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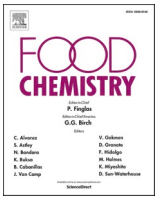

Ketone-phenol reactions and the promotion of aromatizations by food phenolics

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ABSTRACT

Heating of either 3,5-heptadien-2-one or 2,6-heptanedione in the presence of ammonia produced 2,6-dimethylpyridine, and also 3-methylcyclohex-2-en-1-one for the second ketone. When phenolics were present, inhibition of pyridine formation was only observed in mixtures of 3,5-heptadien-2-one and resorcinol. This inhibition was due to the formation of ketone-resorcinol adducts, which were isolated and identified by nuclear magnetic resonance (NMR) and mass spectrometry (MS) as 2,4-dimethyl-5,6-dihydro-4*H*-2,6-methanobenzo[*d*][1,3]dioxocin-9-ol and 1-(7-hydroxy-4-methylchroman-2-yl)propan-2-one. The other assayed phenolics increased pyridine formation. This increase was mainly observed in the presence of oxygen, at slightly basic pH values, depended on time, temperature, and the phenolic concentration, and had an activation energy of 56.8 kJ/mol for the formation of 2,6-dimethylpyridine from 2,6-heptanedione in the presence of orcinol. This increase was a consequence of the promotion by phenolics of a required aromatization step in the pyridine formation pathway. This phenolic function needs to be considered when phenolics are added to food products.

1. Introduction

Phenolic compounds are important constituents of many food products (Rodriguez et al., 2009). They play a major role on sensory food characteristics, including flavor, astringency, and color. In addition, they play a role in the formation of process-induced carbonyl–-amine reaction products, including those formed as a consequence of lipid oxidation. Thus, phenolics have been shown to behave as a triple defensive barrier against the lipid oxidation-induced changes produced in foods (Zamora & Hidalgo, 2016). One of them is related to their ability to chelate the trace metals that initiate the oxidative process (Kostic et al., 2021). In addition, some phenolics are also well-known free radical scavengers that avoid the production of many flavors and off-flavors (Mira-Sanchez, Castillo-Sanchez, & Morillas-Ruiz, 2020). Finally, other phenolics are able to trap carbonyls and to produce the corresponding carbonyl-phenol adducts (Zamora, Aguilar, Granvogl, & Hidalgo, 2016).

These last reactions have been lesser studied than other reactions involving phenolics. However, carbonyl-phenol reactions have been shown to occur with many aldehydes, including those produced in the courses of both Maillard reaction (Peng et al., 2008), and lipid oxidation (Hidalgo & Zamora, 2014). These reactions mainly occur with *m*-diphenols (Hidalgo & Zamora, 2014), many of the produced adducts have been characterized to date (Zamora & Hidalgo, 2018), and several of them have also been determined in food products (Zamora et al., 2016). On the other hand, and to the best of our knowledge, the ability of phenolics to trap the ketones produced in foods as a consequence of oxidative processes is mostly unknown. However, some of these ketones are important food flavors (Mu et al., 2022) and others take part in the formation of process-induced food flavors (Zamora, Lavado-Tena, & Hidalgo, 2020).

In an attempt to clarify the ketone-trapping abilities of phenolic compounds, this manuscript describes the reactions produced when ketones and phenolics are heated together in the presence of ammonia.

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As model ketones, 3,5-heptadien-2-one and 2,6-heptanedione were selected. 3,5-Heptadien-2-one was selected because it is a flavor component in some food products, such as caviar (Xu et al., 2022). In addition, it has a structure that is similar to that of 2,4-alkadienals, and these last compounds both are widely produced in the course of lipid oxidation (Qi, Wang, Zhan, & Tian, 2022), and are trapped by food phenolics (Hidalgo & Zamora, 2018). On the other hand, 3,5-heptadien-2-one is unstable under oxidizing conditions. To avoid this inconvenience, a saturated diketone of the same chain length was also included in the study: 2,6-heptanedione. This compound is present in the essential oils of *Mentha pulegium* L. (Sardashti & Adhami, 2013) and *Theobroma cacao* L. (Chee, Malek, & Ramli, 2005), among others. The hypothesis of this study was that, analogously to aldehydes, ketones should also interact with phenolics in some way. However, ketone reactivity should be reduced in comparison to that of aldehydes because of two main factors: aldehydes are less hindered than ketones, and the carbonyl carbon of aldehydes is generally more electrophilic than that of ketones because of the electron-donating nature of alkyl groups. Therefore, the formation of stable carbonyl-phenol adducts should be more difficulty produced than those previously observed for aldehydes (Zamora & Hidalgo, 2018).

2. Materials and methods

2.1. Materials

Phenolics with different structures were assayed to study their potential reaction with the selected ketones. The assayed phenolics included *o*-diphenols (catechol and 4-methylcathechol), *m*-diphenols (resorcinol, orcinol, and olivetol), *p*-diphenols (hydroquinone and trimethylhydroquinone), and quinones (1,4-benzoquinone, 2-methyl-1,4-benzoquinone, and 2,5-dimethyl-1,4-benzoquinone). Chemical structures for all these compounds are given in Figure S1 of the Supporting Information. Suppliers for employed phenolics are given in Table S1 of the Supporting Information. In addition to phenolic compounds, *tert*-butylhydroperoxide was also employed for comparison.

2,6-Heptanedione, 3,5-heptadien-2-one, 3-methylcyclohex-2-en-1-one, 2,6-dimethylpyridine, and other chemicals used in this research were of the highest available grade and were purchased from reliable commercial sources. Suppliers for main compounds employed in this study are given in Table S1 of the Supporting Information. Sigma-Aldrich/Merck (Darmstadt, Germany), TCI (Tokyo, Japan), and Alfa Aesar (Haverhill, Massachusetts) were the main suppliers.

2.2. Formation of 2,6-dimethylpyridine by thermal heating of 3,5-heptadien-2-one in the presence of ammonia and phenolic compounds

Mixtures of 3,5-heptadien-2-one (20 μmol in 50 μL of methanol), ammonium chloride (20 μmol in 20 μL of water), the phenolic compound (10 μmol in 20 μL of methanol), and 30 μL of 0.3 M sodium phosphate, pH 8, were singly homogenized with 200 mg of 0.0632–0.20 mm silica gel (Macherey-Nagel, Düren, Germany). Mixtures were heated at 150 °C in closed test tubes for 22 h under nitrogen. After cooling, 700 μL of methanol and 30 μL of the internal standard solution (10 μmol of methyl heptanoate per mL of methanol) were added. Suspensions were stirred for 1 min, and the liquid was filtered and centrifuged for 10 min at 16,000 *g*. The supernatant was studied by gas chromatography coupled to mass spectrometry (GC–MS).

