Structure-Activity Relationship (SAR) of Phenolics for the Inhibition of 2-Phenylethylamine Formation in Model Systems Involving Phenylalanine and 13-Hydroperoxide of Linoleic Acid

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ABSTRACT

Lipid hydroperoxides have been shown to produce amino acid decarboxylations. Because thermal decomposition of lipid hydroperoxides produces free radicals and reactive carbonyls, and phenolic compounds have been shown to scavenger both of them, phenolics are expected to inhibit these reactions and this protection should depend on structures of involved phenolics. In this study, the effect of a wide array of phenolics and their mixtures on 2-phenylethylamine formation by phenylalanine degradation in the presence of the 13-hydroperoxide of linoleic acid (LOOH) was studied. LOOH increased considerably the formation of the amine and phenolics mostly exhibited an inhibitory role that depended on their structure. Thus, 1,3-diphenols decreased the formation of 2-phenylethylamine because of their carbonyl trapping abilities. On the contrary, the inhibition of 1,2- and 1,4-diphenols was lower because they could not trap the reactive carbonyls produced by LOOH decomposition. In addition, their free radical scavenging was likely accompanied by the formation of quinones, which acted as reactive carbonyls. The function of all other phenolics could be calculated by adding the individual functions of the different diphenols present in their structures. In fact, experimental values obtained for both mixtures of phenolics and complex phenolics correlated well with the calculated values obtained from their constituting diphenols. All these results suggest that, when reaction mechanisms are known, it is possible to predict the behavior of complex phenolics based on their structure.

KEYWORDS: *Biogenic amines; Lipid hydroperoxides ; Lipid oxidation; Maillard reaction; Phenolics; 2-Phenylethylamine; Reactive carbonyls*

INTRODUCTION

Amino acid degradation pathways play a crucial role in both the sensory properties and the safety of foods. Although these pathways can have a microbiological origin, most of produced amino acid degradation products can also be formed as a consequence of reactive carbonyl-induced chemical degradations.[1,2] Thus, for example, it is well-known the role of Maillard reaction on the formation of Strecker aldehydes as a consequence of food processing,[3,4] and the inhibitory role exhibited by phenolics in these reactions.[5] Something similar has been described for the formation of heterocyclic aromatic amines,[6] which have also been shown to be inhibited by phenolics.[7–9]

Differently to Strecker aldehydes and heterocyclic aromatic amines, chemical pathways leading to amino acid decarboxylations, and their control by antioxidants, are lesser known. However, generated 'biogenic' amines have been shown to be produced in parallel to Strecker aldehydes,[10,11] and their chemical formation seems to be a consequence of presence of reactive carbonyls in foods.[12] Because reactive carbonyls are trapped by food phenolics,[13–17] phenolics can be hypothesized to play a role in the formation of these amines. In fact, Tassoni et al.[18] compared biogenic amine and polyphenol profiles of red and white wines, and concluded that red wines, rich in anthocyanins, had less biogenic amines than white wines. These observations were related to both the higher content of free amino acids usually present in white wines and the inhibition of lactic acid bacteria growth by phenolics. However, the contribution that chemical reactions involving phenolics might be playing in obtained results was not considered.

In an attempt to clarify the inhibitory role of phenolics on amino acid decarboxylations produced by lipid oxidation products, this study analyzes the formation of 2-

phenylethylamine in model systems involving phenylalanine and 13-hydroperoxide of linoleic acid (LOOH) in the presence of a wide array of phenolic compounds.

MATERIALS AND METHODS

Chemicals. LOOH was prepared by oxidation of linoleic acid with lipoxygenase following a previously described procedure.[19] Twenty-seven simple phenolic compounds (or analogues) were employed in this study. They were: catechol (**1**), 4-methylcatechol (**2**), toluene (**3**), *p*-cresol (**4**), 4-methoxycatechol (**5**), 3-(3,4-dihydroxyphenyl)propanoic acid (**6**), 3,4-dihydroxybenzoic acid (**7**), caffeic acid (**8**), ferulic acid (**9**), hydroxytyrosol (**10**), tyrosol (**11**), resorcinol (**12**), 2-methylresorcinol (**13**), orcinol (**14**), 2,5-dimethylresorcinol (**15**), 2,6-dihydroxybenzoic acid (**16**), hydroquinone (**17**), trimethylhydroquinone (**18**), *tert*-butylhydroquinone (**19**), 2,5-dihydroxybenzoic acid (**20**), methoxyhydroquinone (**21**), pyrogallol (**22**), gallic acid (**23**), methyl gallate (**24**), propyl gallate (**25**), phloroglucinol (**26**) and 2-phenyl-1-(2,4,6-trihydroxyphenyl)ethan-1-one (**27**). Their structures are collected in Figure 1. These phenolics can be grouped into five families of simple phenolics and analogues: 1,2-diphenols (compounds **1**, **2**, **5**–**8**, and **10**), 1,3-diphenols (compounds **12**–**16**), 1,4-diphenols (compounds **17**–**21**), 1,2,3-triphenols (compounds **22**–**25**), and 1,3,5-triphenols (compounds **26**–**27**). Compounds **3**, **4**, **9** and **11** are not polyphenols, but they have been included for comparison with compounds **2**, **2**, **8** and **10**, respectively. These compounds are grouped as non-polyphenolics to be distinguished from the other families. In addition, fourteen complex phenolics, usually found in foods, were also studied. These compounds were: resveratrol (**28**), piceatannol (**29**), oxyresveratrol (**30**), gnetol (**31**), naringenin (**32**), catechin (**33**), epicatechin (**34**), epigallocatechin (**35**), chrysin (**36**), baicalein (**37**), kaempferol (**38**), quercetin (**39**), morin (**40**), and myricetin (**41**). Their structures are collected in Figure 2. These compounds can be grouped into five families: stilbenoids (compounds **28**–**31**),

flavanones (compound **32**), flavanols (compounds **33**–**35**), flavones (compounds **36**–**37**), and flavonols (compounds **38**–**41**). Sources and purity of tested phenolics are collected in Table S1 of the Supporting Information. All 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), Merck (Darmstadt, Germany), and Alfa Aesar (Thermo Fischer GmbH, Karlsruhe, Germany).

Formation of 2-Phenylethylamine in Phenylalanine/LOOH/Phenolic Model Systems. Mixtures of phenylalanine, LOOH, and the phenolic compound(s) (10 μmol of each, unless otherwise indicated) in 500 μL of 0.3 M sodium phosphate buffer, pH 8, were heated under nitrogen for 1 h at 180 ºC. At the end of this heating, samples were cooled to room temperature and the 2-phenylethylamine content determined by using LC-MS/MS.

Formation of Aldehydes in LOOH and Phenylalanine/LOOH Mixtures in Thermally Treated Solutions. LOOH or phenylalanine/LOOH mixtures (10 μmol of each) in 500 μL of 0.3 M sodium phosphate buffer, pH 8, were heated under nitrogen for 1 h at 180 ºC. At the end of this heating, samples were cooled to room temperature and derivatized with 400 μL of a freshly prepared solution of *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (10 mg/mL in methanol). The resulting solution was stirred for 1 h at 37 ºC. Finally, reactions were studied by GC-MS. For identification purposes, selected aldehydes were derivatized in the same way.

Determination of 2-Phenylethylamine. 2-Phenylethylamine was determined as described previously.[12] Briefly, incubated and cooled mixtures were diluted with water (650 μL) and treated successively with the internal standard (50 μL of a solution of 23.29 mg of cyclohexylamine in 25 mL of methanol), sodium carbonate (200 μL of a saturated

97 solution in water), and dansyl chloride (1 mL of a solution of 10 mg/mL in acetone). The
98 mixture was incubated for 1 h at 60 ºC and, then, extracted three times with 1 mL of ethyl
99 acetate. The combined organic extracts were then washed with water (1.5 mL), taken to
100 dryness under nitrogen, re-dissolved in 300 μL of acetone, and analyzed by LC-MS/MS.

101 **LC and MS/MS Conditions.** The LC-MS/MS equipment consisted of an Agilent
102 liquid chromatography system (1200 Series) coupled to a triple-quadrupole API 2000
103 mass spectrometer (Applied Biosystems, Foster City, CA, USA) using an electrospray
104 ionization interface in positive ionization mode (ESI^+). Chromatographic separation was
105 carried out on a Zorbax Eclipse XDB-C18 (150 mm × 4.6 mm, 5 μm) column from
106 Agilent (Agilent Technologies, Santa Clara, CA, USA). Chromatographic and multiple
107 reaction monitoring (MRM) conditions were described previously.[12]

108 **Quantitation of 2-Phenylethylamine.** Quantitation of 2-phenylethylamine was
109 carried out by preparing standard curves of this compound in 500 μL of 0.3 M sodium
110 phosphate buffer (pH 8) and following the whole procedure described above. For each
111 curve, six different concentration levels of 2-phenylethylamine (0–10 μmol) were used.
112 2-Phenylethylamine content was directly proportional to the 2-phenylethylamine/internal
113 standard area ratio ($r > 0.995$, $p < 0.001$). The coefficients of variation at the different
114 assayed concentrations were < 10%.

115 **GC-MS Analyses.** GC-MS analyses were conducted with an Agilent 7820A gas
116 chromatograph coupled with an Agilent 5977 mass selective detector (quadrupole type)
117 using a fused-silica HP-5MS UI capillary column (30 m length, 0.25 mm inner diameter,
118 0.25 μm coating thickness) from Agilent. One microliter of sample was injected in the
119 pulsed splitless mode. Working conditions were as follows: carrier gas, helium (1 mL/min
120 at constant flow); injector, 250 ºC; transfer line to mass selective detector, 280 ºC;
121 electron ionization (EI), 70 eV; ion source temperature, 230 ºC; and mass range, 28-550

amu. Oven temperature conditions were from 40 ºC (3 min) to 300 ºC at 10 ºC/min and then held at 300 ºC for 1 min.

