are still very poorly understood, which difficulties the prediction of the evolution of food aromas.

Among the different flavor-related substances produced in foods as a consequence of Maillard reaction, 5-hydroxymethylfurfural (HMF) is an important compound commonly produced to significant extents. Its flavor has been described as caramel, waxy, fatty, musty, or cardboard. HMF is considered as an endogenous contaminant because of its potential adverse effects,[7] and it is also a quality indicator as its levels in foods provide evidence of overheating during processing or inadequate storage conditions.[8] In addition, it has also been associated with color development in some foods.[9] This compound has been shown to be thermally unstable. Thus, its conversion upon heating into 5,5'-oxy-dimethylene-*bis*(2-furaldehyde), 5-methylfurfural, 2,5-furandicarboxaldehyde, or 2-acetyl-5-methylfuran, among others, has been described.[10,11] In addition, diverse chemical syntheses suggest that conversion of HMF into 6-(hydroxymethyl)pyridin-3-ol is possible. To this respect, Müller *et al*.[12] showed that brief exposure to bromine in water-methanol at 0 ºC smoothly and effectively converts hydroxymethylfurfurylamines, originated from either fructose or inuline sources, into 6-substituted pyridin-3-ols. Furthermore, formation of pyridin-3-ols by ring expansion 2-acylfurans is well-known in

organic synthesis.[13,14] Nevertheless, to the best of our knowledge, a relationship between pyridin-3-ols, like 6-(hydroxymethyl)pyridin-3-ol, and 2-oxofurans, like HMF, has not been established so far in foods. However, 6-(hydroxymethyl)pyridin-3-ol, like other pyridin-3-ols, has been found in the hydrolysis of melanoidins[15] or as a product of Maillard reaction.[16]

Pyridines are typical food flavors, which are produced as a consequence of processing.[17] The formation pathways of alkylpyridines seem to be related to the cyclizations and oligomerizations suffered by lipid-derived aldehydes in the presence of ammonia-producing compounds.[18] In addition, formation of alkylpyridiniums has been described in roasted coffee by trigonelline decomposition.[19] On the other hand, the formation pathways of pyridin-3-ols are lesser known in spite of being pyridin-3-ol a common flavor in coffee,[20] tea,[21] or caramel colors,[22] for example. In an attempt to explore the role of 2-oxofurans in the appearance of pyridin-3-ols in foods, this study investigates the formation of 6-(hydroxymethyl)pyridin-3-ol, pyridin-3-ol, and 2-methylpyridin-3-ol in honeys and model systems.

MATERIALS AND METHODS

Materials and chemicals. As model food products, two kinds of honeys were studied. One of them was a high quality multi-floral honey. The other one was a traditional plant syrup, namely ‘miel de caña’ (sugarcane honey or sugarcane syrup). This syrup is traditionally manufactured in Andalusia (Spain) from crushed sugarcane (*Saccharum officinarum*) and, although produced only in limited quantities, it is highly appreciated by consumers. For its preparation, the fresh cane juice is heated rapidly for decantation, then filtered, and, finally, slowly heated until it looks like dark honey. A characterization of this syrup was described previously.[23] Both products were purchased at local supermarkets.

The tested pyridin-3-ols were pyridin-3-ol, 2-methylpyridin-3-ol, and 6-(hydroxymethyl)pyridin-3-ol. As 2-oxofurans, HMF, furfural, and 2-acetylfuran were employed. These and other chemicals employed in this study had the highest available grade and were purchased from reliable commercial sources, including Sigma-Aldrich (St. Louis, MO), Fluka (Buchs, Switzerland), and Alfa Aesar (Thermo Fischer GmbH, Karlsruhe, Germany).

Conversion of 2-Oxofurans into Pyridin-3-ols in Model Systems. Mixtures of the 2-oxofuran (10 μmol dissolved in 20 μL of methanol) and an ammonia-producing compound (30 μmol in 50 μL of water) were singly homogenized with 200 mg of 0.063–0.200 mm silica gel (Macherey-Nagel, Düren, Germany), and 30 μL of 0.3 M buffer, pH 3–10. Samples were heated in closed test tubes for the indicated times (0–22 h) and temperatures (100-180 ºC). The employed buffers were sodium citrate, pH 3–6, sodium phosphate, pH 6–8, and sodium borate, pH 8–10. Times and temperatures employed in each experience are indicated in the respective figure legends.

At the end of the heating period, samples were cooled and 1 mL of methanol and 20 μL of the internal standard solution (13 mg of 1-octadecanol in 25 mL of methanol) were added. Suspensions were stirred for 1 min and centrifuged for 5 min at 2000 *g*. The supernatant was collected, evaporated to dryness, and the residue was successively treated with 200 μL of *N*,*O*-*bis*(trimethylsilyl)trifluoroacetamide (BSTFA), heated for 30 min at 60 ºC, and studied by gas chromatography coupled to mass spectrometry (GC-MS).

The 2-oxofurans tested were HMF, furfural, and 2-acetylfuran. The assayed ammonia-producing compounds were ammonia and ammonium chloride.

Formation of Pyridin-3-ols in Honeys. Honeys (1 g) were heated in closed test tubes for 0–10 h at either 60 or 100 ºC. After cooling, 100 μL of water was added and the

mixture was stirred. Then, 4 mL of acetonitrile and 20 μL of the internal standard solution (13 mg of 1-octadecanol in 25 mL of methanol) were added. Mixtures were successively stirred for 1 min, sonicated for 10 min, stirred for 1 min, and the acetonitrile layer was decanted and taken to dryness. The residue was treated with 300 μL of *N*,*O*-*bis*(trimethylsilyl)trifluoroacetamide (BSTFA), heated for 30 min at 60 ºC, and studied by gas chromatography coupled to mass spectrometry (GC-MS).

GC-MS Analyses. GC-MS analyses were conducted with an Agilent 7820A gas chromatograph coupled with an Agilent 5977 mass selective detector (quadrupole type) using a fused-silica HP-5MS UI capillary column (30 m length, 0.25 mm inner diameter, 0.25 μm coating thickness) from Agilent. One microliter of sample was injected in the pulsed splitless mode. Working conditions were as follows: 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. Oven temperature conditions were from 80 ºC (1 min) to 140 ºC at 20 ºC/min, then to 300 ºC at 50 ºC/min, and finally held at 300 ºC for 4 min.