2.3. Formation of 2,6-dimethylpyridine and 3-methylcyclohex-2-en-1-one by thermal heating of 2,6-heptanedione in the presence of ammonia and phenolic compounds

The reaction of 2,6-heptanedione with ammonia in the presence of phenolic compounds was carried out analogously to the above described reaction of 3,5-heptadien-2-one. Briefly, mixtures 2,6-heptanedione (20 μmol in 50 μL of methanol), ammonium chloride (20 μmol in 20 μL of water), the phenolic compound (10 μmol in 20 μL of methanol), and 30 μL of 0.3 M sodium phosphate, pH 8, were singly homogenized with 200 mg of 0.0632–0.20 mm silica gel (Macherey-Nagel, Düren, Germany). Mixtures were heated at 150 °C in closed test tubes for 1 h. After cooling, 700 μL of methanol and 30 μL of the internal standard solution (10 μmol of methyl heptanoate per mL of methanol) were added. Suspensions were stirred for 1 min, and the liquid was filtered and centrifuged for 10 min at 16,000 *g*. The supernatant was studied by GC–MS.

2.4. Determination of 2,6-dimethylpyridine and 3-methylcyclohex-2-en-1-one

Compounds formed in the heated mixtures of the ketone (either 3,5-heptadien-2-one or 2,6-heptanedione) and ammonia were fractionated, identified, and quantified in an Agilent 7820A gas chromatograph coupled with an Agilent 5977B mass selective detector (MSD), quadrupole type (Agilent Technologies, Santa Clara, CA). One microliter of sample was injected in the pulsed splitless mode and 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 following conditions were employed: carrier gas, helium (1 mL/min at constant flow); injector, 250 °C; transfer line to mass selective detector, 280 °C; electron ionization (EI), 70 eV; ion source temperature, 230 °C; and mass range, 28–550 amu. The oven was programmed from 40 °C (4 min) to 200 °C at 15 °C/min, and then held at 200 °C for 2 min.

2,6-Dimethylpyridine and 3-methylcyclohex-2-en-1-one were identified according to their retention times, mass spectra, and co-elution with authentic standards. Both compounds were quantified by preparing standard curves of 2,6-dimethylpyridine and 3-methylcyclohex-2-en-1-one in 200 mg of silica gel and following the same procedure described above (without heating). Seven amounts of both compounds (0–10 μmol) were used. 2,6-Dimethylpyridine and 3-methylcyclohex-2-en-1-one contents were directly proportional to compound/internal standard area ratio ($r^2 = 0.995$, $p < 0.001$). RSD was < 10 %.

2.5. Detection of adducts produced in ketone-phenol reactions

In addition to determining 2,6-dimethylpyridine and 3-methylcyclohex-2-en-1-one, all assayed reaction mixtures were also screened for the formation of ketone-phenol adducts. This screening was carried out by GC–MS using the same equipment and experimental conditions described in the previous section. Only oven programming was different. The programming employed was: from 50 °C (1 min) to 300 °C at 15 °C/min, and then held at 300 °C for 5 min.

2.6. Isolation and characterization of the carbonyl-phenol adducts produced in the reaction of 3,5-heptadien-2-one with resorcinol

Mixtures of 3,5-heptadien-2-one (50 μmol in 50 μL of methanol), ammonium chloride (50 μmol in 20 μL of water), resorcinol (25 μmol in 20 μL of methanol), and 30 μL of 0.3 M sodium phosphate, pH 8, were singly homogenized with 200 mg of 0.0632–0.20 mm silica gel (Macherey-Nagel, Düren, Germany). Mixtures were heated at 150 °C in closed test tubes for 22 h under nitrogen. After cooling, each mixture was treated with 700 μL of acetonitrile, stirred for 1 min, and the liquid was filtered and centrifuged for 10 min at 16,000*g*. All mixtures (41 reactions in total) were mixed and fractionated by semi-preparative high-performance liquid chromatography.

The isolation of the produced adducts was carried out on an Agilent Technologies 1260 Infinity II liquid chromatograph (Agilent Technologies, Santa Clara, CA) composed by a G7129A autosampler, a G1311C quaternary pump, a G4212 diode array detector, and a G1346F fraction collector. Samples (250 μL) were fractionated on a Zorbax Eclipse XDB-C18 semi-preparative column (9.4 × 250 mm, 5 μm), from Agilent, held at ambient temperature (22 °C). Ketone-resorcinol adducts were

separated at a flow rate of 3.0 mL/min using a gradient of acetonitrile in water. The gradient employed was from 30 to 80 % acetonitrile over 20 min. Formed adducts were detected at 210 nm. Fractions containing the different adducts were collected and those corresponding to the same adduct (identical retention time) were combined. Formed adducts were characterized by mono- and bi-dimensional NMR and MS. In addition, some derivatives were prepared for additional confirmation of their structures. Prepared derivatives were the corresponding trimethylsilyl and carbonate derivatives.

Trimethylsilyl derivatives were prepared by dissolving five hundred micrograms of the corresponding adduct in 200 μL of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and heating for 30 min at 60 °C. The produced trimethylsilyl derivative was studied by GC–MS using the equipment and the conditions described in section 2.5.

Carbonate derivatives were prepared by treating a solution of five hundred micrograms of the adduct in 100 μL of methanol with 100 μL of 1 M NaOH, 35 μL of pyridine, and 20 μL of methyl chloroformate. The reaction mixture was stirred and, after 20 s, other 20 μL of methyl chloroformate was added. The reaction mixture was stirred again and, after 2 min, 400 μL of 0.1 M potassium bicarbonate was added. The reaction mixture was newly stirred and 700 μL of dichloromethane was added. After a final stirring, the mixture was centrifuged at 1000 *g* for 10 min and the organic layer was studied by GC–MS using the equipment and the conditions described in section 2.5.

