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Amino acid decarboxylations produced by lipid-derived reactive carbonyls in amino acid mixtures

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ABSTRACT

The formation of 2-phenylethylamine and phenylacetaldehyde in mixtures of phenylalanine, a lipid oxidation product, and a second amino acid was studied to determine the role of the second amino acid in the degradation of phenylalanine produced by lipid-derived reactive carbonyls. The presence of the second amino acid usually increased the formation of the amine and reduced the formation of the Strecker aldehyde. The reasons for this behavior seem to be related to the α-amino group and the other functional groups (mainly amino or similar groups) present in the side chain of the amino acid. These groups are suggested to modify the lipid-derived reactive carbonyl but not the reaction mechanism because the E_a of formation of both 2-phenylethylamine and phenylacetaldehyde remained unchanged in all studied systems. All these results suggest that the amine/aldehyde ratio obtained by amino acid degradation can be modified by adding free amino acids during food formulation.

Keywords:

Amino acid degradation; Biogenic amines; Carbonyl-amine reactions; Lipid oxidation; Maillard reaction; Strecker degradation

Chemical compounds studied in this article:

Phenylalanine (PubChem ID: 6140); phenylacetaldehyde (PubChem ID: 998); 2-phenylethylamine (PubChem ID: 1001); 2-octenal (PubChem ID: 5283324); 2,4-heptadienal (PubChem ID: 5283321); 2,4-decadienal (PubChem ID: 5283349); 4,5-epoxy-2-heptenal (PubChem ID: 6444055); 4,5-epoxy-2-decenal (PubChem ID: 15825667); 4-oxo-2-nonenal (PubChem ID: 6445537); 4-hydroxy-2-nonenal (PubChem ID:5283344).

1. Introduction

Microbial catabolism of amino acids plays an important role in both the aroma formation of numerous food products, including cheese, wine and fermented sausages (Ardö, 2006; Feng, Su, Zhao, Cai, Cui, Sun-Waterhouse, & Zhao, 2015), and the formation of compounds, such as biogenic amines, that may compromise the health of consumers (Toro-Funes, Bosch-Fuste, Latorre-Moratalla, Veciana-Nogues, & Vidal-Carou, 2015; Shumilina, Ciampa, Capozzi, Rustad, & Dikiy, 2015).

In addition to the amino acid catabolism produced by microorganisms, amino acids can also be degraded chemically as a consequence of food processing (Hidalgo & Zamora, in press). These reactions play a major role in the formation of taste and flavor compounds as well as food-process toxicants. Thus, for example, many volatiles are produced in the course of the Strecker degradation of amino acids (Rizzi, 2008). Among them, Strecker aldehydes, pyrazines, pyridines, pyrroles, and oxazoles are important flavor compounds. On the other hand, production of amines by amino acid decarboxylation is a cause of concern because they are potentially toxic compounds and they have been pointed out as precursors in the formation of vinylogous derivatives of amino acids, including styrene or acrylamide (Granvogl, Bugan, & Schieberle, 2006; Hidalgo & Zamora, 2007).

Both classes of compounds, amino acid-derived Strecker aldehydes and amines, are produced simultaneously in food products by parallel pathways through the same key intermediates. These degradations are initiated by a reactive carbonyl compound and a recent study has shown that the proportion in which both classes of compounds (aldehydes and amines) are produced depends on the carbonyl compound involved and the reaction conditions, including the reaction pH, the amount of oxygen in the reaction atmosphere, the time, and the temperature, among others (Zamora, León, & Hidalgo,

2015). That study showed that it is possible to direct amino acid degradations toward either the formation of flavors or the formation of amines by employing different reaction conditions. However, that study was carried out by employing only one amino acid (phenylalanine) in the presence of different lipid-derived reactive carbonyls, and the presence of other amino acids was not considered. Nevertheless, some free amino acids have been shown to effectively contribute to the formation of Strecker aldehydes through the reactive carbonyl compounds produced by their thermal degradation (Hidalgo, Alcón, & Zamora, 2013). Therefore, the presence of some additional amino acids might play a role in the preferential formation of either Strecker aldehydes or amino acid-derived amines by amino acid degradation in the presence of reactive carbonyl compounds.