Identification of 6-(Hydroxymethyl)pyridin-3-ol, Pyridin-3-ol, 2-Methylpyridin-3-ol and HMF. Identification of the three pyridin-3-ols and HMF was carried out by comparison of retention indexes and mass spectra, and by co-elution with authentic standards, which were derivatized analogously. Mass spectra of the assayed compounds as well as that of the internal standard are collected in the Supporting Information. The following ions (M^+ – methyl) were employed for quantitation purposes: *m/z* 254 for 6-(hydroxymethyl)pyridin-3-ol, *m/z* 152 for pyridin-3-ol, *m/z* 166 for 2-methylpyridin-3-ol, *m/z* 183 for HMF, and *m/z* 327 for the IS.

Quantitation of 6-(Hydroxymethyl)pyridin-3-ol, Pyridin-3-ol, 2-Methylpyridin-3-ol and HMF. Quantitation of the three pyridin-3-ols and HMF in model systems and

honeys was carried out by preparing standard curves of those compounds in the corresponding assayed systems and following the whole procedures described above (without heating). For each curve, six different concentration levels of the three pyridin-3-ols and HMF were used. Pyridin-3-ols and HMF contents were directly proportional to the corresponding compound/internal standard area ratios ($r > 0.99$, $p < 0.001$). The coefficients of variation at the different assayed concentrations were < 10%.

Statistical Analysis. All data given are mean ± standard deviation (SD) of at least three independent experiments. Analysis of variance was employed to compare the different groups. When significant F values were obtained, group differences were evaluated by the Tukey test.[24] These studies were conducted using Origin version 7.0 (OriginLab Corp., Northampton, MA, USA). The significance level is $p < 0.05$ unless otherwise indicated.

RESULTS AND DISCUSSION

Effect of Reaction Conditions on the Conversion of HMF into 6-(Hydroxymethyl)pyridin-3-ol. When HMF was heated in the presence of either ammonia or ammonium chloride, the formation of 6-(hydroxymethyl)pyridin-3-ol was observed. The conversion yield depended on the reaction conditions, including reaction pH, reactant concentrations, time and temperature.

Figure 1 shows the effect of pH on both the formation of 6-(hydroxymethyl)pyridin-3-ol and the disappearance of HMF. As observed, HMF disappearance and 6-(hydroxymethyl)pyridin-3-ol formation increased when the pH increased from 3 to 7. The maximum amount of 6-(hydroxymethyl)pyridin-3-ol was produced at pH 6–8 with sodium phosphate buffer. At higher pH values and in the presence of borate buffer, the amount of the produced 6-(hydroxymethyl)pyridin-3-ol was much lower. For some

unknown reason, HMF disappearance also occurred at pH 8–10 in the presence of sodium borate buffer, but this disappearance did not produce the pyridine to a high extent. Between pH 3 and pH 8 the formation of 6-(hydroxymethyl)pyridin-3-ol and the disappearance of HMF were correlated ($r = 0.96$, $p = 0.002$). The conversion yield of the furan into the pyridine depended on the pH and ranged from 0.4% at pH 3 to 1.3% at pH 7–8. Although there was not a significant difference among the amounts of 6-(hydroxymethyl)pyridin-3-ol produced at either pH 7 or pH 8, pH 8 was selected for the rest of this study because the minimum amount of HMF was recovered at this pH.

6-(Hydroxymethyl)pyridin-3-ol formation also depended on the HMF/ammonia ratio. Figure 2 shows the effect of increasing amounts of HMF on the formation of 6-(hydroxymethyl)pyridin-3-ol in the presence of ammonium chloride. The maximum amount of the pyridine was achieved when 10 μmol of HMF were heated in the presence of 30 μmol of ammonium chloride. Nevertheless, the conversion yield was higher when smaller amounts of HMF were employed. Thus, conversion yield was 3% with 2.5 μmol of HMF, and this yield decreased when higher amounts of HMF were employed. Most of this study was carried out with 10 μmol of HMF because the highest amount of 6-(hydroxymethyl)pyridin-3-ol was obtained by using these conditions.

Conversion yield also depended on the concentration of the ammonia-producing compound. Figure 3 shows the effect of increasing amounts of ammonia and ammonium chloride on the formation of 6-(hydroxymethyl)pyridin-3-ol. The amount of this pyridine increased linearly ($r > 0.9993$, $p < 0.0007$) between 0 and 20 μmol of the amino compound and it remained constant when more than 30 μmol of the amino compound was added. The behavior was inverse for the HMF recovered. It decreased linearly ($r = 0.996$, $p < 0.004$) between 0 and 20 μmol of the amino compound and it remained constant when

more than 30 μmol of the amino compound was added. In fact, formation of 6-(hydroxymethyl)pyridin-3-ol and disappearance of HMF were correlated ($r = 0.98$, $p < 0.0001$). Moreover, the behavior of the two amino compounds assayed (ammonia and ammonium chloride) was very similar. Both of them produced analogous disappearance of HMF, although the conversion yield for 6-(hydroxymethyl)pyridin-3-ol seemed to be slightly higher when ammonium chloride was employed. For that reason, ammonium chloride was employed as ammonia-producing compound in most of the experiments carried out in this study. In addition, 30 μmol of ammonium chloride was selected because this was the minimum amount of the ammonia-producing compound that produced the pyridine to a higher extent when starting from 10 μmol of HMF.

Finally, time and temperature also played a major role on the conversion of HMF into 6-(hydroxymethyl)pyridin-3-ol. Figure 4 shows the time-courses for the formation of 6-(hydroxymethyl)pyridin-3-ol at 100–160 ºC. As observed, the amount of the pyridine increased linearly ($r = 0.91$, $p < 0.04$) as a function of heating time. Furthermore, formation rates, obtained from the slopes of the lines of the best fit, increased with temperature. In addition, there was a small lag period, which was higher when temperature was lower. Reaction rates for this formation were employed in an Arrhenius plot to obtain the activation energy (E_a) for the formation of 6-(hydroxymethyl)pyridin-3-ol from HMF. The obtained plot is shown in Figure 5. The activation energy was obtained from the slope of the line of best fit and resulted to be 74 ± 3 kJ/mol.

Similarly, time-courses for the disappearance of HMF at 100–160 ºC are shown in Figure 6. Recovered HMF decreased linearly ($r > 0.99$, $p < 0.04$) as a function of heating times and disappearance rates increased with temperature. Disappearance rates were employed in an Arrhenius plot for determining the E_a for HMF disappearance. It was 43

191 ± 4 kJ/mol. This value was lower than that found above for 6-(hydroxymethyl)pyridin-3-
192 ol formation. This explains that HMF is easily decomposed, as observed previously,[10] and
193 it is only partially converted into 6-(hydroxymethyl)pyridin-3-ol.