2,4-Dimethyl-5,6-dihydro-4*H*-2,6-methanobenzo[*d*][1,3]dioxocin-9-ol (**8**). ^{1}H NMR (500 MHz, CD_3OD): δ 1.10 (d, 3H, $J = 6.2$ Hz, H1‴), 1.49 (s, 3H, H1″), 1.60 (m, 2H, H5), 1.77 (ddd, 1H, $J = 1.5$ Hz, $J = 3.6$ Hz, $J = 12.7$ Hz, H2′a), 2.01 (dd, 1H, $J = 2.8$ Hz, $J = 12.7$ Hz, H2′b), 3.06 (m, 1H, H6), 3.72 (m, 1H, H4), 6.25 (d, 1H, $J = 2.4$ Hz, *H*10), 6.34 (dd, 1H, $J = 2.4$ Hz, $J = 8.2$ Hz, H8), and 6.88 (d, 1H, $J = 8.2$ Hz, H7). ^{13}C NMR (125.7 MHz, CD_3OD): δ 20.29 (C1‴), 26.83 (C1″), 29.86 (C6), 34.08 (*C*2′), 39.50 (C5), 65.15 (C4), 97.66 (*C*2), 101.13 (C10), 107.51 (C8), 116.99 (C6′), 128.07 (C7), 156.15 (C10′), and 156.82 (C9). MS, *m/z* (%, ion structure): 220 (59, M^+), 205 (31, $M^+ - CH_3$), 177 (24, $M^+ - CH_3CO$), and 163 (100, $M^+ - CH_3COCH_2$). MS of the trimethylsilyl derivative [((2,4-dimethyl-5,6-dihydro-4*H*-2,6-methanobenzo[*d*][1,3]dioxocin-9-yl)oxy)trimethylsilane], *m/z* (%, ion structure): 292 (67, M^+), 277 (49, $M^+ - CH_3$), 249 (56, $M^+ - CH_3CO$), and 235 (100, $M^+ - CH_3COCH_2$). MS of the carbonate [2,4-dimethyl-5,6-dihydro-4*H*-2,6-methanobenzo[*d*][1,3]dioxocin-9-yl methyl carbonate], *m/z* (%, ion structure): 278 (90, M^+), 263 (65, $M^+ - CH_3$), 260 (65, $M^+ - H_2O$), 245 (16, 260 – CH_3), 235 (9, $M^+ - CH_3CO$), 221 (100, $M^+ - CH_3COCH_2$), and 219 (38, $M^+ - CH_3OCO$).

1-(7-Hydroxy-4-methylchroman-2-yl)propan-2-one (**10**). ^{1}H NMR (500 MHz, CD_3OD): δ 1.28 and 1.30 (d, 3H, $J = 6.8$ Hz, H1‴), 2.03 and 2.05 (m, 2H, H3), 2.25 (s, 3H, H3″), 2.73 and 2.88 (m, 2H, H1″), 2.87 and 2.93 (m, 1H, H4), 4.44 and 4.52 (m, 1H, H2), 6.15 and 6.17 (d, 1H, $J = 2.5$ Hz, H8), 6.33 and 6.34 (dd, 1H, $J = 2.4$ Hz, $J = 8.3$ Hz, H6), and 6.94 and 7.04 (dd, 1H, $J = 0.5$ Hz or 1.0 Hz, and $J = 8.3$ Hz, H5). ^{13}C NMR (125.7 MHz, CD_3OD): δ 19.47 and 22.89 (C1‴), 27.37 and 28.73 (C4), 29.25 and 29.31 (C3″), 34.38 and 37.36 (C3), 48.44 and 48.97 (C1″), 67.84 and 72.29 (*C*2), 102.41 and 102.50 (C8), 107.87 and 108.03 (C6), 118.25 and 118.28 (C4′), 129.31 and 129.41 (C5), 154.40 and 154.91 (C8′), 156.12 (C7), and 207.9 and 208.00 (*C*2″). MS of the isomer A, *m/z* (%, ion structure): 220 (32, M^+), 161 (98, 4-methyl-4*H*-chromen-7-ol – H), 147 (100, $M^+ - CH_3$), and 137 (36, $M^+ - C_5H_7O$). MS of the isomer B, *m/z* (%, ion structure): 220 (54, M^+), 161 (58, 4-methyl-4*H*-chromen-7-ol – H), 147 (100, 161 – CH_3), and 137 (75, $M^+ - C_5H_7O$). MS of the trimethylsilyl derivative, *m/z* of the isomer A [1-(4-methyl-7-((trimethylsilyl)oxy)chroman-2-yl)propan-2-one] (%, ion structure): 292 (73, M^+), 277 (15, $M^+ - CH_3$), 233 (46, trimethyl((4-methyl-4H-chromen-7-yl)oxy)silane – H), 219 (100, 233 – CH_3), and 209 (36, $M^+ - C_5H_7O$); *m/z* of the isomer B [6-(2,4-bis((trimethylsilyl)oxy)phenyl) hept-3-en-2-one] (%, ion structure): 364 (3, M^+), 349 (6, $M^+ - CH_3$), and 281 (100, $M^+ - C_5H_7O$). MS of the carbonate [methyl (4-methyl-2-(2-oxopropyl)chroman-7-yl) carbonate], *m/z* of the isomer A (%, ion structure): 278 (31, M^+), 220 (34, methyl (4-methyl-4*H*-chromen-7-yl) carbonate), 219 (100, 220 – H), 205 (76, 220 – CH_3), 195 (23, $M^+ - C_5H_7O$); *m/z* of the isomer B (%, ion structure): 278 (51, M^+), 220 (27, methyl (4-methyl-4*H*-chromen-7-yl) carbonate), 219 (63, 220 – H), 205 (100, 220 – CH_3), and 195 (62, $M^+ - C_5H_7O$).

2.7. NMR spectroscopy

NMR spectra were obtained by using a Bruker Advance III spectrometer operating at 500 MHz for protons. Acquisition parameters used for ^{1}H and ^{13}C monodimensional experiments were described previously (Zamora et al., 2016). In addition, bidimensional experiments (mainly COSY, HMQC, and HMBC) were carried out as a help for structural determinations. All experiments were performed at 22 °C.

2.8. Statistical analysis

All data given are mean ± SD values of, at least, three independent experiments. Analysis of variance was used to compare mean values. When *F* values were significantly different, group differences were evaluated by the Tukey test (Snedecor & Cochran, 1980). Statistical comparisons were carried out by 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. Effect of phenolics on the formation of 2,6-dimethylpyridine by cyclization of 3,5-heptadien-2-one in the presence of ammonia

When 3,5-heptadien-2-one (**1**) was heated in the presence of ammonia, 2,6-dimethylpyridine (**4**) was produced as shown in Fig. 1A (Zamora et al., 2020). Thus, the reaction between the ketone and ammonia produces the corresponding imine (**2**) in a first step. Then, the imine (**2**) cyclizes by addition of the nitrogen to the gamma-delta carbon–carbon double bond of the ketone to produce a dihydropyridine (**3**). Finally, the dihydropyridine (**3**) is aromatized to 2,6-dimethylpyridine (**4**).

In the presence of phenolics, some changes in the amount of the produced pyridine were observed (Fig. 1B). According to the previous research carried out with aldehydes, *m*-diphenols are able to trap carbonyl compounds, and the amount of the produced pyridine should decrease. As shown in Fig. 1B, this was only observed in the presence of resorcinol. Furthermore, although a small decrease was observed, this decrease was not significant ($p < 0.05$). Nevertheless, 3,5-heptadien-2-one was trapped by resorcinol to some extent and the corresponding ketone-phenol adducts were isolated by semipreparative HPLC and identified by NMR and MS.

The adducts produced were identified as 2,4-dimethyl-5,6-dihydro-4*H*-2,6-methanobenzo[*d*][1,3]dioxocin-9-ol (**8**) and 1-(7-hydroxy-4-methylchroman-2-yl)propan-2-one (**10**), respectively. Both compounds **8** and **10** had a molecular weight that resulted from the addition of one molecule of the ketone and one molecule of the resorcinol.