In an attempt to find out factors that determine whether amino acid degradations will mainly produce either Strecker aldehydes or amino acid-derived amines, this study investigates the simultaneous formation of phenylacetaldehyde (PAC) and 2-phenylethylamine (PEA) in ternary mixtures of phenylalanine, lipid-derived reactive carbonyls, and a second amino acid.

2. Materials and methods

2.1. Materials

Different lipid oxidation products were employed in this study. They included lipid hydroperoxides (13-hydroperoxyoctadeca-9,11-dienoic acid, methyl 13-hydroperoxyoctadeca-9,11-dienoate, and methyl 13-hydroperoxyoctadeca-9,11,15-trienoate) as well as lipid-derived reactive carbonyls: alkenals (2-octenal), 2,4-alkadienals (2,4-heptadienal and 2,4-decadienal), 4,5-epoxy-2-alkenals (4,5-epoxy-2-heptenal and 4,5-epoxy-2-decenal), 4-oxo-2-alkenals (4-oxo-2-hexenal and 4-oxo-2-nonenal), and 4-hydroxy-2-alkenals (4-hydroxy-2-nonenal). 2-Alkenals and 2,4-

alkadienals were purchased from Aldrich (Milwaukee, WI). All other compounds were prepared in the laboratory. Their syntheses were described previously. Thus, hydroperoxides were prepared by fatty acid oxidation with lipoxygenase (Hidalgo & Zamora, 1995); epoxyalkenals were obtained from alkadienals by epoxidation (Zamora, Gallardo, & Hidalgo, 2006); oxoalkenals were obtained from 2-alkyfurans by ring opening (Zamora, Alcon, & Hidalgo, 2013); and 4-hydroxy-2-nonenal was obtained from 3-nonenol by epoxidation and later oxidation with Dess-Martin periodinane (Hidalgo, Gallardo, & Zamora, 2005).

The other chemicals employed in this study were purchased from Aldrich, Sigma (St. Louis, MO), Fluka (Buchs, Switzerland), or Merck (Darmstadt, Germany) and were of the highest analytical grade available.

2.2. Formation of PEA and PAC in ternary mixtures of phenylalanine, oxidized lipids, and amino acids

Mixtures of phenylalanine, the lipid derivative, and the second amino acid (10 μmol of each) were singly homogenized with sand (50-70 mesh, 600 mg, obtained from Aldrich), 0.3 M sodium citrate buffer (30 μL), pH 3, and water (80 μL). Samples were heated in closed test tubes under a controlled atmosphere. Heating times and temperatures are indicated in the text for each experiment, but most experiments were carried out for 1 h at 140 ºC under nitrogen. At the end of heating, samples were cooled (5 min at room temperature and 15 min at –30 ºC), and the addition of the internal standard (20 μL of a solution containing 24.09 mg of 2-ethylpyridine in 50 mL of methanol) and 1 mL of methanol-water (80:20) was carried out. The obtained mixture was stirred for 1 min and then centrifuged for 10 min at 2000 × *g*. The supernatant (700 μL) was collected and reduced with 1 mg of sodium borohydride for 30 min. Reduction

was finished by adding 500 µL of acetone and the mixture was centrifuged for 10 min at 2000 × *g*. The produced compounds were determined by GC–MS.

GC-MS analyses were conducted as described previously (Zamora, León, & Hidalgo, 2015) and the ions monitored for the quantitation of the studied analytes were: $[C_7H_8N]^+$ = 106 for the internal standard, $[C_7H_7]^+$ = 91 for the PEA, and $[C_8H_{10}O]^+$ = 122 for the PAC (determined as 2-phenylethanol).

Quantitation of PEA and PAC (as 2-phenylethanol) was also described previously (Zamora, León, & Hidalgo, 2015).

Additional experiments were also carried out with either methylguanidine or butylamine. Reactions were carried out as described above but the second amino acid was replaced by either methylguanidine or butylamine.