Statistical Analysis. All data given are mean ± standard deviation (SD) of at least three independent experiments. Statistical comparisons among different groups were made using analysis of variance. When significant *F* values were obtained, group differences were evaluated by the Tukey test.[20] 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 LOOH and Phenolics on 2-Phenylethylamine Formation by Thermal Degradation of Phenylalanine. Heating of phenylalanine in phosphate buffer, pH 8, for 1 h at 180 ºC always produced a small amount of 2-phenylethylamine (Table 1). When the model system also included simple phenolics, small changes in the amount of formed 2-phenylethylamine were produced, which were not significant for most phenolics. Only addition of 4-methylcatechol (**2**), 3-(3,4-dihydroxyphenyl)propanoic acid (**6**), and ferulic acid (**9**) increased significantly ($p < 0.05$) the amount of 2-phenylethylamine produced, which increased by 106–143%. Besides, addition of trimethylhydroquinone (**17**), methyl gallate (**24**), and propyl gallate (**25**) decreased significantly ($p < 0.05$) the amount of 2-phenylethylamine produced, which was reduced by 66–69%. Figure 3A shows the effect of addition of phenolics on the 2-phenylethylamine produced as a function of the kind of phenolic family involved. As can be observed, significant differences were not found among the different families of phenolics analyzed.

The presence of LOOH increased considerably the amount of 2-phenylethylamine produced in the control (the amount of 2-phenylethylamine produced increased from 1.12

146 nmol of 2-phenylethylamine per μmol of phenylalanine to 93.06 nmol/μmol). In addition,
147 most phenolics reduced significantly ($p < 0.05$) the amount of 2-phenylethylamine
148 produced (Table 1). Differences of behavior among the different families of phenolics are
149 shown in Figure 3B. As can be seen in the figure, most families exhibited a similar
150 protective effect with the exception of 1,3,5-triphenols. The presence of phenolics of this
151 last family produced significantly ($p < 0.05$) less 2-phenylethylamine compared to 1,2-
152 diphenols, 1,4-diphenols, or non-polyphenols. However, differences were not significant
153 ($p < 0.05$) between 1,3,5-triphenols and either 1,3-diphenols or 1,2,3-triphenols. Most
154 phenolics within each family exhibited a homogeneous behavior, although there were
155 significant exceptions. In the family of 1,2-diphenols, two phenolics had an anomalous
156 behavior, and inhibited the formation of 2-phenylethylamine to a much higher extent than
157 other members of the family. These phenolics were 4-methoxycatechol (**5**) (most likely
158 because of the presence of the methoxy group, see below) and 3-(3,4-
159 dihydroxyphenyl)propanoic acid (**6**) (for some, at present, unknown reason). These two
160 phenolics were excluded from the box family and are shown in Figure 3B as individual
161 compounds. Other exception was observed in the family of 1,4-diphenols. Thus,
162 contrarily to other family members, trimethylhydroquinone (**18**) increased slightly the
163 amount of 2-phenylethylamine produced by the control, although this increase was not
164 significant. This compound was also excluded from the box family and is shown in the
165 figure as an individual compound. In addition, methoxyhydroquinone (**21**) exhibited a
166 much higher protection than other 1,4-diphenols, most likely because of the presence of
167 the methoxy group. This methoxy group seems to function like an additional hydroxyl
168 group and, for that reason, when LOOH was present, compound **5** exhibited a higher
169 effect than other 1,2-diphenols, compound **21** exhibited a higher effect than other 1,4-
170 diphenols, and ferulic acid (**9**) had a similar effect to caffeic acid (**8**). When LOOH was

171 absent, the methoxy group seemed to act as a 2-phenylethylamine enhancer. As an
172 example, compound **9** produced more 2-phenylethylamine than compound **8**.

173 To understand these results, the different reactions that take place in the system should
174 be considered.[1] A general scheme of these reactions is shown in Figure 4. As indicated in
175 the figure, LOOH is decomposed into free radicals (FR in the figure) and reactive
176 carbonyls (RCO in the figure). A wide variety of reactive carbonyls are produced by the
177 thermal decomposition of lipid hydroperoxides. As an example of the reactive carbonyls
178 produced under the reaction conditions employed in this study, Fig. S1 of the Supporting
179 Information shows the formation of hexanal (Fig. S1A), 2-octenal (Fig. S1C), ad 2,4-
180 decadienal (Fig. S1C).

181 Both free radicals and reactive carbonyls are able to decarboxylate the amino acid and
182 produce the amine. The mechanism for amino acid degradation initiated by reactive
183 carbonyls was described previously.[12] Although many lipid-derived carbonyls are able to
184 degrade amino acids, the different reactive carbonyls have a different reactivity among
185 them. As observed in Fig. S1 of the Supporting Information, when LOOH was heated in
186 the presence of phenylalanine, the ratio among the different aldehydes produced was
187 different to that observed in the absence of the amino acid. Thus, the area of hexanal was
188 very similar in the absence (Fig. S1A) and in the presence (Fig. S1B) of the amino acid.
189 Because 2-alkenals are more reactive than alkanals, the area of 2-octenal was lower in the
190 presence of amino acid (Fig. S1D) than in its absence (Fig. S1C). Finally, 2,4-alkadienals
191 were the most reactive aldehydes in comparison to alkanals and 2-alkenals, and 2,4-
192 decadienal was almost absent in the heated mixture of LOOH and phenylalanine (Fig.
193 S1F). In fact, 2,4-decadienal is a strong amino acid decarboxylation carbonyl
194 compound.[12]

Differently to amino acid decarboxylations produced by reactive carbonyls, amino acid decarboxylations produced by lipid-derived radicals are lesser known, most likely because of the difficulty of separating the effects of free radicals and reactive carbonyls produced because of hydroperoxide decomposition. Nevertheless, hydroxyl radicals have been shown to decarboxylate amino acids in basic aqueous solutions,[21,22] and a similar mechanism can also be hypothesized for amino acid degradation produced by lipid radicals (Fig. S2 of the Supporting Information).

Both free radicals and reactive carbonyls can be scavenged by phenolic compounds. However, both scavenging reactions are different, and the involved phenolics in each reaction require different structural characteristics. Reactive carbonyl trapping is carried out by phenolics having a high electronic density in aromatic carbons because the reaction takes place by addition of the phenolic compound to the reactive carbonyl.[17] The structural requirements for this high electronic density usually occurs in phenolics having two hydroxyl groups in *meta* positions (examples of the carbonyl-phenol adducts expected to be produced in these reactions are shown in Fig. S3 of the Supporting Information).[22] On the other hand, free radical scavenging is carried out by phenolics that can delocalize the free electron of the radical. This occurs mainly in phenolics having two hydroxyl groups in *ortho* or *para* positions.[23] However, free-radical scavenging phenolics are converted into quinones as a consequence of this reaction, and quinones function as reactive carbonyls favoring amino acid degradations.[24] Nevertheless, conversion of suitable phenolics into quinones only occurs in the presence of free radicals or under oxidizing conditions. Because in this study the conversion of phenylalanine into 2-phenylethylamine was carried out under nitrogen, contribution of quinones to a significant extent should only be expected to occur when LOOH was present in the reaction mixture.

220 Thus, in the absence of LOOH, very small amounts of 2-phenylethylamine were
221 produced and significant differences were not observed among the different families of
222 phenolics assayed (Figure 3A). This is likely a consequence of the lack of free radicals
223 and reactive carbonyls in the reaction. In addition, non-polyphenols produced an amount
224 of 2-phenylethylamine close to that produced by the control.

225 The addition of LOOH produced differences of behavior among the different
226 phenolics. However, most of them decreased the 2-phenylethylamine produced (Table 1).
227 This is consequence that all of them either scavenged the formed free radicals or trapped
228 the reactive carbonyls produced by LOOH decomposition. Differences among families
229 were a consequence of both, the differences in the free radical-scavenging and carbonyl-
230 trapping abilities among the different phenolics, and the different contribution of free
231 radicals and reactive carbonyls to 2-phenylethylamine formation. Thus, 1,2-diphenols,
232 1,4-diphenols, and non-polyphenolics were the families that least reduced the 2-
233 phenylethylamine produced; 1,3,5-triphenols were the phenolics that most reduced the 2-
234 phenylethylamine produced; and 1,3-diphenols and 1,2,3-triphenols had an intermediate
235 behavior (Figure 3B). These behaviors can be explained according to the different
236 reactions shown in Figure 4.

237 1,2- and 1,4-Diphenols can scavenge free radicals, but they cannot trap reactive
238 carbonyls to a high extent. In addition, free radical scavenging produces quinones that
239 also contribute to the formation of 2-phenylethylamine. The effect observed for 1,2- and
240 1,4-diphenols was a decrease in the formation of 2-phenylethylamine in relation to the
241 control, but 2-phenylethylamine was produced to a significant extent because the reactive
242 carbonyls produced by LOOH degradation were present. In addition, the contribution of
243 these reactive carbonyls seemed to be more efficient than free radicals to 2-

phenylethylamine formation because 1,2- and 1,4-diphenols were the phenolics that decreased 2-phenylethylamine formation to a lowest extent.

Differently to 1,2- and 1,4-diphenols, 1,3-diphenols can scavenge reactive carbonyls and, as discussed above, reactive carbonyls seem to contribute more than free radicals to 2-phenylethylamine formation. For this reason, 1,3-diphenols had a more relevant role than 1,2- and 1,4-diphenols in avoiding 2-phenylethylamine formation.

Polyphenols might be considered as a mixture of diphenols. Thus, 1,2,3-triphenols have three hydroxyl groups, and their relative positions are one *meta* and two *ortho*. For that reason they might be considered as the mixture of one 1,3-diphenol with two 1,2-diphenols. Therefore, they should have the ability of trapping reactive carbonyls and, also, of scavenging free radicals. However, the presence of two 1,2-diphenols might favor the formation of quinones and the promotion of 2-phenylethylamine. The result of these different processes was that they protected analogously to 1,3-diphenols, and, although differences were not significant, they seemed to protect better than most 1,2- and 1,4-diphenols. Finally, 1,3,5-triphenols have three phenolic groups placed in *meta* positions. Therefore, they might be considered as the mixture of three 1,3-diphenols. This means that they should be very efficient carbonyl scavengers and, because carbonyl trapping is more important than free radical scavenging to avoid 2-phenylethylamine formation, they should reduce considerably the 2-phenylethylamine produced. In fact, they were significantly ($p < 0.05$) more efficient 2-phenylethylamine inhibitors than 1,2- and 1,4-diphenols, and also, although the difference was not significant, they seemed to protect better than 1,3-diphenols and 1,2,3-triphenols.