The analysis of the NMR spectra of compound **8** showed that the carbonyl group had disappeared, the skeleton of the ketone in this compound had changed from CH_3-C-CH-CH-CH-CH-CH_3 to CH_3-C-CH_2-CH-CH_2-CH-CH_3, and the oxygen at C3 and the C4 of resorcinol were bonded to *C*2 and C4 of the ketone, respectively. HMBC spectrum confirmed the proposed structure, which was also in agreement with the MS of the compound and the MS of the produced derivatives.

The structural determination of adduct **10** resulted more complex because two isomers were produced. For this adduct, NMR spectra showed that the carbonyl group was present, the skeleton of the ketone in compound **10** had changed analogously to that observed in compound **8**, and the oxygen at C3 and the C4 of resorcinol were bonded to C4 and C6 of the ketone, respectively. HMBC spectrum confirmed the proposed

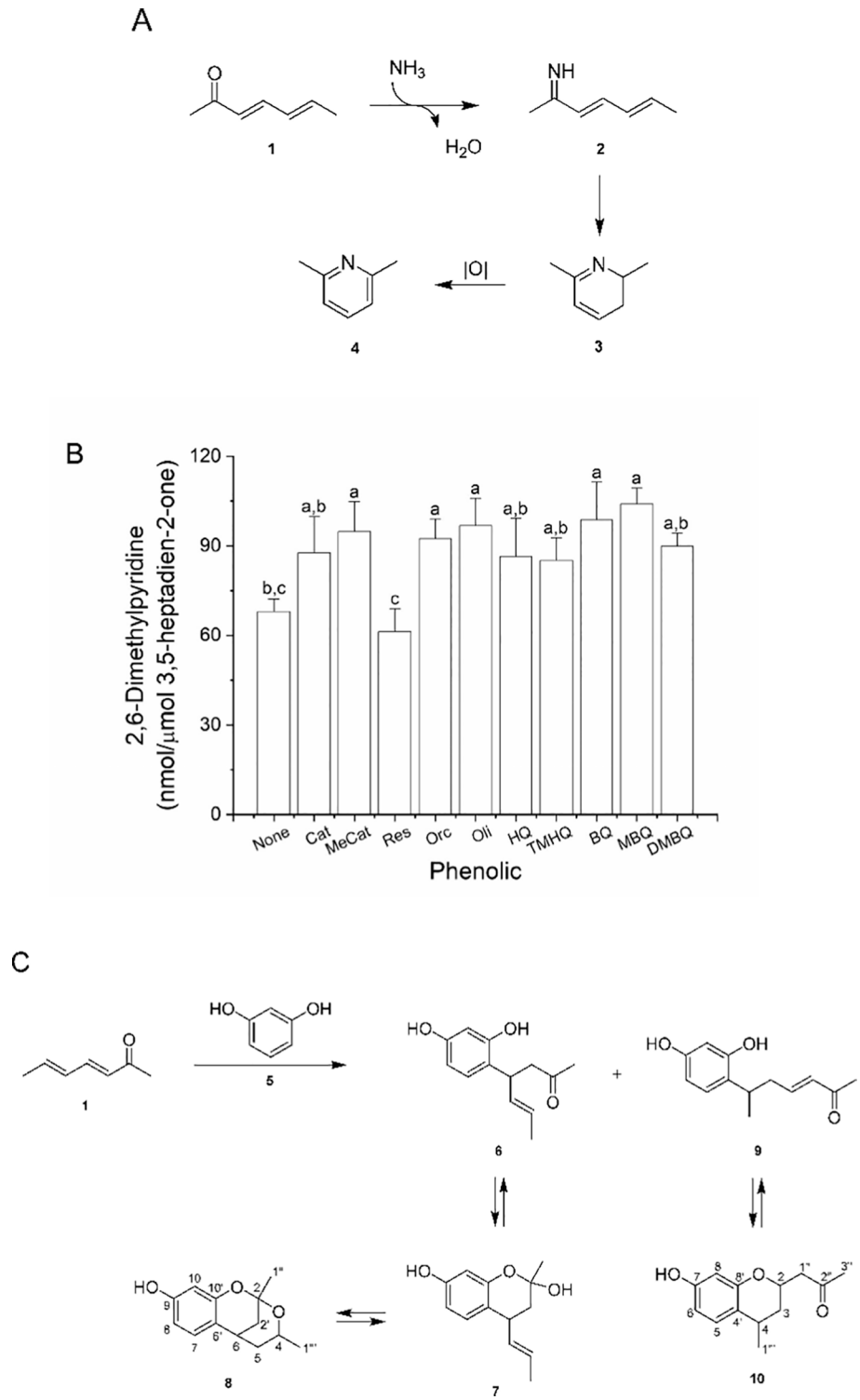

Fig. 1. (A) Proposed pathway for the formation of 2,6-dimethylpyridine (**4**) by cyclization of 3,5-heptadien-2-one (**1**) in the presence of ammonia. (B) Formation of 2,6-dimethylpyridine by cyclization of 3,5-heptadien-2-one in the presence of ammonia and phenolics. Reaction mixtures were heated at 150 °C for 22 h at pH 8 under nitrogen. Means with different letters are significantly ($p < 0.05$) different. Abbreviations: Cat, catechol; MeCat, 4-methylcatechol; Res, resorcinol; Orc, orcinol; Oli, olivetol; HQ, hydroquinone; TMHQ, trimethylhydroquinone; BQ, benzoquinone; MeBQ, methylbenzoquinone; and DMBQ, 2,5-dimethylbenzoquinone. (C). Proposed pathway for the formation of 2,4-dimethyl-5,6-dihydro-4*H*-2,6-methanobenzo[*d*][1,3]dioxocin-9-ol (**8**) and 1-(7-hydroxy-4-methylchroman-2-yl)propan-2-one (**10**) from 3,5-heptadien-2-one (**1**) and resorcinol (**5**).

structure, which was also in agreement with the MS of the compound and the MS of the produced derivatives. The only significant difference between MS of compound **10** and that of compound **8** was that compound **10** was partially opened in the presence of BSTFA and the corresponding derivative with two silyl groups was observed (in addition to the derivative with only one silyl group). The reason for the presence of two isomers in compound **10** is the presence of two chiral carbons in this adduct (carbons *C*2 and C4). Therefore, the two isomers observed by both NMR and GC–MS should correspond to the two pairs of diastereomers. Analogously, compound **8** has three chiral carbons (*C*2, C4, and C6). However, only one isomer (or one pair of stereoisomers) was observed by NMR and GC–MS. The reason might be related to the tight structure of compound **8**. Because of steric reasons, only the formation of the detected adduct should be favored.