194 **Conversion of 2-Oxofurans into Pyridin-3-ols**. The conversion of HMF into the
195 corresponding pyridin-3-ol is not exclusive for this furan. To confirm that other 2-
196 oxofurans can suffer analogous transformations, the conversion of other two products of
197 the Maillard reaction (furfural[25] and 2-acetylfuran[26]) into the corresponding pyridin-3-ols
198 (pyridin-3-ol and 2-methylpyridin-3-ol, respectively) was studied. Figure 7 shows the
199 formation of the corresponding pyridin-3-ols after 3 and 22 h at 100 ºC. The figure also
200 shows the formation of 6-(hydroxymethyl)pyridin-3-ol by heating of HMF under the
201 same reaction conditions for comparison purposes. As expected, the corresponding
202 pyridin-3-ols were always produced, although the conversion yield depended on the
203 employed 2-oxofuran. Thus, the highest yield was observed for 6-
204 (hydroxymethyl)pyridin-3-ol. This pyridine was produced with a yield of 0.5% after 3 h
205 at 100 ºC, and the yield increased to 1.3% after 22 h at 100 ºC. The yields of the other
206 two pyridines were similar among them and were lower than those of 6-
207 (hydroxymethyl)pyridin-3-ol. The pyridine that was produced to a higher extent was
208 pyridin-3-ol (0.03% and 0.11% after 3 and 22 h, respectively). This pyridine has been
209 found in different foods that had suffered Maillard reaction.[16–18] The yield for 2-
210 methylpyridin-3-ol was 0.01% and 0.06% after 3 and 22 h, respectively.

211 **Proposed Pathway for the Conversion of 2-Oxofurans into Pyridin-3-ols**. Above
212 described results suggest that, in the presence of ammonia and moderate temperatures, 2-
213 oxofurans are decomposed and the formation of pyridin-3-ols is produced. A pathway
214 that explains this transformation is shown in Figure 8. This reaction pathway is based on
215 that previously published by Gruber.[14] Thus, in the presence of ammonia, the

corresponding imine should be produced in a first step. Then, the addition of a second molecule of ammonia would produce the opening of the ring with the formation of a compound derived from the dicarbonyl precursor of the furan. This attack is consequence of the polarization of the C–O bond in the furan ring because of the difference of electronegativities between both atoms.[27] Finally, cyclization of this intermediate with the exit of a molecule of ammonia would produce the corresponding pyridine derivative.

This reaction only requires the presence of an oxo group at position 2 of the furan ring. This means that it should be produced with any other 2-oxofuran that might be present. This explains, for example, the presence of both 6-methylpyridin-3-ol and 5-methylfurfural in coffee aroma.[28] In addition, it could also be expected to be produced in analogous 2-oxoheterocycles. For example, 2-acetylthiophene is a well-known Maillard reaction product.[29] According to the proposed reaction pathway, it should be converted to a certain extent into 2-methylpyridin-3-thiol. To the best of our knowledge, this flavor compound has not been yet identified in foods. However, other Maillard reaction product, 2-thiophenecarboxaldehyde, which is present in coffee[30] or pork broth,[31] for example, should produce pyridin-3-thiol. This last pyridine has been identified in soy sauce, which is also a source of 2-acetylthiophene.[32]

Although the presence of the oxo group is a requisite for the reaction, reaction yield also depended on the existence of substituents in the carbon at position 5 of the furan ring. The hydroxyl group of the hydroxymethyl substituent at position 5 of HMF has a negative inductive effect. This effect should contribute to increase the electron deficiency of carbon at position 5 of the furan ring, therefore converting this atom into a better electrophile. For this reason, HMF is converted more easily into 6-(hydroxymethyl)pyridin-3-ol than furfural into pyridin-3-ol. This suggests that, although pyridin-3-ol is more frequently described than 6-(hydroxymethyl)pyridin-3-ol in most

241 foods, this last pyridine is likely to be present to a higher extent, but it has mostly gone
242 unnoticed until now. To confirm this last hypothesis, and to test the formation of pyridin-
243 3-ols in food products, the effect of thermal heating on commercial honeys was studied.
244 Obtained results will be discussed in the next subsection.

245 **Formation of Pyridin-3-ols in Honeys**. Two different types of honeys were studied.
246 One of them was a high quality honey, which had not suffered any previous thermal
247 overheating. The other one was a sugarcane honey. As described in the Materials and
248 Methods section, the production of this last honey requires thermal treatment that is likely
249 to promote Maillard reaction to a high extent.

250 Figure 9 shows the effect of heating on the formation of both HMF and 6-
251 (hydroxymethyl)pyridin-3-ol in the studied honey. As expected, because of its high
252 quality, very small amounts of HMF and 6-(hydroxymethyl)pyridin-3-ol were detected in
253 this honey at the initial step. However, when the honey was heated, both compounds were
254 produced to a significant extent. On the other hand, formation of pyridin-3-ol was not
255 observed, and only trace amounts of 2-methylpyridin-3-ol were detected when the honey
256 was heated at 100 ºC for long heating periods. This indicated that, similarly to that
257 observed in model systems, 6-(hydroxymethyl)pyridin-3-ol is produced to a higher extent
258 than other pyridines. In addition, HMF and 6-(hydroxymethyl)pyridin-3-ol formation
259 time-courses were different. Thus, HMF increased exponentially as a function of heating
260 time, as observed previously.[33] On the other hand, 6-(hydroxymethyl)pyridin-3-ol
261 concentration increased linearly ($r = 0.94$, $p < 0.0002$). This difference of time-courses
262 does not necessarily mean that 6-(hydroxymethyl)pyridin-3-ol is not produced by ring
263 expansion of HMF. As observed in the studied model systems (Figure 2), increased
264 amounts of HMF do not always produce increasing amounts of 6-
265 (hydroxymethyl)pyridin-3-ol. This conversion requires the presence of ammonia (or other

ammonia-producing compound) that can be the limiting factor in this conversion. In addition, and analogously to that observed in model systems, HMF disappearance should produce other compounds in addition to 6-(hydroxymethyl)pyridin-3-ol.

Results obtained with the sugarcane honey studied were someway different. Because this food product suffers a heating process during its production, both HMF and 6-(hydroxymethyl)pyridin-3-ol were already present in the initial sample. In addition, both of them increased linearly ($r > 0.93$, $p < 0.0008$) when the sugarcane honey was heated. In fact, there was a correlation between them at both 60 ºC ($r > 0.97$, $p = 0.005$) and 100 ºC ($r > 0.97$, $p < 0.0001$). Surprisingly, 6-(hydroxymethyl)pyridin-3-ol/HMF ratio was higher at 60 ºC (~0.27) than at 100 ºC (~0.034). This suggests that disappearance of HMF at low temperature mainly produces the pyridine. However, higher temperatures favor the transformation of HMF into other compounds.