Effect of LOOH and Mixtures of Phenolics on 2-Phenylethylamine Formation by Thermal Degradation of Phenylalanine. Conclusions obtained in the previous section

suggested that observed results in polyphenols could be the consequence of a combination of the effects of the different groups present in their structures. To confirm this hypothesis, the effect of the combined action of two phenolics on the 2-phenylethylamine produced was also studied. These mixtures always included one 1,3,5-triphenol [either phloroglucinol (**26**) or 2-phenyl-1-(2,4,6-trihydroxyphenyl)ethan-1-one (**27**)] for two reasons. On one hand, 1,3,5-triphenols had a significantly different behavior in relation to other phenolics for 2-phenylethylamine formation when LOOH was present (Figure 3B). In addition, one 1,3,5-triphenol (or analogue) is usually present in the A-ring of many flavonoids. Therefore, compounds **26** or **27** were mixed with representative phenolics of the other families. The compounds selected for these mixtures were catechol (**1**) and 4-methylcatechol (**2**) as 1,2-diphenols, resorcinol (**12**) and orcinol (**14**) as 1,3-diphenols, hydroquinone (**17**) and trimethylhydroquinone (**18**) as 1,4-diphenols, and pyrogallol (**22**) as 1,2,3-triphenol. In addition, mixtures of compounds **26** or **27** with toluene (**3**) and *p*-cresol (**4**) were also studied for comparison purposes. The results obtained are collected in Table 2. As can be observed, in the absence of LOOH, most mixtures decreased significantly ($p < 0.05$) the 2-phenylethylamine produced. This significant decrease ranged from 34% of the mixture between compounds **26** and **14** to 79% of the mixture between compounds **27** and **2**. To compare the behavior of the mixtures between different families, Figure 5A shows a box plot of the mixture of 1,3,5-triphenols with the other four phenolic families and the non-polyphenolic group. As can be observed, there were not significant differences among the different phenolic families.

When LOOH was present, mixtures always decreased significantly ($p < 0.05$) the amount of 2-phenylethylamine produced. In addition, percentages of variation in the presence of LOOH were higher than in its absence (Table 2). Thus, in the presence of LOOH, decreases ranged from 44% of the mixture **27** + **3** to 90% of the mixture **26** + **14**.

In addition, differences among the different phenolic families were also observed. As shown in Figure 5B, the presence of mixtures involving 1,3,5-triphenols and either 1,3-diphenols or 1,2,3-triphenols produced significantly ($p < 0.05$) less 2-phenylethylamine than the presence of mixtures involving 1,3,5-triphenols and either 1,4-diphenols or non-polyphenolics. The difference with mixtures involving 1,2-diphenols was not significant, but the behavior of the mixtures including these phenolics was closer to that of mixtures including 1,4-diphenols than to the behavior of mixtures including 1,3-diphenols.

These results can also be interpreted considering the reactions shown in Figure 4. In the absence of LOOH, most of studied mixtures (Table 2) produced an amount of 2-phenylethylamine similar to that produced by either compound **26** or **27** when added independently (Table 1) and there was not any significant difference among the different phenolic families (Figure 5A). This suggests that 1,3,5-triphenols scavenged any small amount of reactive carbonyls that might promote 2-phenylethylamine formation. Therefore, differences among families were mostly suppressed.

Differently to the observed in the absence of LOOH, the presence of free radicals and reactive carbonyls produced by LOOH decomposition changed the behavior of the mixtures. Thus, 1,3,5-triphenols are very efficient carbonyl scavengers and the addition of either 1,3-diphenols or 1,2,3-triphenols did not change the residual amount of 2-phenylethylamine produced. However, when 1,3,5-triphenols were mixed with either 1,2- or 1,4-diphenols, the amount of 2-phenylethylamine produced was reduced in relation to the production of 2-phenylethylamine in the presence of only either 1,2- or 1,4-diphenols but it was higher than that produced in the presence of only 1,3,5-triphenols. This should be interpreted considering that 1,3,5-triphenols are able to trap reactive carbonyls, but not the quinones produced by oxidation of 1,2- and 1,4-diphenols. Once else, 1,2,3-triphenols had a behavior similar to that of 1,3-diphenols. Thus, mixtures of 1,3,5-triphenols and

318 1,2,3-triphenols had a behavior close to that of mixtures of 1,3,5-triphenols and 1,3-
319 diphenols.

320 To confirm that the observed effects were the result of a combination of the effects of
321 the individual components in the mixtures, the experimental values were compared with
322 the calculated values of 2-phenylethylamine formation. These values were calculated by
323 adding the amounts of 2-phenylethylamine produced when the phenolics in the mixture
324 were added individually (Table 1). Figure 6 shows the plot of the experimental vs. the
325 calculated amount of 2-phenylethylamine produced in the absence (Figure 6A) and in the
326 presence (Figure 6B) of LOOH. No relationship between experimental and calculated
327 data was observed for mixtures incubated in the absence of LOOH (Figure 6A). However,
328 experimental and calculated values were correlated ($r = 0.90$, $p < 0.0001$) in mixtures
329 involving LOOH, therefore suggesting that the effect of phenolics in these mixtures was
330 additive.

331 The lack of correlation in Figure 6A is likely a consequence of the small amounts of
332 2-phenylethylamine produced in the absence of LOOH. On the contrary, the correlation
333 observed in Figure 6B can be explained according to the reaction mechanism given in
334 Figure 4. Thus, in the presence of LOOH, each phenolic played a specific role and this
335 role was maintained in the mixture. Therefore, the function observed for a mixture was
336 the addition of the effects of the components in the mixture.

337 **Effect of LOOH and Complex Phenolics on 2-Phenylethylamine Formation by**
338 **Thermal Degradation of Phenylalanine.** In addition to simple phenolics (Table 1) and
339 some of their mixtures (Table 2), the role of some complex phenolics on 2-
340 phenylethylamine formation by phenylalanine degradation in the presence of LOOH was
341 also studied. As shown in Table 3, in the absence of LOOH, many of assayed phenolics

342 increased significantly ($p < 0.05$) the amount of 2-phenylethylamine produced by the
343 control. The behavior of the different families of complex phenolics is shown in Figure
344 7A. As can be observed, most families increased the formation of 2-phenylethylamine in
345 relation to the value of control (1.12 nmol of 2-phenylethylamine per μmol of
346 phenylalanine). In addition, stilbenoids (compounds **28**–**31**) and flavones (compounds
347 **36**–**37**) produced significantly ($p < 0.05$) more 2-phenylethylamine than flavanols
348 (compounds **33**–**35**), and flavonols (compounds **38**–**41**).

349 Differently to the results obtained in the absence of LOOH, when LOOH was present
350 all assayed phenolics decreased significantly ($p < 0.05$) the amount of 2-
351 phenylethylamine produced (Table 3). In addition, differences in the behavior of the
352 different phenolics within their family increased. In particular, quercetin (**39**) protected
353 considerably less than other flavonols. Therefore, this compound was excluded from the
354 box family and is shown in Figure 7B as an individual compound. As observed in Figure
355 7B all families decreased the amount of 2-phenylethylamine produced by the control
356 (93.06 nmol of 2-phenylethylamine per μmol of phenylalanine). Nevertheless, Figures
357 7A and 7B were quite similar. Thus, flavones, which were the phenolics that most
358 increased 2-phenylethylamine formation in the absence of LOOH were the phenolics that
359 least decreased 2-phenylethylamine formation in its presence. In fact, and analogously to
360 that observed in Figure 7A, 2-phenylethylamine was produced significantly ($p < 0.05$) to
361 a higher extent in the presence of flavones than in the presence of flavanols and flavonols.

362 Analogously to that discussed in previous sections, these results could be interpreted
363 based on the reactions involved (Figure 4) and the structural characteristics of the
364 different phenolics, which could be analyzed as a function of their constituting parts.
365 Thus, for example, studied complex phenolics (**28**–**41**) were composed of a 1,3-diphenol
366 (stilbenoids) or an 1,3,5-triphenol (flavonoids) in ring A and a benzene, a phenol, a 1,2-

diphenol, a 1,3-diphenol, or a 1,2,3-triphenol in ring B. In addition, they had other groups (carbon-carbon double bond, carbonyl or hydroxyl groups) in the C-ring of flavonoids or the bridge of stilbenoids. In the absence of LOOH, stilbenoids and flavones increased the amount of 2-phenylethylamine produced. The reason for this promoting effect of stilbenoids should be related to the presence of the carbon-carbon double bond conjugated to both A- and B-rings. The reason for the promoting effect of flavones should be related to the presence of the conjugated and unsaturated carbonyl carbon in C-ring, which should act similarly to quinones. An analogous conjugated and unsaturated carbonyl carbon in C-ring is also present in flavonols, but the presence of the additional hydroxyl group at position 3 should play some inhibitory role in the formation of 2-phenylethylamine.

When LOOH was present, all phenolics protected, which is likely due to the presence of either an 1,3-diphenol or an 1,3,5-triphenol in the A-ring of stilbenoids or flavonoids, respectively. Thus, this ring should trap any reactive carbonyl produced during LOOH decomposition. Therefore, the amount of 2-phenylethylamine produced should decrease because, as commented previously, the carbonyl trapping ability is the function that mainly decreases 2-phenylethylamine formation. This means that differences among phenolic families should be mostly related to the different configuration of rings B and C, although the A-ring of stilbenoids is likely less protective than the A-ring of flavonoids. Thus, flavones were the phenolics that reduced less the formation of 2-phenylethylamine. The reason should be mainly related to the presence of the α,β-unsaturated carbonyl group in C-ring. The flavanone studied [naringenin (**32**)] had a behavior similar to that of flavones, more likely because of the presence of the carbonyl carbon. In relation to the stilbenoids, there were two different behaviors: resveratrol (**28**) and piceatannol (**29**) decreased 2-phenylethylamine formation less than oxyresveratrol (**30**) and gnetol (**31**), most likely because these last two compounds had a 1,3-diphenol as

B-ring. Something similar occurred in flavonols. Quercetin (**39**) decreased 2-phenylethylamine formation less than other phenolics in its family most likely because of the existence of a 1,2-diphenol as B-ring.