Compounds **8** and **10** are suggested to be produced according to the pathway shown in Fig. 1C. Because the ketone has two carbon–carbon double bonds, the phenolic C4 of the resorcinol can added to either the alpha–beta or the gamma-delta carbon–carbon double bond of the ketone. In the first case, it would produce the corresponding adduct **6**. This adduct is in equilibrium with other structures that are more stable. Thus,

it would cyclize intramolecularly to the hemiacetal **7** to protect the carbonyl group, and then, the produced hydroxyl group could be added to the initial gamma-delta carbon–carbon double bond to produce adduct **8** in which all reactive groups are protected in some way. The alternative possibility is the addition of the C4 of the resorcinol to the gamma-delta carbon–carbon double bound to produce the adduct **9**. In this case, the formation of the hemiacetal is not possible, and the only possibility of stabilization for compound **9** is the addition of the hydroxyl group to the initial alpha–beta carbon–carbon double bond of the ketone to produce adduct **10**.

Differently to resorcinol, the formation of ketone-phenol adducts was not observed for all other assayed phenolics, which included *ortho*-, *meta*-, and *para*-diphenols, and quinones. Furthermore, the presence of these phenolics increased the amount of the pyridine produced (Fig. 1B). This suggested that most assayed phenolics played a role with ketones different to the expected carbonyl-trapping, which was previously observed in aldehyde-phenolic reaction mixtures (Zamora & Hidalgo, 2018). This different role was less evident for resorcinol, most likely because of two reasons: it has an enhanced carbonyl-trapping ability because it is a *m*-diphenol (Hidalgo & Zamora, 2014), and it has less steric hindrance than other assayed *m*-diphenols. To confirm that this promoting effect of carbonyl–amine reactions is general for other ketones, the cyclization reaction of 2,6-heptanedione in the presence of ammonia and phenolics was studied.

3.2. Formation of 3-methylcyclohex-2-en-1-one and 2,6-dimethylpyridine by cyclization of 2,6-heptanedione in the presence of ammonia and phenolics

Similar results to those described for the cyclization of 3,5-heptadien-2-one (**1**) were also obtained for the cyclization of 2,6-heptanedione (**11**). However, some differences were also observed because both reactions are different. Thus, when 2,6-heptanedione (**11**) was heated in the presence of ammonia, two different compounds were produced: 2,6-dimethylpyridine (**4**) and 3-methylcyclohex-2-en-1-one (**13**) (Fig. 2). Both compounds can be hypothesized to be produced by two alternative routes that compete between them as shown in Fig. 2. In the first one, ammonia can abstract one proton of the ketone, and the produced carbanion would cyclize. After dehydration of the produced cyclohexanone **12**, 3-methylcyclohex-2-en-1-one (**13**) would be formed. This is a typical intramolecular aldol reaction, which does not involve any oxidative step. Alternatively, a second reaction can be produced. Thus, the corresponding imine **14** can be produced between 2,6-heptanedione and ammonia, and this compound would be able to cyclize. The resulting cyclic adduct **15** can be dehydrated to the corresponding dihydropyridine **16**. Finally, the aromatization of this dihydropyridine **16** would be the origin of 2,6-dihydropyridine (**4**).

Both routes compete between them and the formation of 2,6-dimethylpyridine should be promoted in the presence of compounds that favor the aromatization step. Table 1 shows the results obtained when 2,6-heptanedione was heated in the presence of ammonia and several phenolic compounds under three different atmospheres.

In the presence of nitrogen, 2,6-heptanedione was almost quantitatively converted into 3-methylcyclohex-2-en-1-one and only trace

Table 1
Formation of 3-methylcyclohex-2-en-1-one and 2,6-dimethylpyridine by thermal heating of 2,6-heptanedione in the presence of ammonia and phenolics.

	3-methylcyclohex-2-en-1-one (nmol/μmol of 2,6-heptanedione)			2,6-dimethylpyridine (nmol/μmol of 2,6-heptanedione)		
phenolic	nitrogen	air	oxygen	nitrogen	air	oxygen
None	982 ± 54 a A	877 ± 75 a A	660 ± 12 b A	1 ± 1 a D	28 ± 2 b E	62 ± 14 c D
BuOOH	428 ± 50 a D	468 ± 45 a B	441 ± 16 a B	110 ± 5 a C	116 ± 11 a E	123 ± 1 a D
Cat	988 ± 29 a A	431 ± 21 b B	136 ± 31 c C,D	24 ± 1 a D	367 ± 22 b C,D	349 ± 31 b C
4-Methylcat	975 ± 45 a A	119 ± 25 b C	52 ± 22 b D,E	29 ± 12 a D	673 ± 6 b A	580 ± 16 c A
Resorcinol	985 ± 8 a A	511 ± 61 b B	240 ± 36 c C	17 ± 2 a D	385 ± 11 b C,D	412 ± 9 b C
Orcinol	992 ± 4 a A	458 ± 17 b B	240 ± 38 c C	20 ± 19 a D	387 ± 28 b C,D	406 ± 44 b C
Olivetol	851 ± 45 a A,C	430 ± 56 b B	234 ± 28 c C	20 ± 3 a D	319 ± 8 b D	441 ± 25 c B,C
Hydroquinone	924 ± 31 a A	189 ± 37 b C	51 ± 25 c D,E	27 ± 16 a D	377 ± 50 b C,D	425 ± 16 b C
TrimethylHQ	711 ± 59 a C	208 ± 10 b C	140 ± 5 b C,D	115 ± 14 a C	495 ± 53 b B,C	391 ± 23 b C
Benzoquinone	473 ± 26 a D	56 ± 6 b C	23 ± 5 b E	241 ± 51 a B	654 ± 83 b A	525 ± 80 b A, B
MethylBQ	462 ± 58 a D	56 ± 11 b C	22 ± 1 b E	226 ± 48 a B	499 ± 75 b B,C	457 ± 3 b B,C
2,5-DimethylBQ	309 ± 56 a D	60 ± 5 b C	49 ± 9 b D,E	374 ± 20 a A	588 ± 19 b A,B	553 ± 27 b A, B

*Means in the same row (for either 3-methylcyclohex-2-en-1-one or 2,6-dimethylpyridine) with different lower-case letter are significantly ($p < 0.05$) different. Means in the same column with different capital letter are significantly ($p < 0.05$) different. Abbreviations: BuOOH, *tert*-butyl hydroperoxide; BQ, benzoquinone; Cat, catechol; HQ, hydroquinone.