2.3. Statistical analysis

All data given are mean ± SD values of, at least, three independent experiments. Statistical comparisons were carried out using Origin® v. 7.0 (OriginLab Corporation, Northampton, MA). Group differences were evaluated by the Tukey test (Snedecor & Cochran, 1980). The significance level is $p < 0.05$ unless otherwise indicated.

3. Results

3.1. Effect of a second amino acid in PEA and PAC formation by phenylalanine degradation in the presence of 2,4-decadienal

To study the role of a second amino acid in amino acid degradations, 2,4-decadienal was employed because it is a good producer of both PEA and PAC, and the mechanisms by which these two compounds are produced have been previously studied (Zamora, Delgado, & Hidalgo, 2012; Zamora, Gallardo, & Hidalgo, 2007). Thus, when

phenylalanine was heated in the presence of 2,4-decadienal, the formation of both PEA and PAC was observed (Table 1). As described previously (Zamora, León, & Hidalgo, 2015), the amount of the produced compounds depended on the reactions conditions, including the presence or not of oxygen in the reaction atmosphere. As shown in Table 1, PEA was produced to a higher extent when the reaction was carried out under nitrogen than under air, and the contrary effect was observed in the formation of PAC.

When a second amino acid was added, the amount of the produced PEA usually increased independently of the presence of oxygen or not (Table 1). The exceptions were the addition of methionine or threonine under nitrogen or the addition of methionine, tyrosine, asparagine, or glutamine under air. In these last reactions, the amount of the formed PEA did not increase significantly ($p < 0.05$) in relation to control. On the other hand, arginine was the amino acid that produced the highest increase in PEA formation under nitrogen (309% increase in relation to control) followed by cysteine and lysine (about 200% increase in relation to control). When oxygen was present, the amino acids that most increased PEA formation were lysine, glycine, cysteine, arginine, and alanine (124–151% increase in relation to control).

Differently to the increases produced in the formation of PEA, addition of a second amino acid usually decreased the formation of PAC. Only the addition of serine, aspartic acid, glutamic acid or arginine under nitrogen, or serine under air, did not change significantly ($p < 0.05$) the PAC produced by the control. The amino acids that most decreased the formation of PAC were tryptophan, cysteine, and proline both under nitrogen and under air. The observed decreases in relation to control were 75–88% under nitrogen and 75–90% under air. In both cases the amino acid that produced the highest decrease was tryptophan.

3.2. Effect of lipid oxidation products on PEA and PAC formation by phenylalanine degradation in the presence of arginine

Analogously to the study of the effect of a second amino acid in phenylalanine degradation by 2,4-decadienal described in the previous section, the effect of lipid oxidation products in phenylalanine degradation in the presence of a second amino acid was also studied. This study was carried out under nitrogen to avoid oxidations. As second amino acid, arginine was selected because it was the amino acid that most increased the formation of PEA without producing high decreases in the formation of PAC (Table 1). Therefore, it is the amino acid that most degraded phenylalanine into either PEA or PAC under nitrogen. Thus, the addition of arginine increased the conversion of phenylalanine into either PEA or PAC by 152% in relation to control.

Formation of both PEA and PAC in mixtures of phenylalanine and arginine was mainly a consequence of the presence of the lipid oxidation product (Table 2). However, not all lipid oxidation products produced the amino acid degradation to the same extent and some lipid oxidation products promoted the formation of the amine more than the formation of the aldehyde and vice versa. Thus, PEA formation was mainly promoted by 2,4-decadienal and methyl 13-hydroperoxyoctadeca-9,11,15-trienoate, which increased the formation of this amine more than twenty times. On the other hand, the lipids that produced a lower increase in PEA formation were 2-pentenal and 4-hydroxy-2-nonenal. In the presence of these oxidized lipids, PEA formation did not increase significantly ($p < 0.05$).

Formation of PAC also increased in the presence of oxidized lipids, but the lipids that most favored the formation of this aldehyde were different to those that increased the formation of PEA. Thus, addition of either methyl 13-hydroperoxy-9,11,15-trienoate or 4,5-epoxy-2-decenal increased the formation of PAC more than seven hundred times

in relation to the formation of this compound by the control. On the other hand, 13-hydroperoxyoctadeca-9,11-dienoic acid, 2-pentenal, 2-octenal, and 4-hydroxy-2-nonenal did not increase significantly ($p < 0.05$) the PAC produced by the control.