The possibility that the effect observed for the different complex phenolics was the addition of the effects of their different constituent parts was also investigated. Thus, for example, among stilbenoids, resveratrol (**26**) can be considered the addition of orcinol (**14**) plus *p*-cresol (**4**), piceatannol (**27**) can be considered the addition of orcinol (**14**) plus 4-methylresorcinol, oxyresveratrol (**28**) can be considered the addition of orcinol (**14**) plus 4-methylresorcinol, and gnetol (**29**) can be considered the addition of orcinol (**14**) plus 2-methylresorcinol (**13**). Values employed to determine the calculated 2-phenylethylamine production in the presence of complex phenolics were taken from Table 1. When one value was not available (for example, that of 4-methylresorcinol), the closest available value was employed (for example, the value of 2-methylresorcinol (**13**) was employed in the place the non-existing value of 4-methylresorcinol). Figure 8 shows the amount of 2-phenylethylamine determined experimentally vs. the calculated using the values for the simple phenolics that could be considered constituting parts of these complex phenolics. Similarly to that obtained for mixtures of phenolics (Figure 6A), experimental data obtained in the absence of LOOH were not correlated with calculated values (Figure 8A). However, a correlation ($r = 0.75$, $p = 0.002$) was observed between experimental and calculated values when LOOH was present (Figure 8B).

The absence of correlation between experimental and calculated values when LOOH was not present and the worse correlation observed in Figure 8B than that observed in Figure 6B are likely related to the contribution of the C-ring, which could not be considered when adding the effects of rings A and B.

The results obtained in this study show that 2-phenylethylamine formation in the presence of LOOH is inhibited by phenolics, which act in an additive way. Thus, the presence of 1,3-diphenols decreased the formation of 2-phenylethylamine because of their carbonyl trapping abilities. On the contrary, the contribution of 1,2- and 1,4-diphenols was lower because they could not trap the reactive carbonyls produced by LOOH decomposition. In addition, their free radical scavenging was likely accompanied by the formation of quinones, which acted as reactive carbonyls. The behavior of all other phenolics could be interpreted based on the action of these diphenols because complex phenolics can be considered as a mixture of them. Therefore, these results suggest that, when reaction mechanisms are known, it is possible to predict the behavior of phenolics based on their structure.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Table S1. Sources and purity of employed phenolics

Figure S1. Selected carbonyl compounds produced by thermal degradation of LOOH and LOOH/phenylalanine mixtures

Figure S2. Proposed phenylethylamine formation pathway by phenylalanine degradation initiated by free radicals

Figure S3. Carbonyl-phenol adducts produced in the reactions of 2-alkenals and 2,4-alkadienals with quercetin

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445 **Notes**

446 The authors declare no competing financial interest.

447

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FIGURE CAPTIONS

Figure 1. Structures of simple phenolics (and analogues) employed in this study. They were: catechol (**1**), 4-methylcatechol (**2**), toluene (**3**), *p*-cresol (**4**), 4-methoxycatechol (**5**), 3-(3,4-dihydroxyphenyl)propanoic acid (**6**), 3,4-dihydroxybenzoic acid (**7**), caffeic acid (**8**), ferulic acid (**9**), hydroxytyrosol (**10**), tyrosol (**11**), resorcinol (**12**), 2-methylresorcinol (**13**), orcinol (**14**), 2,5-dimethylresorcinol (**15**), 2,6-dihydroxybenzoic acid (**16**), hydroquinone (**17**), trimethylhydroquinone (**18**), *tert*-butylhydroquinone (**19**), 2,5-dihydroxybenzoic acid (**20**), methoxyhydroquinone (**21**), pyrogallol (**22**), gallic acid (**23**), methyl gallate (**24**), propyl gallate (**25**), phloroglucinol (**26**) and 2-phenyl-1-(2,4,6-trihydroxyphenyl)ethan-1-one (**27**). These phenolics have been grouped into five families for comparison purposes: 1,2-diphenols (compounds **1**, **2**, **5**–**8**, and **10**), 1,3-diphenols (compounds **12**–**16**), 1,4-diphenols (compounds **17**–**21**), 1,2,3-triphenols (compounds **22**–**25**), and 1,3,5-triphenols (compounds **26**–**27**). Compounds **3**, **4**, **9** and **11** are not polyphenols, they have been included for comparison with other phenolics, and they have been grouped as non-polyphenolics to be distinguished from the other families.

Figure 2. Structures of complex phenolics employed in this study. They were: resveratrol (**28**), piceatannol (**29**), oxyresveratrol (**30**), gnetol (**31**), naringenin (**32**), catechin (**33**), epicatechin (**34**), epigallocatechin (**35**), chrysin (**36**), baicalein (**37**), kaempferol (**38**), quercetin (**39**), morin (**40**), and myricetin (**41**). These compounds have been grouped into five families for comparison purposes: stilbenoids (compounds **28**–**31**), flavanones (compound **32**), flavanols (compounds **33**–**35**), flavones (compounds **36**–**37**), and flavonols (compounds **38**–**41**).

Figure 3. 2-Phenylethylamine production by phenylalanine degradation in the presence of different families of phenolics and: A, the absence of LOOH; and, B, the presence of

LOOH. Abbreviations: D12, 1,2-diphenols; D13, 1,3-diphenols; D14, 1,4-diphenols; T123, 1,2,3-triphenols; T135; 1,3,5-triphenols; NPP, non-polyphenols; LOOH, 13-hydroperoxide of linoleic acid; Phe, phenylalanine. Boxes with different letters are significantly ($p < 0.05$) different. Composition of the different families are given in Figure 1.

Figure 4. Chemical reactions produced as a consequence of the heating of phenylalanine in the presence of LOOH and phenolics. Abbreviations: LOOH, 13-hydroperoxide of linoleic acid; FR, free radicals; RCO, reactive carbonyls.

Figure 5. 2-Phenylethylamine production by phenylalanine degradation in the presence of mixtures of 1,3,5-triphenols with other families of phenolics and: A, the absence of LOOH; and, B, the presence of LOOH. Abbreviations: D12, 1,2-diphenols; D13, 1,3-diphenols; D14, 1,4-diphenols; T123, 1,2,3-triphenols; NPP, non-polyphenols; LOOH, 13-hydroperoxide of linoleic acid; Phe, phenylalanine. Boxes with different letters are significantly ($p < 0.05$) different. Composition of the different families are given in Figure 1.

Figure 6. Plot of the experimental vs. the calculated amount of 2-phenylethylamine produced by phenylalanine degradation in the presence of mixtures of 1,3,5-triphenols with other phenolic families and: A, the absence of LOOH; and, B, the presence of LOOH. Abbreviations: PEA, 2-phenylethylamine; Phe, phenylalanine.

Figure 7. 2-Phenylethylamine production by phenylalanine degradation in the presence of different phenolic families and: A, the absence of LOOH; and, B, the presence of LOOH. Abbreviations: LOOH, 13-hydroperoxide of linoleic acid; Phe, phenylalanine. Boxes with different letters are significantly ($p < 0.05$) different. Composition of the different families are given in Figure 2.

Figure 8. Plot of the experimental vs. the calculated amount of 2-phenylethylamine produced by phenylalanine degradation in the presence of either stilbenoids or flavonoids and: A, the absence of LOOH; and, B, the presence of LOOH. Abbreviations: PEA, 2-phenylethylamine; Phe, phenylalanine.

Table 1. Effect of Simple Phenolics and LOOH on 2-Phenylethylamine Formation in Thermally Treated Phenylalanine Solutions[a]

phenolic	without LOOH		with LOOH	
	PEA produced	variation (% vs. control)	PEA produced	variation (% vs. control)
None	1.12 ± 0.22 c,d,i		93.06 ± 14.96 a,b	
1	1.37 ± 0.30 c,d	22	80.04 ± 2.84 b,c,d,e	–14
2	2.38 ± 0.52 a,b	112	80.88 ± 3.16 b,c,d	–13
3	1.55 ± 0.32 b,c	27	84.27 ± 10.82 a,c	–9
4	1.29 ± 0.33 c,f	6	85.50 ± 4.65 a,c	–8
5	1.55 ± 0.24 b,c	38	14.55 ± 3.38 l,m	–84
6	2.73 ± 0.69 a	143	16.92 ± 3.22 l,m	–82
7	0.61 ± 0.13 d,e,f,j	–46	68.16 ± 8.56 c,d,f,g	–27
8	1.36 ± 0.29 c,e	21	55.52 ± 9.96 d,i	–40
9	2.32 ± 0.50 a	106	43.43 ± 5.56 g,h,i,j,k	–53
10	0.78 ± 0.17 c,d,j	–30	52.35 ± 10.10 d,i,j	–44
11	1.15 ± 0.22 c,d,g,h	2	50.74 ± 9.96 d,i,j	–45
12	0.64 ± 0.12 d,e,f,j	–43	52.31 ± 13.66 e,f,h,i,j	–44
13	0.41 ± 0.07 g,i,j	–63	37.74 ± 5.99 h,i,j,k,m	–59
14	0.52 ± 0.06 d,j	–54	44.88 ± 9.13 g,h,i,j	–52
15	0.51 ± 0.12 d,f,j	–55	47.82 ± 10.34 g,h,i,j	–49
16	1.10 ± 0.17 c,d,j	–2	32.64 ± 2.32 i,m	–65
17	0.38 ± 0.07 h,j	–66	69.86 ± 13.09 c,d,f	–25
18	1.04 ± 0.24 c,d,j	–8	109.36 ± 7.06 a	18
19	0.65 ± 0.09 d,f,j	–42	52.70 ± 10.38 f,h,i,j	–43
20	0.63 ± 0.04 d,e,f,j	–44	66.86 ± 12.33 c,d,h	–28
21	1.27 ± 0.25 c,d,g	13	28.39 ± 3.81 j,m	–69
22	0.89 ± 0.16 c,d,j	–21	40.37 ± 4.15 g,h,i,l	–57
23	1.06 ± 0.14 c,d,j	–6	51.11 ± 12.28 d,i,j	–45
24	0.35 ± 0.03 g,j	–69	46.01 ± 8.19 f,h,i,j	–51
25	0.38 ± 0.09 g,i,j	–67	43.93 ± 8.45 f,h,i,j,k	–53
26	0.62 ± 0.05 d,e,f,j	–45	9.69 ± 2.39 m	–90
27	0.56 ± 0.07 d,j	–50	19.31 ± 3.98 k,m	–79

Data (mean ± SD of, at least, three independent experiments) are given in nmol of 2-phenylethylamine per μmol of phenylalanine. Means in the same column with the same letter are not significantly ($p < 0.05$) different. Structures and names for phenolic compounds and families are given in Figure 1.