Differently to the studied honey, the studied sugarcane honey also contained the other analyzed pyridines (pyridin-3-ol and 2-methylpyridin-3-ol) at the initial step. In addition, concentrations of both pyridines increased linearly ($r > 0.90$, $p < 0.002$) as a function of heating. However, and analogously to the observed in the studied model systems and the studied honey, the concentrations of both pyridines were always lower than that of 6-(hydroxymethyl)pyridin-3-ol.

Obtained results suggest that, analogously to that observed in honey, sugarcane honey, and model systems, conversion of 2-oxofurans into pyridin-3-ols is unavoidable when 2-oxofurans are submitted to thermal heating and ammonia (or an ammonia-producing compound) is present. This suggests that, although not frequently found in foods, pyridin-3-ols should be common components of foods in which Maillard reaction has occurred, even in those foods submitted to soft heating or stored for a limited time period.

Obtained results also show that 6-(hydroxymethyl)pyridin-3-ol is an additional, previously unknown in foods, transformation product of HMF. Some authors have shown doubts about the usefulness of using HMF as a quality indicator of inadequately processed foods because of its instability,[9] although in many food systems, after a short lag time, a constant increase in HMF concentration is observed, which indicates a faster formation than degradation. Nevertheless, because aromaticity of pyridines is usually higher than that of furans,[35] 6-(hydroxymethyl)pyridin-3-ol is expected to be more stable than HMF. Therefore, although reaction yield of 6-(hydroxymethyl)pyridin-3-ol is much lower than that of HMF, the potential use of 6-(hydroxymethyl)pyridin-3-ol as an alternative marker of thermal stress in some foods might be suggested.

Finally, a last consequence of the described reaction is related to the observed changes in the antioxidant activity of honeys submitted to thermal heating.[34,35] As described above, 6-(hydroxymethyl)pyridin-3-ol increases upon heating, and pyridin-3-ols have been shown to be efficient chain-breaking antioxidants.[36] The potential contribution of 6-(hydroxymethyl)pyridin-3-ol to the observed increase of antioxidant activity in honey might be hypothesized.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at

Mass spectra of trimetylsilyl derivatives of pyridin-3-ol, 2-methylpyridin-3-ol, 6-(hydroxymethyl)pyridin-3-ol, 5-hydroxymethylfurfural (HMF), and 1-octadecanol (PDF)

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319 **Notes**

320 The authors declare no competing financial interest.

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428

429

FIGURE CAPTIONS

Figure 1. Effect of pH on the formation of 6-(hydroxymethyl)pyridin-3-ol (circles) and on the disappearance of 5-hydroxymethylfurfural (HMF, up pointing triangles). HMF (10 μmol) was heated in the presence of ammonium chloride (30 μmol) and 30 μL 0.3 M buffer for 22 h at 100 ºC. Three buffers were assayed: sodium citrate buffer (open symbols), sodium phosphate buffer (half-closed symbols), and sodium borate buffer (closed symbols). Data are given in nmol of the produced 6-(hydroxymethyl)pyridin-3-ol per μmol of the amount of HMF added to the reaction mixture.

Figure 2. Effect of 5-hydroxymethylfurfural (HMF, △) concentration on the formation of 6-(hydroxymethyl)pyridin-3-ol (○). HMF was heated in the presence of ammonium chloride (30 μmol) and 0.3 M sodium phosphate buffer, pH 8, for 22 h at 100 ºC.

Figure 3. Effect of amine compound concentration on the formation of 6-(hydroxymethyl)pyridin-3-ol (○ and ◇) and on the disappearance of 5-hydroxymethylfurfural (HMF, △ and ▽). HMF (10 μmol) was heated in the presence of the amine compound and 0.3 M buffer for 22 h at 100 ºC. Two amine compounds were assayed: ammonia (◇ and △) and ammonium chloride (○ and ▽). Data are given in nmol of the produced 6-(hydroxymethyl)pyridin-3-ol per μmol of the amount of HMF added to the reaction mixture.

Figure 4. Effect of time and temperature on the formation of 6-(hydroxymethyl)pyridin-3-ol. 5-Hydroxymethylfurfural (HMF, 10 μmol) was heated in the presence of ammonium chloride (30 μmol) and 0.3 M sodium phosphate buffer, pH 8. The assayed temperatures were: 100 (▽), 120 (△), 140 (○), and 160 (□) ºC. Data are given in nmol of the

produced 6-(hydroxymethyl)pyridin-3-ol per μmol of the amount of HMF added to the reaction mixture.

Figure 5. Arrhenius plot obtained for the formation of 6-(hydroxymethyl)pyridin-3-ol (○) and the disappearance of 5-hydroxymethylfurfural (HMF, □). HMF (10 μmol) was heated in the presence of ammonium chloride (30 μmol) and 0.3 M sodium phosphate buffer, pH 8, at the temperatures indicated in Figures 4 and 6.

Figure 6. Effect of time and temperature on the disappearance of 5-hydroxymethylfurfural (HMF). HMF (10 μmol) was heated in the presence of ammonium chloride (30 μmol) and 0.3 M sodium phosphate buffer, pH 8. The assayed temperatures were: 100 (▽), 120 (△), 140 (○), and 160 (□) ºC.

Figure 7. Formation of pyridin-3-ols [6-(hydroxymethyl)pyridin-3-ol (stripped bars), pyridin-3-ol (open bars), and 2-methylpyridin-3-ol (crosshatched bars)] from 2-oxofurans [5-hydroxymethylfurfural (HMF), furfural, and 2-acetylfuran, respectively]. The 2-oxofuran (10 μmol) was heated in the presence of ammonium chloride (30 μmol) and 0.3 M sodium phosphate buffer, pH 8, at 100 ºC for the indicated times. Data are given in nmol of the produced pyridin-3-ol per μmol of the amount of 2-oxofuran added to the reaction mixture.

Figure 8. Proposed pathway for the formation of pyridine-3-ols from the corresponding 2-oxofurans. For furfural and pyridin-3-ol, R_1 = H and R_2 = H. For 2-acetylfuran and 2-methylpyridin-3-ol, R_1 = H and R_2 = CH_3. For 5-hydroxymethylfurfural (HMF) and 6-(hydroxymethyl)pyridin-3-ol, R_1 = CH_2OH and R_2 = H.

Figure 9. Effect of heating time on the formation of 6-(hydroxymethyl)pyridin-3-ol (○) and 5-hydroxymethylfurfural (HMF, △) in honey heated at, A, 60, and B, 100 ºC. Honey (1 g) was heated in closed test tubes for the indicated times and temperatures.