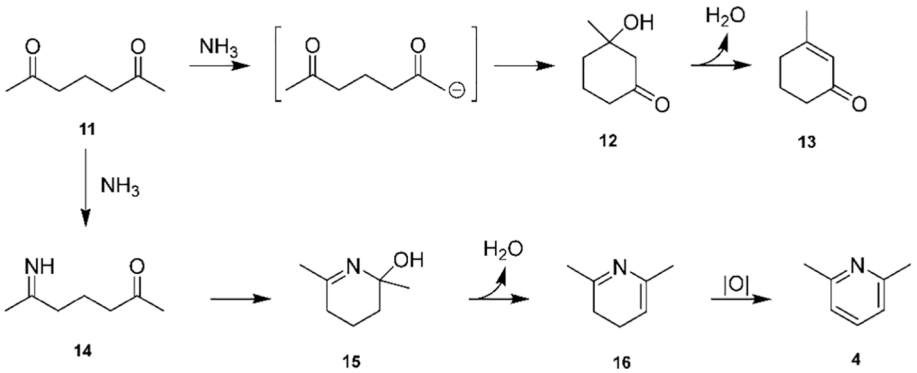

Fig. 2. Proposed pathway for the formation of 2,6-dimethylpyridine (**4**) and 3-methylcyclohex-2-en-1-one (**13**) from 2,6-heptanedione (**11**) in the presence of ammonia.

amounts of 2,6-dimethylpyridine were detected. Similar results were obtained when the assayed *o*-diphenols (catechol and 4-methylcatechol), *m*-diphenols (resorcinol, orcinol, and olivetol), and hydroquinone were present in the reaction mixture. Significant increase of 2,6-dimethylpyridine, and, therefore, significant decrease of 3-methylcyclohex-2-en-1-one was only observed in the presence of trimethylhydroquinone, *tert*-butylhydroperoxide, and the three assayed quinones (benzoquinone, methylbenzoquinone, and 2,5-dimethylbenzoquinone). Particularly, the assayed quinones were the compounds that produced the highest amount of 2,6-dimethylpyridine.

When the nitrogen was substituted by air, many significant changes were observed (Table 1). Thus, the control produced more 2,6-dimethylpyridine and less 3-methylcyclohex-2-en-1-one than when heated under nitrogen. On the contrary, there was not any difference in the results obtained for the samples heated under nitrogen and under air in the presence of *tert*-butylhydroperoxide. Nevertheless, all phenolics increased the formation of 2,6-dimethylpyridine and decreased the formation of 3-methylcyclohex-2-en-1-one when heated under air, and there were not clear differences among different phenolics as a function of the distribution of hydroxyl groups within the aromatic ring. Thus, *m*-diphenols, catechol, and hydroquinone produced a similar amount of 2,6-dimethylpyridine. This amount was lower than that produced by 4-methylcatechol, trimethylhydroquinone, and the different quinones assayed.

The change of air by oxygen increased the amount of 2,6-dimethylpyridine in the control, but not many more significant changes were observed. Only the amount of 2,6-dimethylpyridine decreased slightly in the presence of 4-methylcatechol and increased in the presence of olivetol. On the contrary, the presence of oxygen usually decreased the amounts of 3-methylcyclohex-2-en-1-one produced in relation to those produced in the presence of air, which might be a consequence of parallel oxidative reactions that take place in the presence of oxygen.

These results both confirmed the above described results obtained for 3,5-heptadien-2-one, and pointed out to phenolics as promoters of reactions in which an aromatization step was required.

3.3. Effect of reaction conditions on the formation of 2,6-dimethylpyridine by cyclization of 2,6-heptanedione in the presence of ammonia and orcinol

To further confirm the role of phenolics on aromatization and to determine the conditions by which that reaction is favored, the effect of reaction conditions on the formation of 2,6-dimethylpyridine by cyclization of 2,6-heptanedione in the presence of ammonia and orcinol under air was studied. Fig. 3A shows the effect of pH on the formation of both: 3-methylcyclohex-2-en-1-one and 2,6-dimethylpyridine. As can be observed, the conversion of 2,6-heptanedione into 3-methylcyclohex-2-en-1-one is produced to the same extent at the different assayed pH values. However, the conversion of 2,6-heptanedione into 2,6-dimethylpyridine increased linearly ($r = 0.955$, $p = 0.045$) as a function of pH in the range 5–8.

In addition to the pH, the production of both 3-methylcyclohex-2-en-1-one and 2,6-dimethylpyridine depended on the amount of phenolic present (Fig. 3B). Thus, the conversion of 2,6-heptanedione into 3-methylcyclohex-2-en-1-one decreased linearly ($r = -0.98$, $p = 0.005$) as a function of the concentration of orcinol in the range 0–8 μmol of the phenolic. This decrease was parallel to the increase in the conversion of 2,6-heptanedione into 2,6-dimethylpyridine. Thus, 2,6-dimethylpyridine increased linearly ($r = 0.98$, $p = 0.0004$) as a function of the concentration of orcinol in the range 0–10 μmol of the phenolic. Higher concentrations of orcinol did not produced increased concentrations of the pyridine (or decreased concentrations of 3-methylcyclohex-2-en-1-one).

Conversion of 2,6-heptanedione into both 3-methylcyclohex-2-en-1-one and 2,6-dimethylpyridine also depended on heating time and temperature (Fig. 4A and 4B). In fact, the concentrations of both compounds

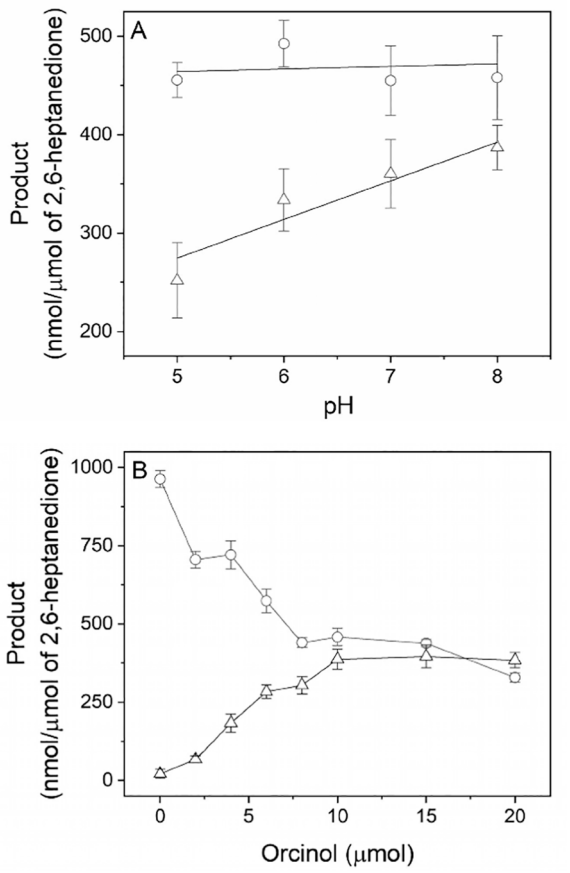

Fig. 3. Effect of: (A), pH; and (B), amount of orcinol, on the formation of 3-methylcyclohex-2-en-1-one (○) and 2,6-dimethylpyridine (Δ) in mixtures of 2,6-heptanedione, ammonia, and orcinol. Reaction mixtures were heated at 150 °C for 1 h under air.

increased linearly ($r > 0.99$, $p < 0.01$) as a function of time within the studied temperature range (90–170 °C). In addition, both products were produced to a higher extent when temperature increased. Reaction rates were obtained from the slopes of the adjusted lines in Fig. 4A and 4B, and were used in an Arrhenius plot (Fig. 4C) to determine the activation energies of the formation of both compounds. These activation energies resulted to be 54.2 ± 1.5 and 56.8 ± 1.9 kJ/mol for 3-methylcyclohex-2-en-1-one and 2,6-dimethylpyridine, respectively, therefore indicating that, in the presence of phenolics, the activation energy of both reactions was similar.