3.3. Effect of the second amino acid concentration in PEA and PAC formation by phenylalanine degradation in the presence of 2,4-decadienal

To try to understand the reasons for the contribution of the second amino acid to the formation of PEA and PAC by phenylalanine degradation in the presence of an oxidized lipid, the amino acid arginine and 2,4-decadienal were selected because their combination produced very high amounts of PEA and, to a lesser extent, also of PAC. In addition, lysine, cysteine, methyl guanidine, and butylamine were also studied for comparison purposes.

The increase observed in the formation of PEA and the decrease observed in the formation PAC when arginine was present depended on the amount of this second amino acid (Fig. 1). Thus, the amount of PEA increased linearly ($r = 0.9993$, $p = 0.0007$) when arginine was added in the range 0–6 μmol, and then continued increasing but to a lesser extent. Analogously, the amount of PAC decreased linearly ($r = –0.997$, $p < 0.0001$) as a function of the amount of arginine added in the whole studied range (0–10 μmol). This was common for other amino acids. Thus, the amount of PEA increased linearly ($r = 0.9991$, $p = 0.023$) for lysine in the range 0–4 μmol and also ($r = 0.998$, $p < 0.0001$) for cysteine in the range 0–10 μmol. Analogously, the amount of PAC decreased linearly ($r = –0.993$, $p = 0.07$) for lysine in the range 0–4 μmol and also ($r = –0.969$, $p = 0.001$) for cysteine in the range 0–8 μmol.

The fact that different amino acids produce different amounts of PAC and PEA should be a consequence of the different side chains of the assayed amino acids. Because the side chain of arginine is an alkylguanidine, the effect of increasing amounts

of methylguanidine in the formation of PEA and PAC by phenylalanine degradation in the presence of 2,4-decadienal was studied. As observed in Fig. 2, the concentration of both PEA and PAC increased linearly ($r = 0.998$, $p < 0.0001$, and $r = 0.9998$, $p < 0.0001$, respectively) as a function of the amount of methylguanidine added. These results suggested that the guanidine group of arginine took part in the degradation of phenylalanine. However, the behavior of methylguanidine was different to that observed for arginine. On one hand, it was more active than arginine for PEA formation. In addition, it increased PAC formation, differently to arginine in which a small reduction was observed.

In addition to guanidine group, the α-amino group of arginine might also play a role in the formation of both PEA and PAC. To determine the potential contribution of this group, the effect of increasing amounts of butylamine in the formation of PEA and PAC by phenylalanine degradation in the presence of 2,4-decadienal was also determined (Fig. 2). As observed in the figure, the concentration of PEA increased linearly ($r = 0.998$, $p < 0.0001$) as a function of the amount of butylamine added, but the concentration of PAC decreased linearly ($r = –0.999$, $p < 0.0001$) as a function of the amount of butylamine added. These results suggested that the amino group of arginine also took part in the degradation of phenylalanine. However, the behavior of butylamine was also different to that of arginine. On one hand, it was less active than arginine for PEA formation. In addition, it decreased PAC formation more than arginine.

As observed in Fig. 2, the effect of arginine is more or less in the middle of the effects of methylguanidine and butylamine. For that reason, when the effects of methylguanidine and butylamine were added, a straight line was obtained with a slope that was quite similar to that of arginine. These results suggested that the effect of arginine is consequence of the presence in its molecule of both a guanidine group and

an amino group. Both groups increased the formation of PEA and had opposite effects for PAC formation.

To confirm whether the found additive effects were also observed in other amino acids, the effect of butylamine concentration on both PEA and PAC formation was compared to that of lysine and cysteine (Fig. 3). As observed in the figure for PEA formation, the slope of the line obtained for butylamine was very similar to that of cysteine, and lower than that of lysine. However, lysine has two amino groups. If the comparison is carried out between lysine and the double concentration of butylamine, the slopes of both lines were very similar, therefore suggesting that the presence of amino groups in lysine and cysteine, and the presence of the amino and guanidine groups in arginine were the responsible for the contribution of these amino acids to PEA formation.