Figure 10. Effect of heating time on the formation of 6-(hydroxymethyl)pyridin-3-ol (○), 5-hydroxymethylfurfural (HMF, △), pyridin-3-ol (◁), and 2-methylpyridin-3-ol (▷) in sugarcane honey heated at, A, 60, and B, 100 ºC. Sugarcane honey (1 g) was heated in closed test tubes for the indicated times and temperatures.

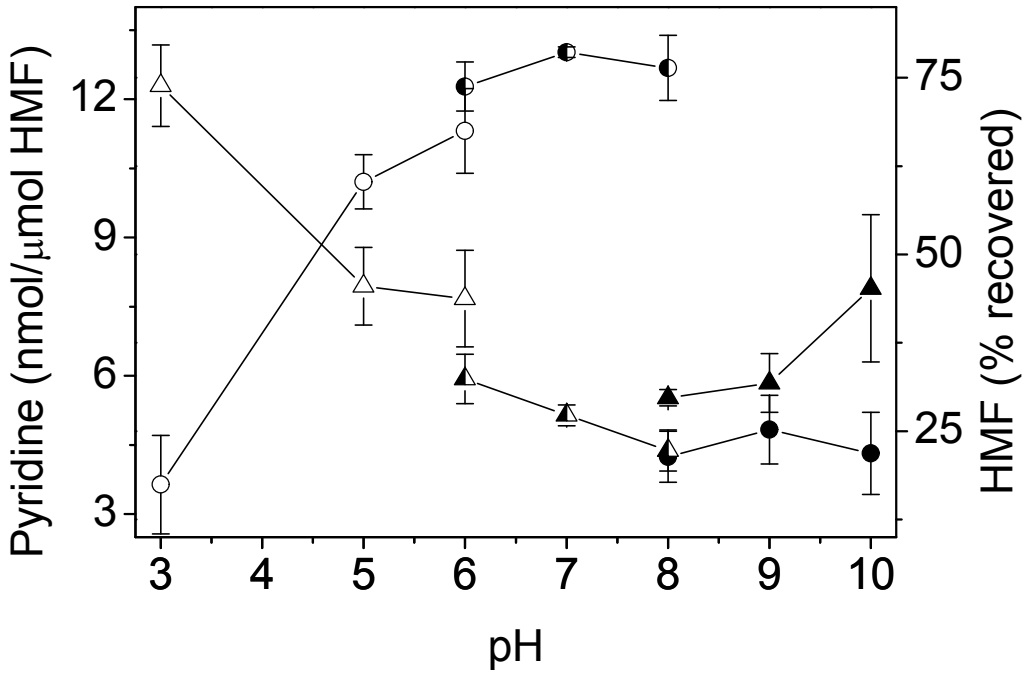
Pyridine (nmol/μmol HMF)
HMF (% recovered)
pH
3
6
9
12
25
50
75
3
4
5
6
7
8
9
10

Figure 1

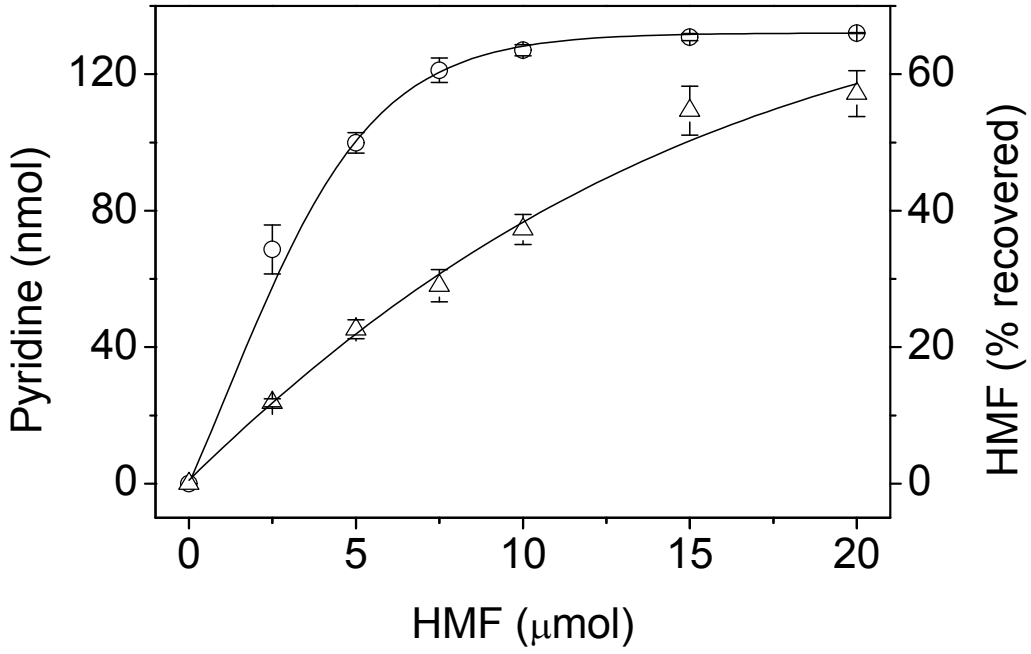
Pyridine (nmol)
HMF (% recovered)
HMF (μmol)
0
40
80
120
0
20
40
60
0
5
10
15
20

Figure 2

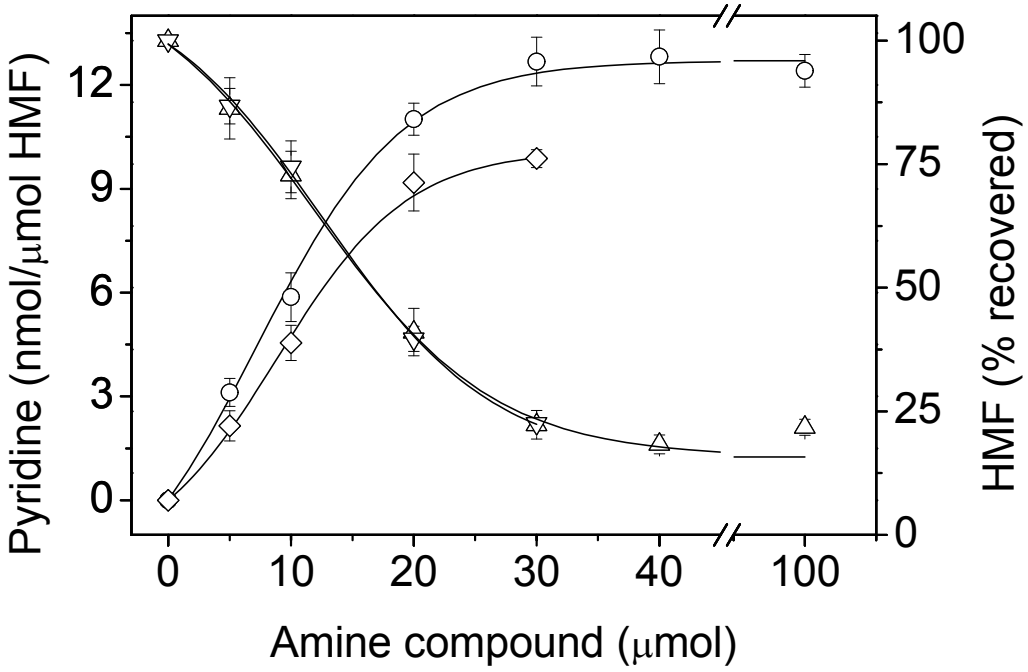
Pyridine (nmol/μmol HMF)
HMF (% recovered)
Amine compound (μmol)
0
3
6
9
12
0
25
50
75
100
0
10
20
30
40
100