3.4. Phenolics as promoters of aromatization reactions

Previous results showed that, differently to aldehydes, most phenolics were not able to trap ketones, more likely because of the reduced reactivity of the carbonyl group of these compounds and the steric hindrance introduced by the additional alkyl group bonded to the carbonyl carbon. Among the two assayed ketones, only 3,5-heptadien-2-one was trapped to some extent and only by resorcinol (the *m*-diphenol with lower steric hindrance). However, although they were not able to trap the ketones, the presence of phenolics played a role in the formation of 2,6-dimethylpyridine by both assayed ketones when they were heated in the presence of ammonia. Both reactions were promoted by phenolics, and this promotion is likely related to their favoring of the aromatization step needed to produce the pyridine.

Aromatization is the chemical process by which a nonaromatic ring is converted into an aromatic ring. This process usually occurs during the formation of numerous compounds involved in food safety and

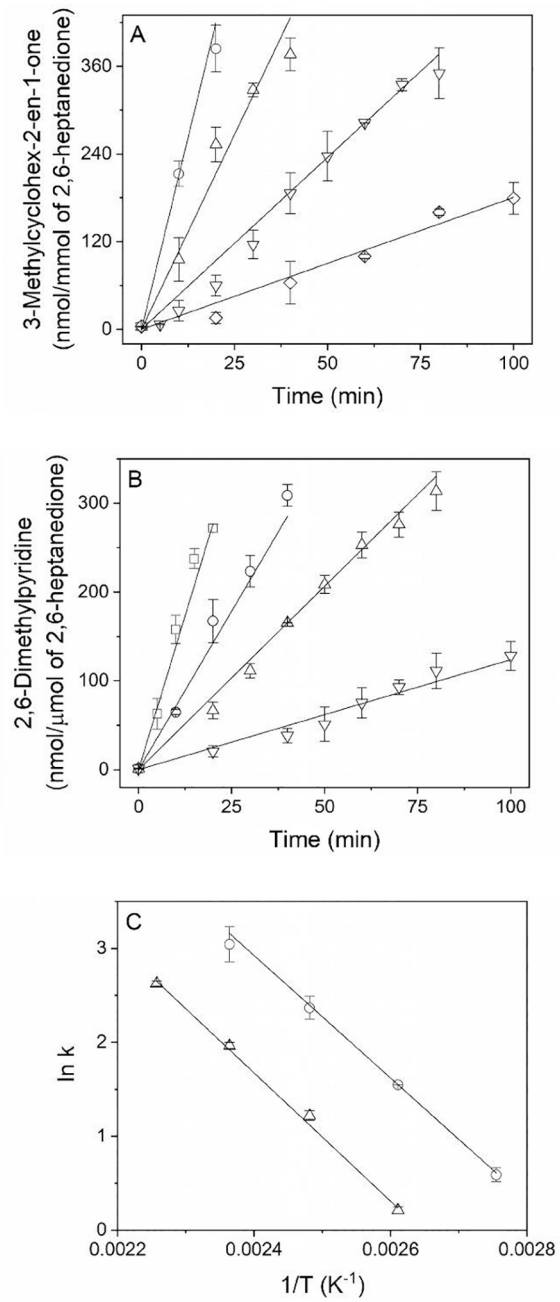

Fig. 4. Time courses of the formation of: (A), 3-methylcyclohex-2-en-1-one; and (B), 2,6-dimethylpyridine in mixtures of 2,6-heptanedione, ammonia, and orcinol heated under air. Five temperatures were assayed: 170 (□), 150 (○), 130 (Δ), 110 (▽), and 90 °C (◇). Panel (C) shows the Arrhenius plot obtained for 3-methylcyclohex-2-en-1-one (○), 2,6-dimethylpyridine (Δ).

quality. As described in Fig. 1A and 2, an aromatization step is required for the formation of 2,6-dimethylpyridine, a compound which is present in the aroma of different food products, including fermented green coffee beans (Kim et al., 2019) or Doubanjiang, a Chinese Traditional Fermented Red Pepper Paste (Li et al., 2019), among others.

In chemical synthesis, aromatizations are produced by means of different oxidants, including catalytic systems, oxidative reagents, radical initiators, etc. (Asikainen, Jauhiainen, Aaltonen, & Harlin, 2013). In addition, aromatizations have also been described to be produced by means of *p*-benzoquinone (Bueno, Brandao, & Gusevskaya, 2008). Therefore, obtained results in Fig. 1B and Table 1 should be able to be explained in that way.

In the absence of oxygen and phenolics, 2,6-heptanedione only produced trace amounts of 2,6-dimethylpyridine (Table 1). However, when air was present, the amount of the pyridine increased, and the highest amount of pyridine was produced when 2,6-heptanedione was heated under oxygen. Therefore, aromatization in this case is likely a consequence of the presence of oxygen and can be hypothesized to be produced as indicated in Fig. 5A. Upon heating, a homolytic cleavage can be produced in the dihydropyridine and the produced free radical can react with oxygen to produce a hydroperoxyl radical, which would later facilitate the aromatization of the ring.

Ring aromatization can also be produced in the absence of oxygen. This is facilitated in the presence of free radicals as occurs in the presence of *t*-butyl hydroperoxide. As indicated in Fig. 5B, the thermal heating of *t*-butyl hydroperoxide produces its homolytic breakage. The produced radical can abstract the two protons of the dihydropyridine to promote its aromatization. This process is likely produced in two steps.

According to Table 1, analogously to *t*-butyl hydroperoxide, quinones do not need the presence of air to produce the conversion of 2,6-heptanedione into 2,6-dimethylpyridine. A possible mechanism that explains this conversion is shown in Fig. 5C. Quinones are able to abstract one hydrogen atom from the dihydropyridine to produce the corresponding radical at the same time that the quinone is converted into a semiquinone radical. The abstraction of a new hydrogen atom by this radical would produce the pyridine.