Differently to the formation of PEA, the formation of PAC did not seem to depend only on the kind and number of amino groups present. Thus, the slopes of both lysine and cysteine were similar among them and smaller than that of butylamine. In addition, the slope for the double concentration of butylamine was much higher. A reason for this behavior might be the reaction of the amino acid with the formed PAC, such as the formation of thiazolidines when cysteine is involved (Kim & Shin, 2011).

3.4. Effect of time and temperature in PEA and PAC formation by phenylalanine degradation in the presence of 2,4-decadienal and a second amino acid

The results obtained in the previous section suggested that the contribution of the second amino acid was a consequence of the presence of both the α-amino group and any other additional group (mainly an amino or similar group) present in the side chain of the amino acid. In an attempt to know whether these additional groups are modifying

254 in some way the pathways by which PEA and PAC are produced, the activation
255 energies (E_a) of formation of both compounds in the absence and in the presence of a
256 second amino acid was determined. These determinations were carried out by studying
257 formation kinetics of both PEA and PAC at different temperatures.

258 In the absence of a second amino acid, the concentrations of PEA and PAC increased
259 linearly as a function of time at the different assayed temperatures when phenylalanine
260 was heated in the presence of 2,4-decadienal (Figs. S-1A and S-1B, respectively,
261 Supplementary data). Reaction rates at the different assayed temperatures were
262 calculated using the following equations:

263 $$[PEA] = [PEA]_0 + kt$$

264 and

265 $$[PAC] = [PAC]_0 + kt$$

266 where $[PEA]_0$ and $[2\text{-}PAC]_0$ represent the intercept, k is the rate constant, and t the time.
267 These rate constants were used in an Arrhenius plot for calculation of the E_a of PEA and
268 PAC formation in the presence of 2,4-decadienal (Figure 4). The values obtained for E_a
269 were 51 kJ/mol for PEA and 78 kJ/mol for PAC.

270 Analogously, the concentrations of PEA and PAC also increased linearly as a
271 function of time at the different assayed temperatures when mixtures of phenylalanine
272 and arginine were heated in the presence of 2,4-decadienal (Figs. S-2A and S-2B,
273 respectively, Supplementary data). The rate constants, calculated using the previous
274 equations, were used in an Arrhenius plot for calculation of the E_a of PEA and PAC
275 formation in the presence of 2,4-decadienal and arginine (Figure 4). The values obtained
276 for E_a were 51 kJ/mol for PEA and 74 kJ/mol for PAC.

277 The concentrations of PEA and PAC also increased linearly as a function of time at
278 the different assayed temperatures when mixtures of phenylalanine and lysine were
279 heated in the presence of 2,4-decadienal (Figs. S-3A and S-3B, respectively,
280 Supplementary data). The obtained rate constants in these figures were also used in an
281 Arrhenius plot for calculation of the E_a of PEA and PAC formation in the presence of
282 2,4-decadienal and lysine (Figure 4). The values obtained for E_a were 54 kJ/mol for
283 PEA and 71 kJ/mol for PAC.

284 Finally, the whole procedure was repeated once again for mixtures of phenylalanine,
285 cysteine and 2,4-decadienal. The concentrations of PEA and PAC also increased
286 linearly as a function of time at the different assayed temperatures (Figs. S-4A and S-
287 4B, respectively, Supplementary data). The obtained rate constants in these figures were
288 also used in an Arrhenius plot for calculation of the E_a of PEA and PAC formation in
289 the presence of 2,4-decadienal and lysine (Figure 4). The values obtained for E_a were 47
290 kJ/mol for PEA and 81 kJ/mol for PAC.