Figure 3

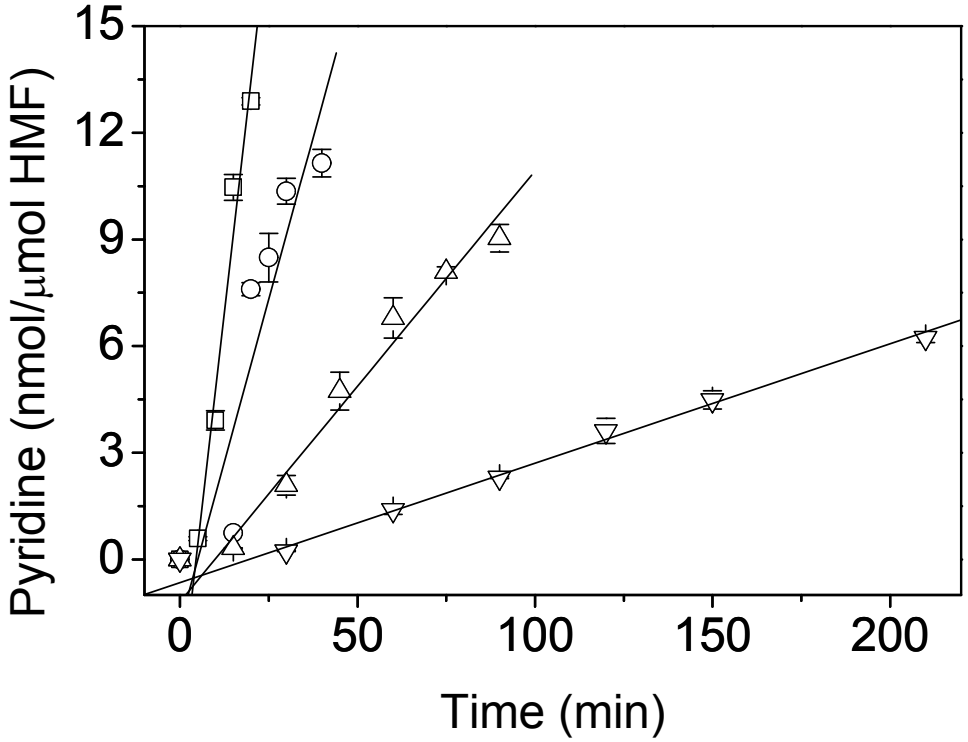

Figure 4

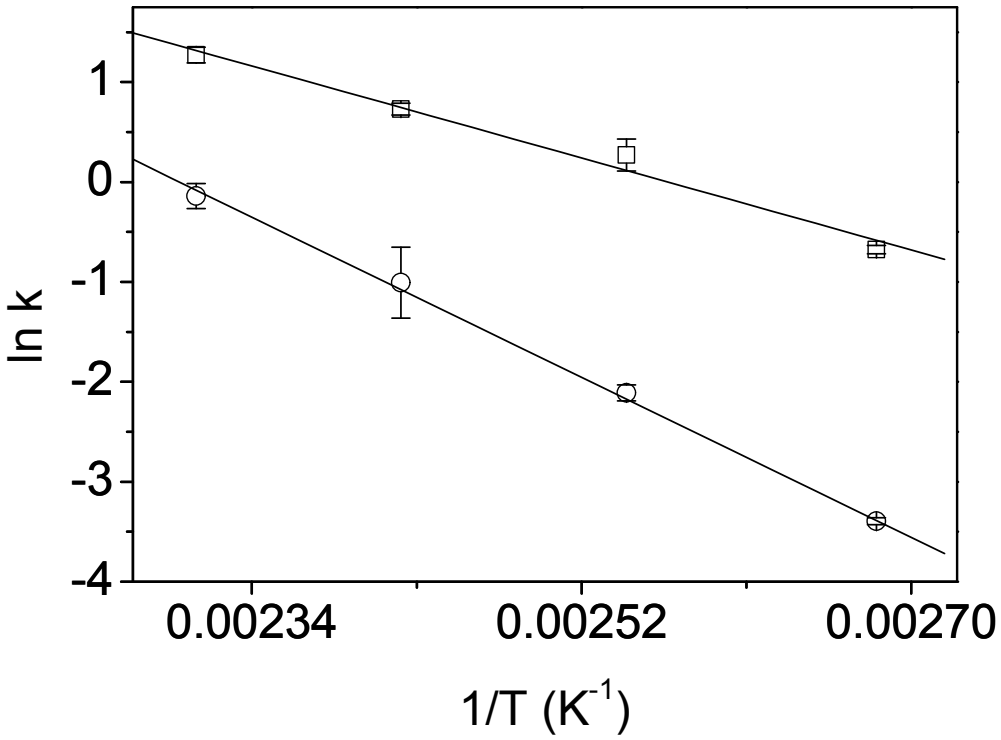

Figure 5

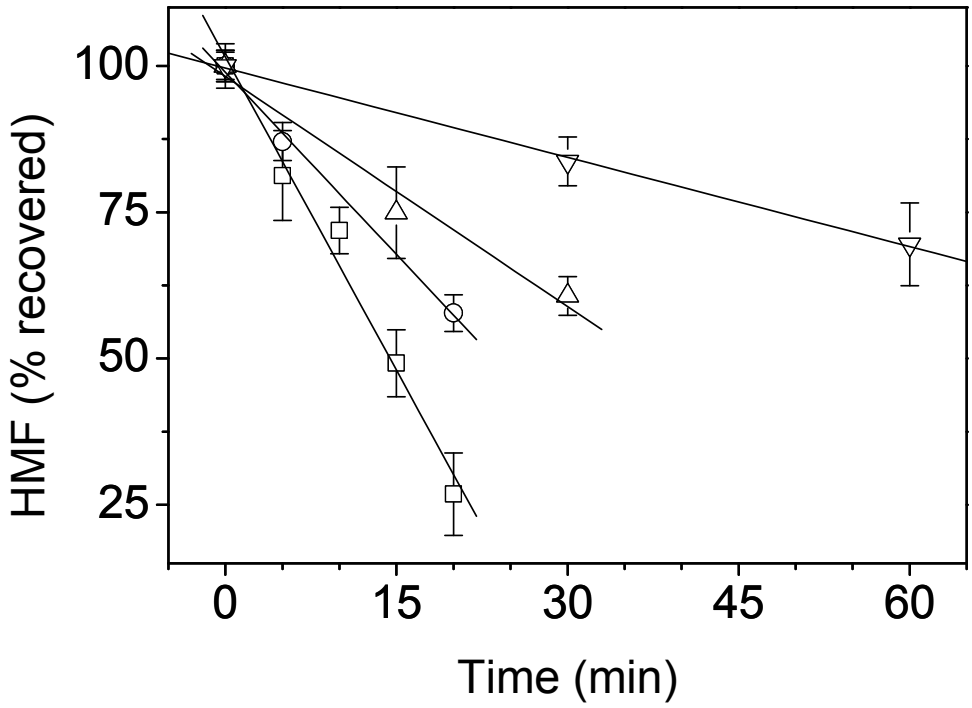

Figure 6

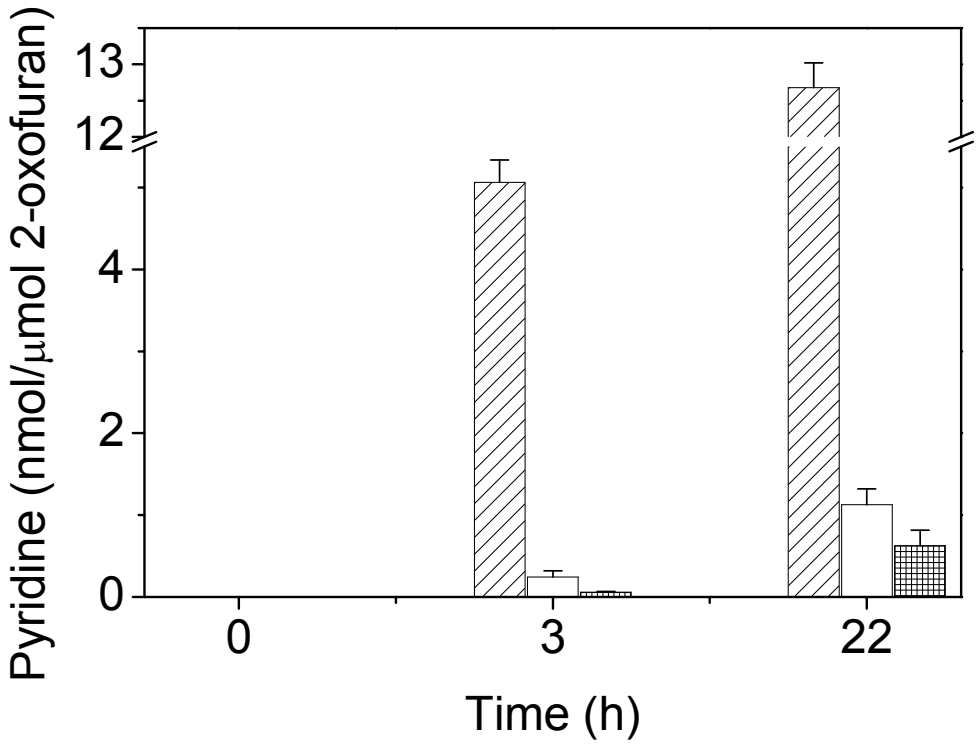

Figure 7