Phenolics can also play a similar role to that of quinones, but the presence of oxygen is usually required, as observed in Table 1. As shown in Fig. 5D, phenolics produce radicals that can abstract the proton from the dihydropyridine. This radical can react then with oxygen to produce the corresponding hydroperoxyl radical which is easily decomposed to produce the pyridine. Although the presence of oxygen facilitates the process, these reactions can also be produced in the absence of oxygen and in the presence of free radicals. This is hypothesized to be produced in the promotion of 2,6-dimethylpyridine formation by heating 3,5-heptadien-2-one with ammonia under nitrogen (Fig. 1B). This ketone is unstable and prone to be oxidized. Therefore, it is expected to produce easily free radicals that can promote its cyclization to 2,6-dimethylpyridine in the presence of ammonia. When phenolics were present, the formation of the pyridine was promoted.

4. Conclusion

Differently to aldehydes, carbonyl-trapping abilities of phenolics are much reduced when ketones are involved. This is consequence of both ketones are more hindered than aldehydes, and the carbonyl carbon of ketones is less electrophilic than that of aldehydes because of the electron-donating nature of the additional alkyl group. However, it is still possible to detect the formation of ketone-phenol adducts. In this study, they were only produced when 3,5-heptadien-2-one was heated with resorcinol, which is the *m*-diphenol with the lowest steric hindrance. In this case, the reaction occurred similarly to the reaction between *m*-diphenols and 2,4-alkadienals (Hidalgo & Zamora, 2018), and the produced adducts could be isolated and characterized as 2,4-dimethyl-5,6-dihydro-4*H*-2,6-methanobenzo[*d*][1,3]dioxocin-9-ol (**8**) and 1-(7-hydroxy-4-methylchroman-2-yl)propan-2-one (**10**). The formation of these adducts reduced the amount of the ketone and inhibited to some extent its conversion into the pyridine. To the best of our knowledge, this is the first time that the formation of ketone-phenolic adducts is described.

Differently to resorcinol, other assayed phenolics did not trap the ketones and did not inhibit the formation of the pyridine. On the contrary, they promoted the formation of this last compound. This promotion is likely related to the ability of phenolics and quinones to promote the aromatization step required for the formation of many carbonyl–amine adducts (including the pyridines described in this study). Therefore, obtained results suggest that, in addition to the recognized chelating, free-radical scavenging, and carbonyl-trapping functions of

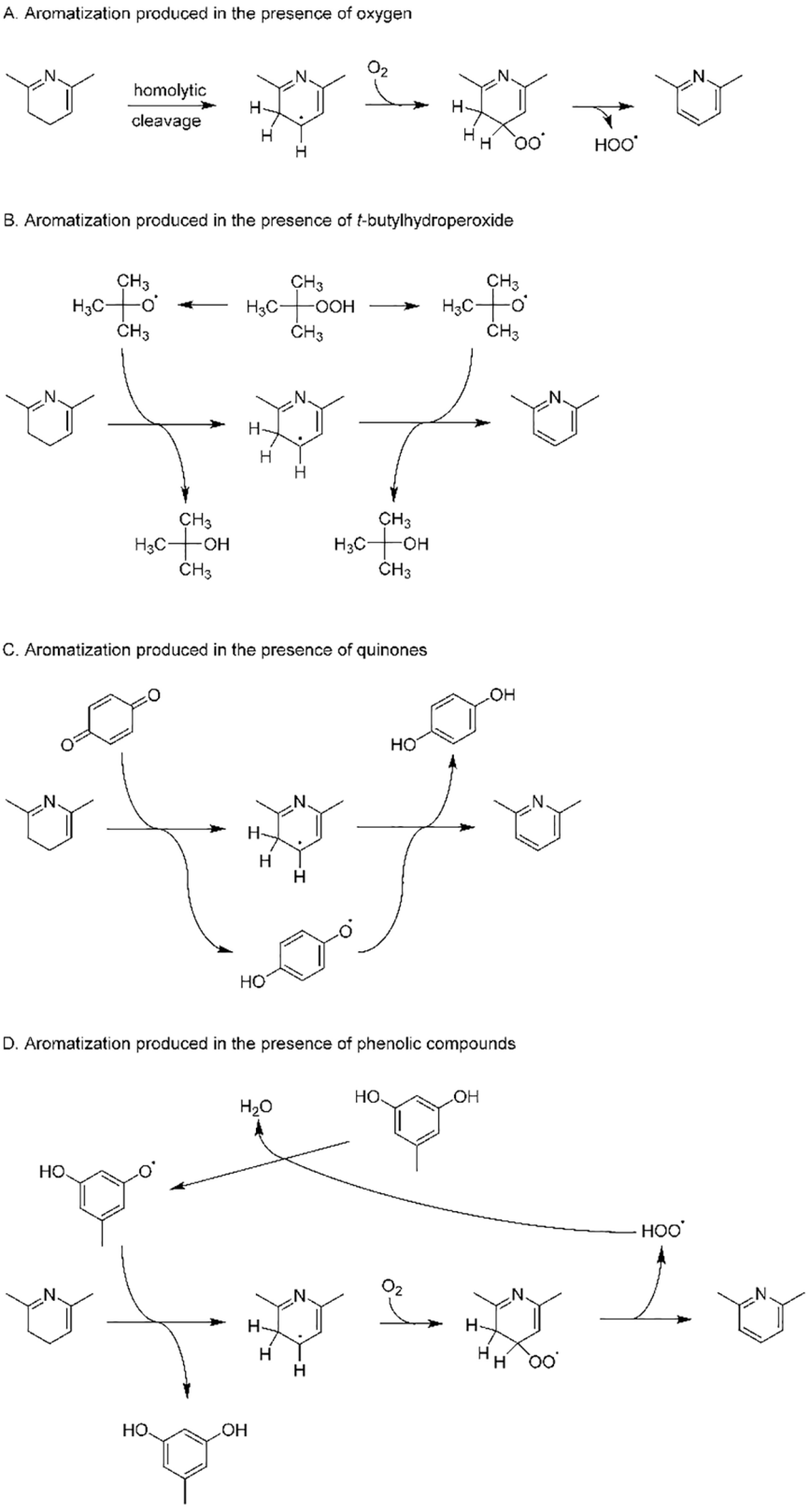

Fig. 5. Proposed pathways for the conversion of 2,6-dimethyl-3,4-dihydropyridine into 2,6-dimethylpyridine in the presence of: (A), oxygen; (B), *t*-butyl hydroperoxide; (C), quinones; and (D), phenolics.

phenolics (Zamora & Hidalgo, 2016), food phenolics can also promote the formation different compounds by facilitating the aromatization step needed to generate many of them. This new function needs to be considered when phenolics are added to food products.

CRediT authorship contribution statement

Francisco J. Hidalgo: Conceptualization, Software, Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Rosario Zamora:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134554.

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