Table 2. Effect of Combined Phenolics and LOOH on 2-Phenylethylamine Formation in Thermally Treated Phenylalanine Solutions[a]

Phenolics	Without LOOH		With LOOH	
	PEA produced	Variation (% vs. control)	PEA produced	Variation (% vs. control)
None	1.12 ± 0.22 a		93.06 ± 14.96 a	
26 + **1**	0.55 ± 0.19 c,d,e	–51	22.17 ± 3.89 c,d,e	–76
26 + **2**	0.37 ± 0.17 d,e	–67	20.12 ± 1.93 d,e	–78
26 + 3	1.00 ± 0.12 a,b	–11	31.55 ± 2.33 b,c,d,e	–66
26 + 4	0.54 ± 0.10 b,c,d,e	–52	25.58 ± 0.52 b,c,d,e	–73
26 + **12**	0.29 ± 0.06 d,e	–74	11.69 ± 3.17 e	–87
26 + **14**	0.74 ± 0.22 b,c,d	–34	9.59 ± 3.95 e	–90
26 + **17**	0.48 ± 0.04 c,d,e	–57	32.83 ± 1.18 b,c,d,e	–65
26 + **18**	0.88 ± 0.22 a,b,c	–21	44.70 ± 1.73 b,c,d	–52
26 + **22**	0.73 ± 0.13 b,c,d	–35	9.68 ± 1.43 e	–90
27 + **1**	0.57 ± 0.13 b,c,d,e	–49	34.14 ± 5.76 b,c,d,e	–63
27 + **2**	0.24 ± 0.01 e	–79	42.48 ± 3.22 b,c,d,e	–54
27 + 3	0.55 ± 0.15 b,c,d,e	–51	52.08 ± 3.49 b	–44
27 + 4	0.43 ± 0.09 c,d,e	–62	47.69 ± 4.82 b,c,d	–49
27 + **12**	0.37 ± 0.07 d,e	–67	23.62 ± 4.09 c,d,e	–75
27 + **14**	0.40 ± 0.09 c,d,e	–64	13.41 ± 0.40 e	–86
27 + **17**	0.76 ± 0.16 a,b,c,d	–32	31.50 ± 5.92 b,c,d,e	–66
27 + **18**	0.64 ± 0.16 b,c,d,e	–43	49.70 ± 4.57 b,c	–47
27 + **22**	0.48 ± 0.11 c,d,e	–57	13.96 ± 3.62 e	–85

Data (mean ± SD of, at least, three independent experiments) are given in nmol of 2-phenylethylamine per μmol of phenylalanine. Means in the same column with the same letter are not significantly ($p < 0.05$) different. Structures and names for phenolic compounds and families are given in Figure 1.

Table 3. Effect of Complex Phenolics and LOOH on 2-Phenylethylamine Formation in Thermally Treated Phenylalanine Solutions[a]

Phenolic	Without LOOH		With LOOH	
	PEA produced	Variation (% vs. control)	PEA produced	Variation (% vs. control)
None	1.12 ± 0.22 e		93.06 ± 14.96 a	
28	2.51 ± 0.22 b,c	124	45.93 ± 5.47 b	–51
29	2.31 ± 0.54 b,c,d	106	40.87 ± 6.95 b,c	–56
30	3.50 ± 0.57 a,b	212	30.37 ± 3.99 b,c	–67
31	3.37 ± 0.87 a,b	201	26.31 ± 4.68 b,c	–72
32	1.33 ± 0.03 c,d,e	19	40.18 ± 2.82 b,c	–57
33	1.63 ± 0.24 c,d,e	46	24.45 ± 3.96 b,c	–74
34	0.97 ± 0.28 e	–13	20.80 ± 2.68 b,c	–78
35	1.13 ± 0.46 d,e	1	18.51 ± 3.20 c	–80
36	3.45 ± 0.05 a,b	208	44.06 ± 3.04 b	–53
37	3.93 ± 0.97 a	251	48.45 ± 0.40 b	–48
38	1.59 ± 0.41 c,d,e	42	25.69 ± 2.77 b,c	–72
39	1.95 ± 0.32 c,d,e	74	42.74 ± 2.48 b,c	–54
40	1.22 ± 0.22 c,d,e	9	24.93 ± 5.19 b,c	–73
41	1.83 ± 0.55 c,d,e	63	21.04 ± 4.21 b,c	–77

Data (mean ± SD of, at least, three independent experiments) are given in nmol of 2-phenylethylamine per μmol of phenylalanine. Means in the same column with the same letter are not significantly ($p < 0.05$) different. Structures and names for phenolic compounds and families are given in Figure 2.