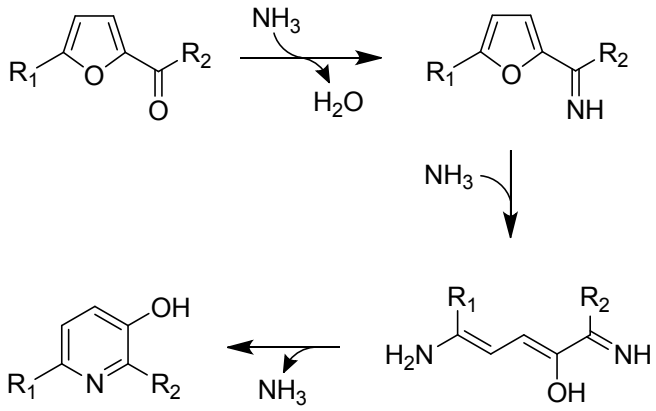

Figure 8

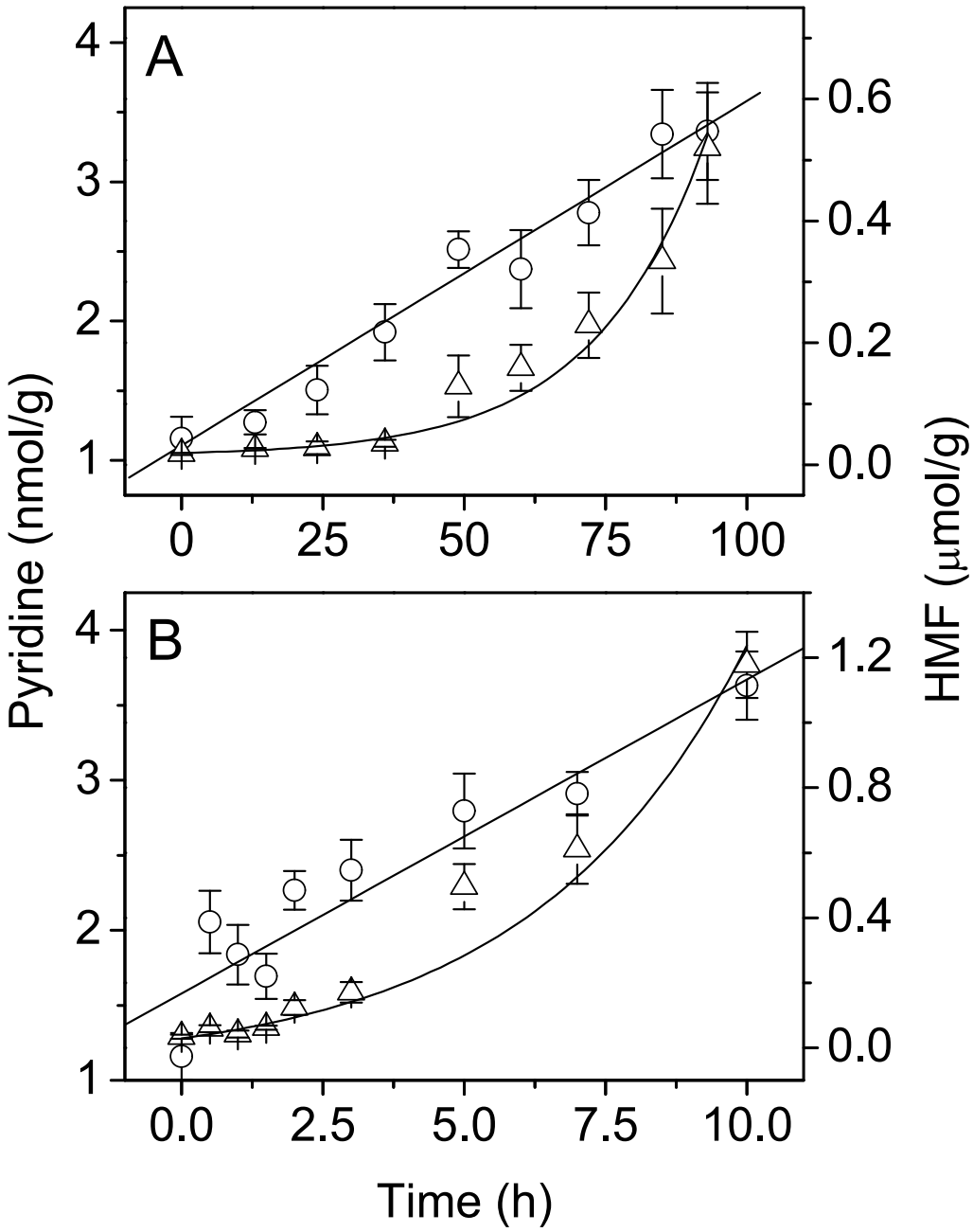
A
B
Pyridine (nmol/g)
HMF (μmol/g)
Time (h)
0
25
50
75
100
0.0
2.5
5.0
7.5
10.0
1
2
3
4
0.0
0.2
0.4
0.6
0.8
1.2

Figure 9