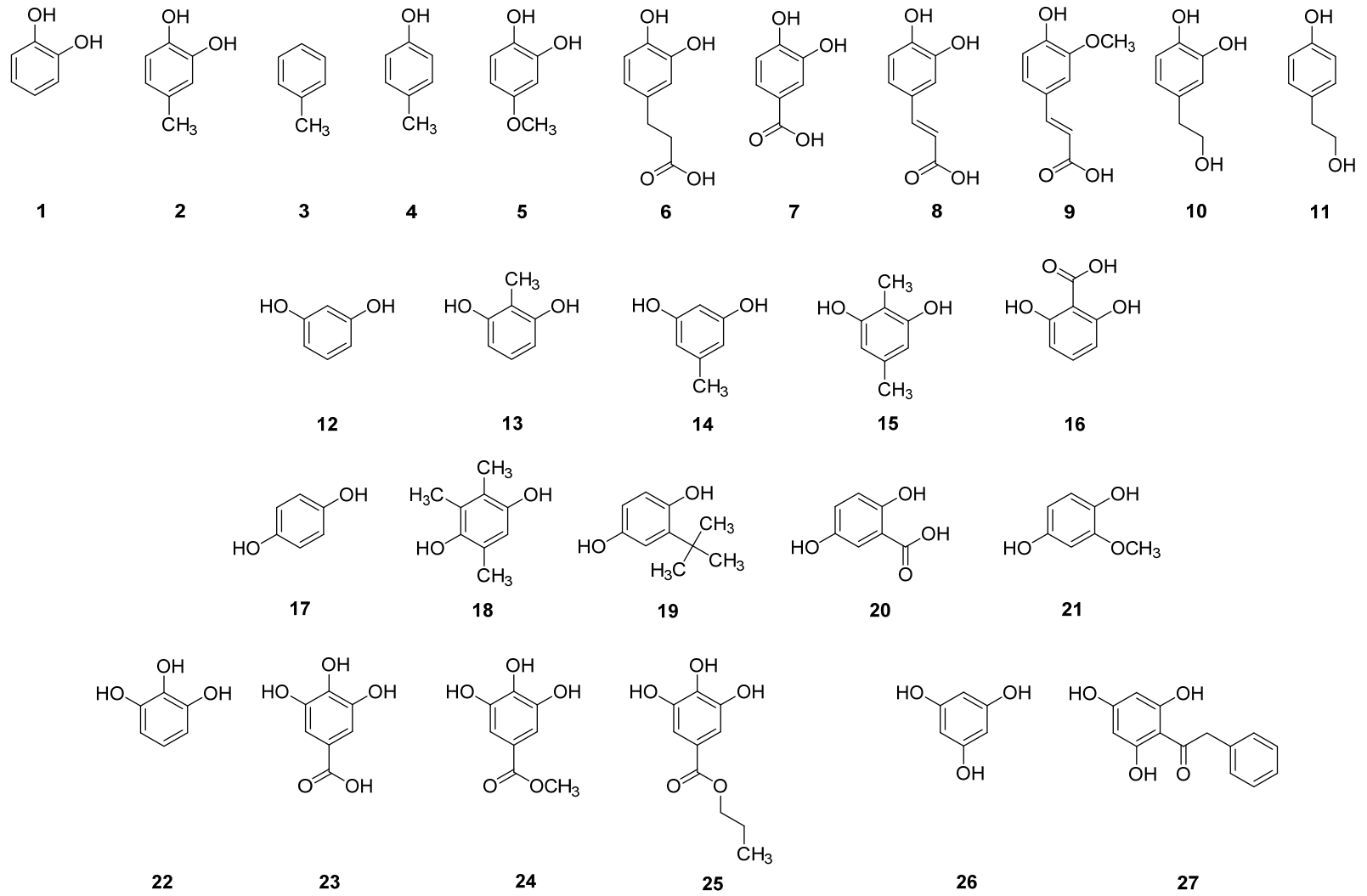


Figure 1

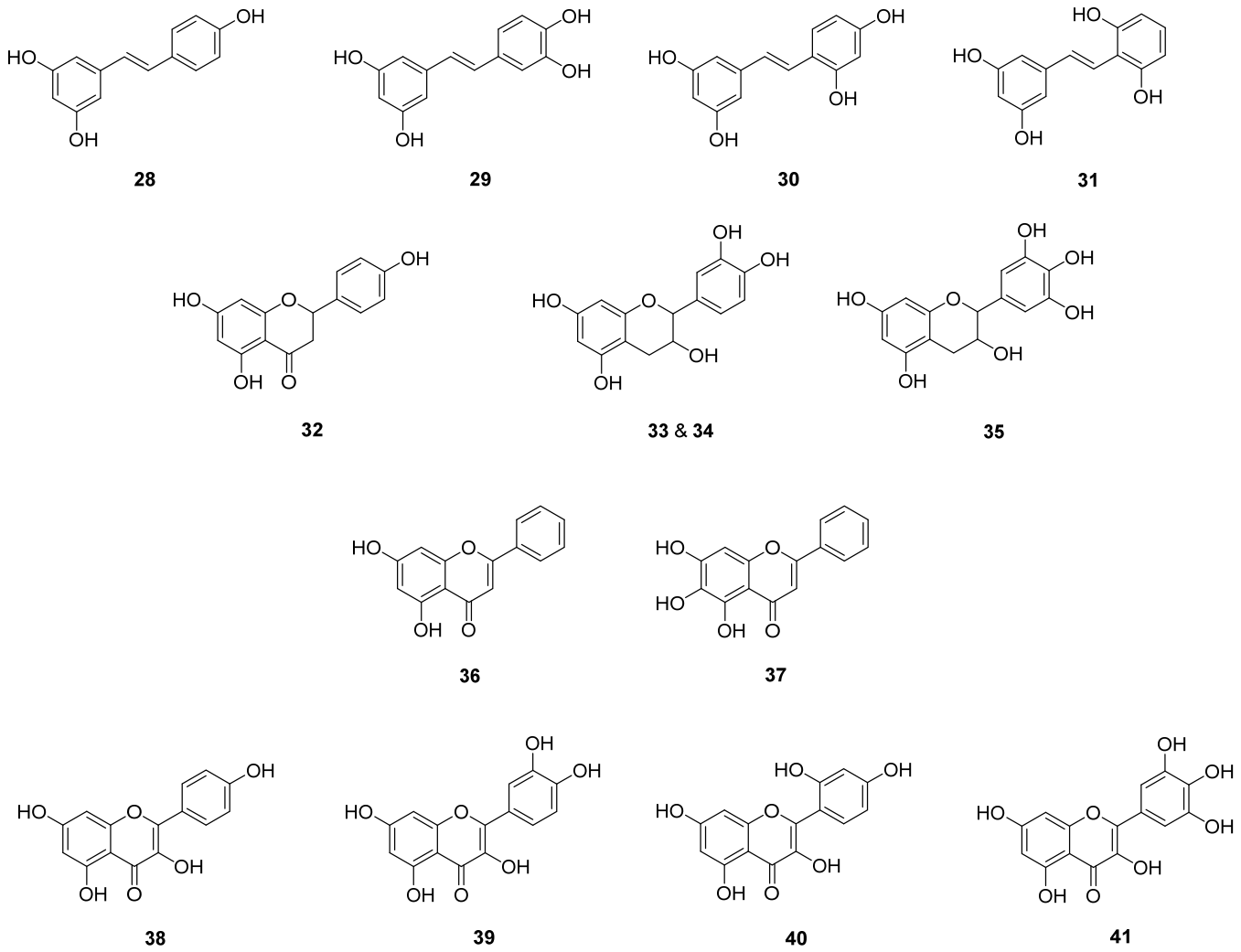
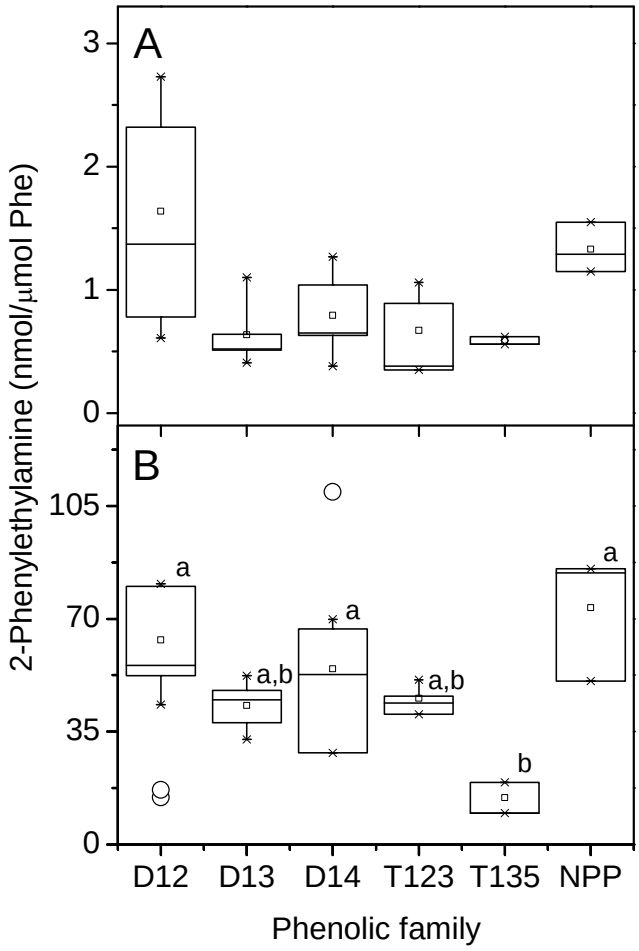


Figure 2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Phenolic family
2-Phenylethylamine (nmol/μmol Phe)

Figure 3

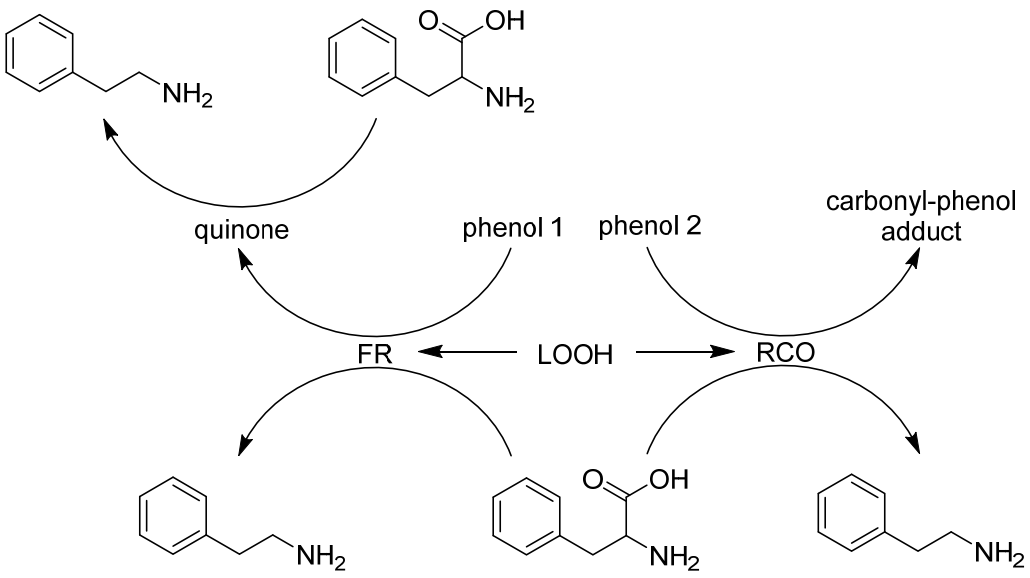
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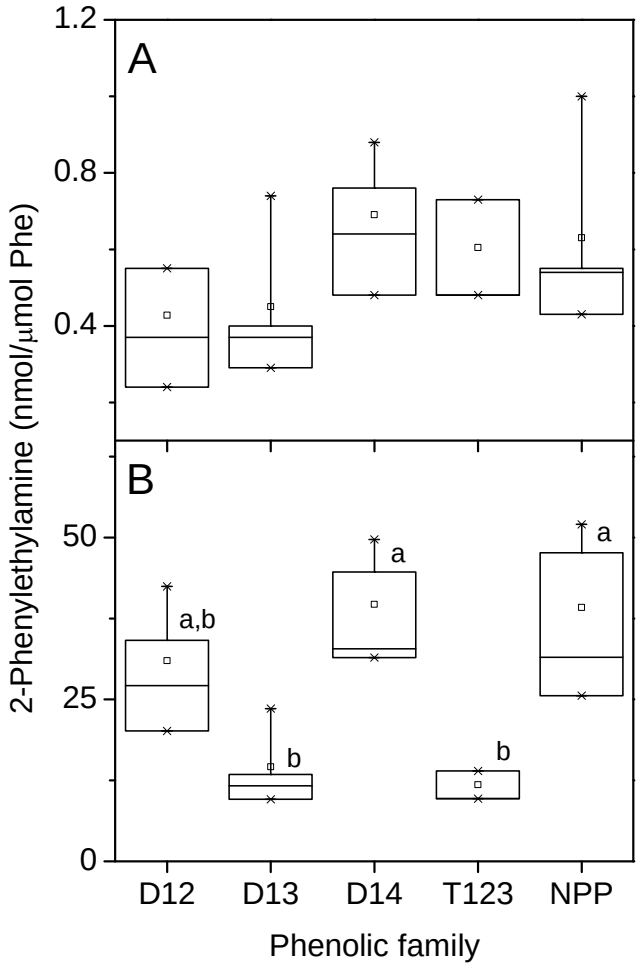
Figure 4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T123
NPP
Phenolic family

Figure 5

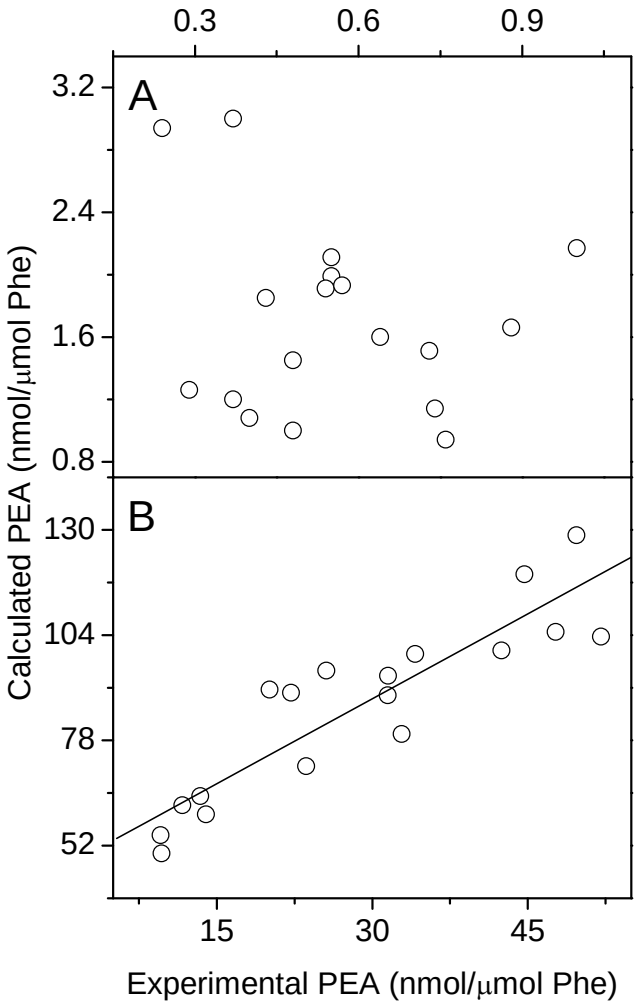
0.3
0.6
0.9
A
3.2
2.4
1.6
0.8
B
130
104
78
52
15
30
45
Calculated PEA (nmol/μmol Phe)
Experimental PEA (nmol/μmol Phe)

Figure 6

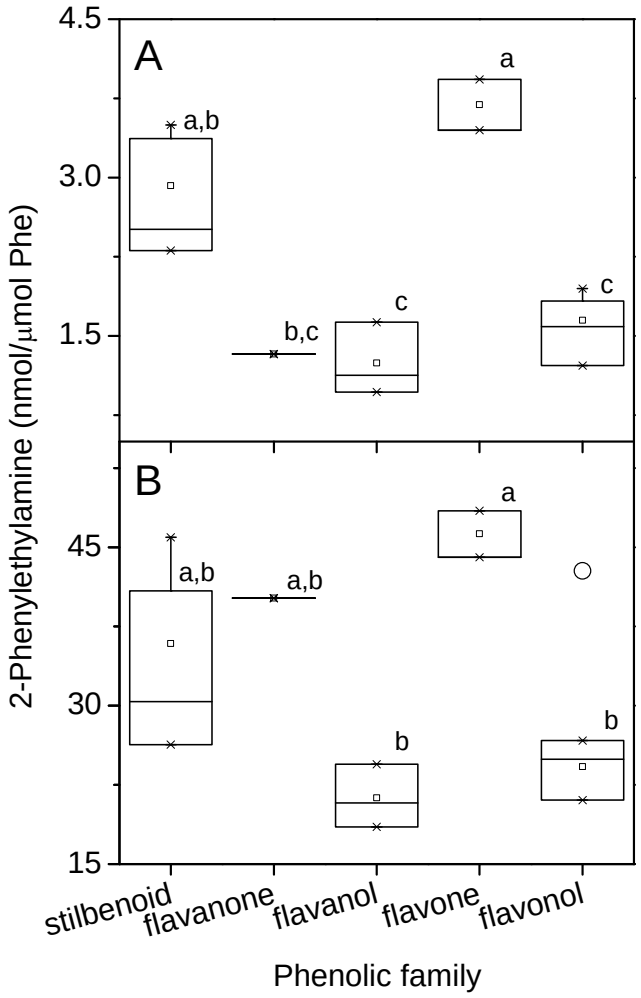
4.5
3.0
1.5
A
a
a,b
b,c
c
c
45
30
15
B
a
a,b
a,b
b
b
stilbenoid
flavanone
flavanol
flavone
flavonol
Phenolic family
2-Phenylethylamine (nmol/μmol Phe)

Figure 7

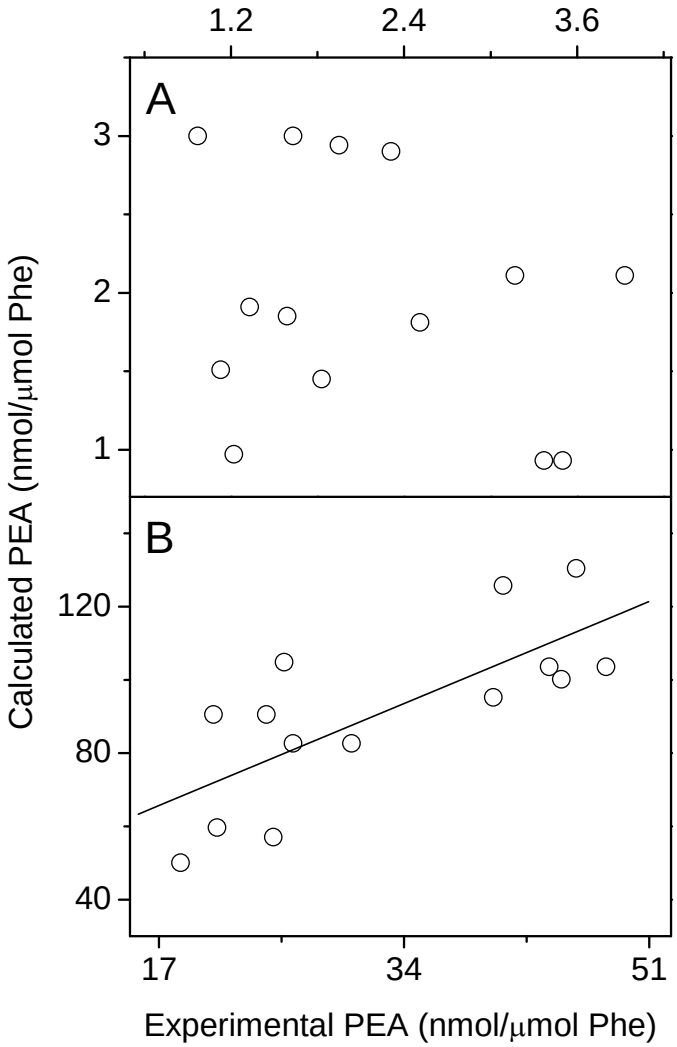
1.2
2.4
3.6
A
3
2
1
B
120
80
40
17
34
51
Calculated PEA (nmol/μmol Phe)
Experimental PEA (nmol/μmol Phe)

Figure 8

SUPPORTING INFORMATION

Structure–Activity Relationship (SAR) of Phenolics for the Inhibition of 2-Phenylethylamine Formation in Model Systems Involving Phenylalanine and the 13-Hydroperoxide of Linoleic Acid

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Table S-1. Information on the Source and Purity of Tested Phenolic (and Analogous) Compounds

No.	Compound name	Source	Purity
1	catechol	Sigma-Aldrich	>99%
2	4-methylcatechol	Aldrich	>95%
3	toluene	Sigma-Alchich	>99%
4	*p*-cresol	Alfa Aesar	99%
5	4-methoxycatechol	Apollo Scientific	>98%
6	3-(3,4-dihydroxyphenyl)propanoic acid	Aldrich	98%
7	3,4-dihydroxybenzoic acid	Aldrich	>97%
8	caffeic acid	Sigma	>98%
9	ferulic acid	Aldrich	99%
10	hydroxytyrosol	Sigma	>98%
11	tyrosol	Aldrich	98%
12	resorcinol	Sigma-Aldrich	99%
13	2-methylresorcinol	Aldrich	98%
14	orcinol	Aldrich	97%
15	2,5-dimethylresorcinol	TCI	>98%
16	2,6-dihydroxybenzoic acid	Aldrich	98%
17	hydroquinone	Sigma	99%
18	trimethylhydroquinone	Aldrich	97%
19	*tert*-butylhydroquinone	Fluka	>98%
20	2,5-dihydroxybenzoic acid	Fluka	99%
21	methoxyhydroquinone	Alfa Aesar	97%
22	pyrogallol	Sigma-Aldrich	99%
23	gallic acid	Aldrich	>98%
24	methyl gallate	Fluka	>98%
25	propyl gallate	Sigma	>98%
26	phloroglucinol	Aldrich	>99%
27	2-phenyl-1-(2,4,6-trihydroxyphenyl)ethan-1-one	Aldrich	97%
28	resveratrol	TCI	>98%
29	piceatannol	TCI	>98%
30	oxyresveratrol	TCI	>95%
31	gnetol	TCI	>97%
32	naringenin	Aldrich	>95%
33	catechin	Sigma	>98%
34	epicatechin	Sigma	>90%
35	epigallocatechin	TCI	>98%
36	chrysin	Aldrich	97%
37	baicalein	Cayman	>95%
38	kaempferol	Cayman	>98%
39	quercetin	Sigma	>98%
40	morin	Sigma	>85%
41	myricetin	TCI	>97%

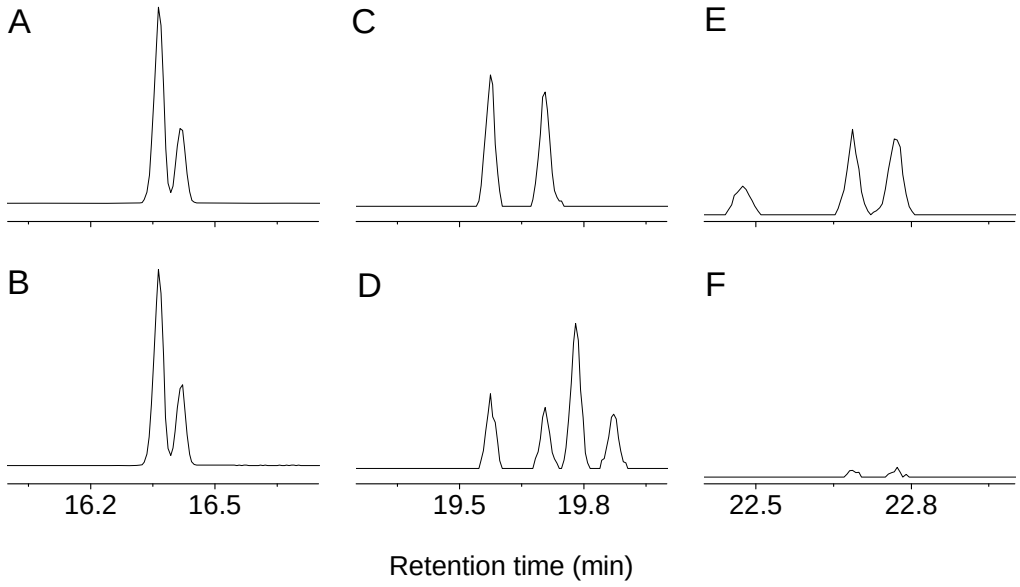

Figure S1. Trace chromatograms of selected aldehydes determined in the thermal degradation of 13-hydroperoxide of linoleic acid (LOOH) (layers A, C, and E), and in the degradation of LOOH in the presence of phenylalanine (Phe) (layers B, D, and F). The selected aldehydes were hexanal (layers A and B), 2-octenal (layers C and D), and 2,4-decadienal (layers E and F). Reactions were carried out under the same conditions. Therefore, differences on peak areas of the same aldehyde in the absence or the presence of Phe correspond to differences in aldehyde concentration. The scales employed for the different aldehydes are different among them and, therefore, they are not comparable.