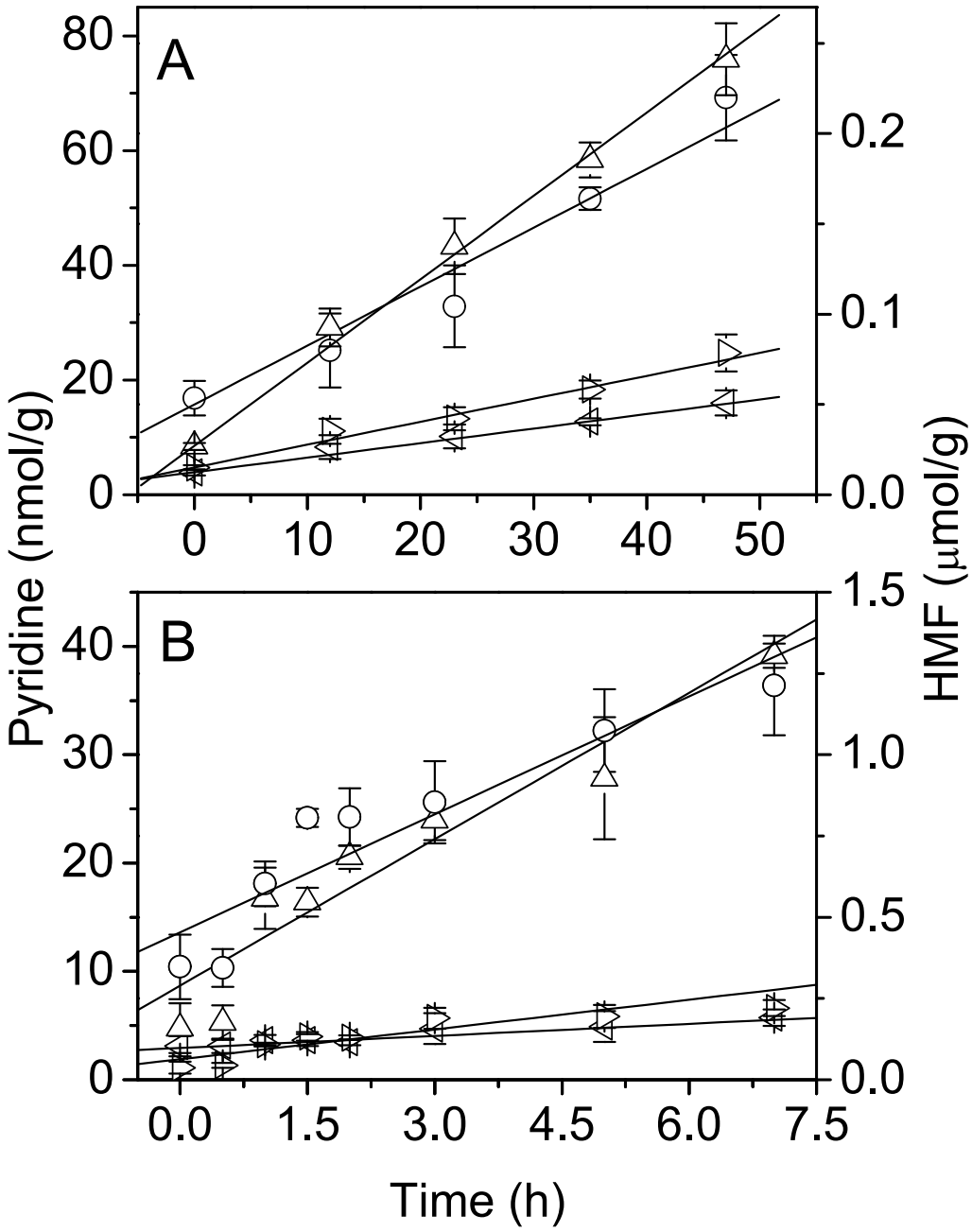
A
B
Pyridine (nmol/g)
HMF (μmol/g)
Time (h)
0
20
40
60
80
0.0
0.1
0.2
0
10
20
30
40
50
0
10
20
30
40
0.0
0.5
1.0
1.5
0.0
1.5
3.0
4.5
6.0
7.5

Figure 10

GRAPHIC FOR TABLE OF CONTENTS