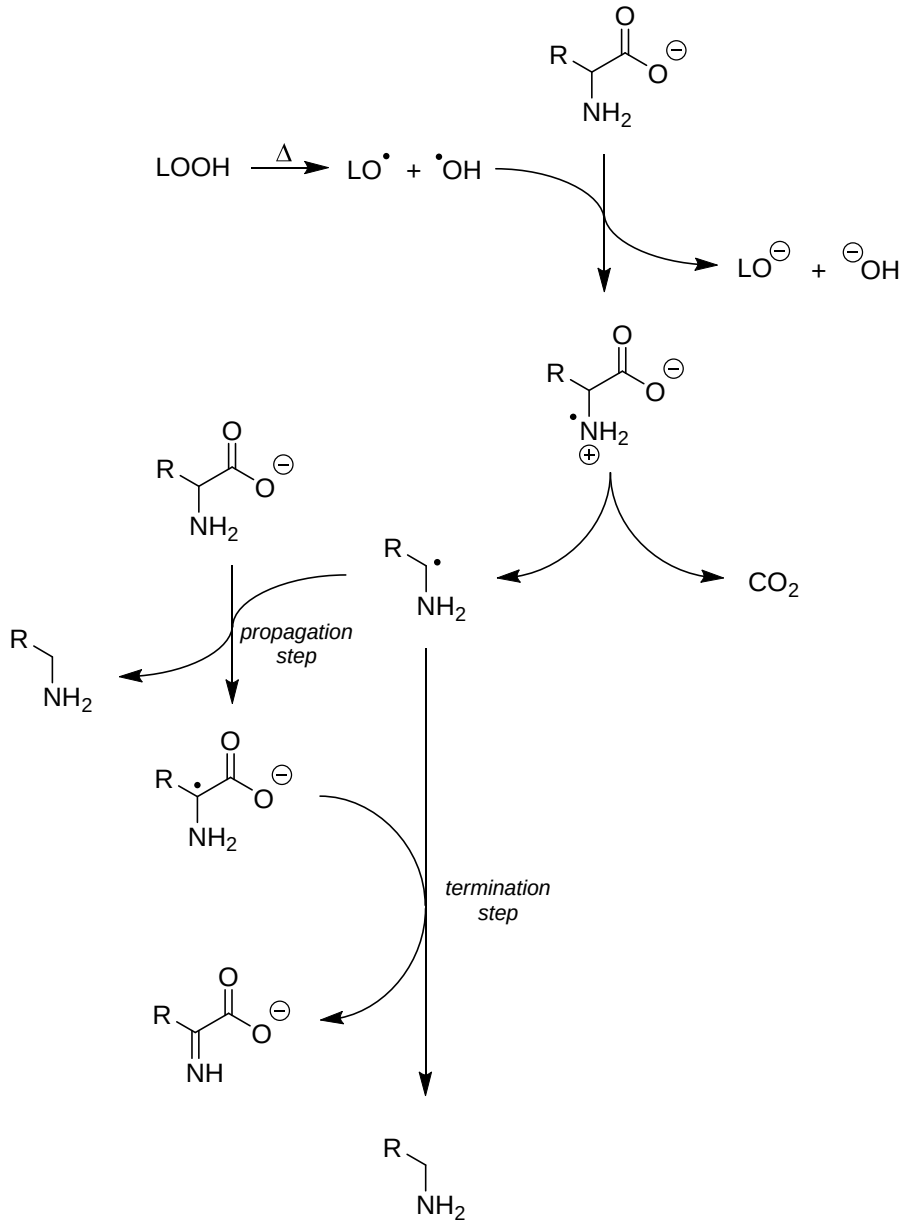

Figure S2. Proposed formation of phenylethylamine from phenylalanine in the presence of lipid hydroperoxides. Both lipid and hydroxyl radicals might be involved in the reaction. This pathway is based on the studies of Monig et al. (1985)[1] and Bonifacic et al. (1998).[2]

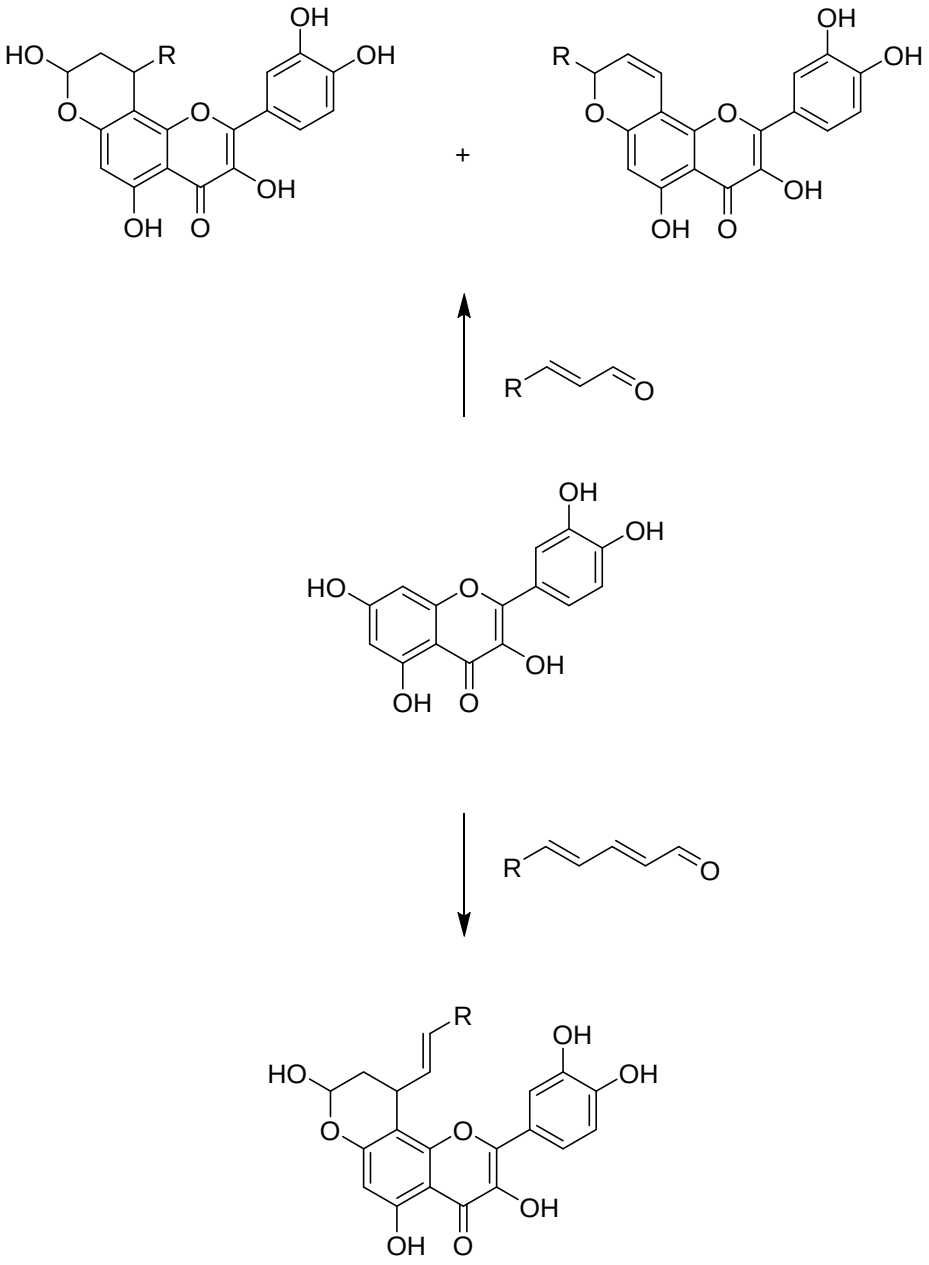

Figure S3. Examples of carbonyl adducts produced by trapping of 2-alkenals (upper part of the scheme) and 2,4-alkadienals (lower part of the scheme) by quercetin as an example of a dietary flavonoid. Mechanism for 2-alkenal trapping by phenolics was described by Hidalgo and Zamora (2014).[3] Characterization of the structures produced between 2-alquenals and quercetin was described by Zamora et al. (2016).[4] Mechanism for 2,4-alkadienal trapping by phenolics was described by Hidalgo and Zamora (2018).[5] The structure of quercetin-alkadienal adduct has been proposed based on the reaction mechanism and the characterization of the structures produced when simple phenolics were involved.

References

(1) Monig, J.; Chapman, R.; Asmus, K.-D. Effect of the Protection State of the Amino Group on the ·OH Radical Induced Decarboxylation of Amino Acids in Aqueous Solution. *J. Phys. Chem.* **1985**, *89*, 3139–3144.

(2) Bonifacic, M.; Stefanic, I.; Hug, G. L.; Armstrong, D. A.; Asmus, K.-D. Glycine Decarboxylation: the Free Radical Mechanism. *J. Am. Chem. Soc.* **1998**, *120*, 9930–9940.

(3) Hidalgo, F. J.; Zamora, R. 2-Alkenal-Scavenging Ability of *m*-Diphenols. *Food Chem.* **2014**, *160*, 118–126.

(4) Zamora, R.; Aguilar, I.; Granvogl, M.; Hidalgo, F. J. Toxicologically Relevant Aldehydes Produced during the Frying Process Are Trapped by Food Phenolics. *J. Agric. Food Chem.* **2016**, *64*, 5583–5589.

(5) Hidalgo, F. J.; Zamora, R. 2,4-Alkenal Trapping by phenolics. *Food Chem.* **2018**, *263*, 89–95.

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