291 **4. Discussion**

292 Amino acid degradations play a major role in food quality (Choe & Min, 2007;
293 Garcia-Torres, Ponagandla, Rouseff, Goodrich-Schneider, & Reyes-De-Corcuera,
294 2009). These degradations are mainly produced by either the amino acid
295 decarboxylation or the decarboxylation together with the conversion of the α-amino
296 group into a carbonyl group. The fact that one of these compounds can be produced to a
297 higher extent than the other is important for food quality because it will determine
298 whether these degradations will increase either the organoleptic properties of the food
299 with the formation of Strecker aldehydes and heterocyclic odorous compounds, or the
300 content of potentially toxic compounds. A previous study showed that the produced
301 amine/aldehyde ratio can be shifted as a function of both the carbonyl compound

responsible for the amino acid degradation and the reaction conditions (Zamora, León, & Hidalgo, 2015). The results obtained in this study extend the factors that will determine the final amine/aldehyde ratio also to free amino acids. Thus, most amino acids increased the formation of the amine and most of them reduced the formation of the Strecker aldehyde. However, not all amino acids increased the formation of the amine or reduced the production of the Strecker aldehyde to the same extend. The amino acids that increased most the formation of the amine were arginine, lysine and cysteine. The amino acids that reduced most the formation of the PAC were tryptophan and cysteine. These results point to cysteine as the amino acid that most will shift the amine/aldehyde ratio towards the formation of the amine under the employed reaction conditions.

The reasons for this behavior are not fully understood, although the results obtained in the present study suggest that they seem to be related to the α-amino group and the other functional groups (mainly amino or similar groups) present in the side chains of the amino acid. It is hypothesized that the participation of these groups would be through the formation of carbonyl–amine adducts with the carbonyl compound responsible for the amino acid degradation (2,4-decadienal in most of the described experiments). This reaction should modify the carbonyl compound in such a way that the obtained adduct would favor the formation of the amine and inhibit the formation of the aldehyde. Nevertheless, these reactions should not change the reaction mechanism because the E_a of formation of both PEA and PAC was always approximately the same and independent of the presence of additional amino acids in the reaction (as can be observed, all the lines in each panel of Fig. 4 had very similar slopes). Thus, under the conditions employed in this study, PEA was produced with an E_a of 47–54 kJ/mol and PAC was produced with an E_a of 71–81 kJ/mol.

In the previous study, we found that the PEA/PAC ratio can be increased by using electron-donating groups in the chain of the carbonyl compound and it was explained according to the reaction mechanism proposed for the formation of both PEA and PAC (Zamora, León, & Hidalgo, 2015). The reaction of a second amino acid with 2,4-decadienal can take place in two ways (Fig. S-5, Supplementary data). On one hand it can produce the imine between the amino group of the amino acid and the carbonyl group of the 2,4-decadienal. This reversible reaction would inhibit the reaction of the aldehyde with the phenylalanine and therefore the formation of both PEA and PAC should decrease. On the other hand, the amino group of the second amino acid can also be added to the 2,4-decadienal. This would produce the corresponding 4- or 5-alkylamino-2-decenal. Therefore, this reaction would convert an alkadienal into an alkenal with an alkylamino group as substituent, and an alkylamino group is a more electron-donating group than a carbon-carbon double bond. This would favor the formation of the PEA over the PAC, as observed. This reaction would also explain the higher or lower effects of some amino acids in relation to others, which might be related to their different reactivity with 2,4-decadienal.

All these results confirm that the amine/aldehyde ratio obtained by amino acid degradation in the presence of carbonyl compounds can be modified as a function of reaction conditions and food formulation, including the addition of some free amino acids.

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Appendix A. Supplementary data

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

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Figure legends

Fig. 1. Effect of the concentration of a second amino acid in the formation of: A, 2-phenylethylamine (PEA); and B, phenylacetaldehyde (PAC), by phenylalanine (Phe) degradation in the presence of 2,4-decadienal. The assayed amino acids were arginine (○), lysine (△), and cysteine (□).

Fig. 2. Effect of the concentration of a second amino compound in the formation of: A, 2-phenylethylamine (PEA); and B, phenylacetaldehyde (PAC), by phenylalanine (Phe) degradation in the presence of 2,4-decadienal. The assayed amino compounds were arginine (○), methylguanidine (▽), and butylamine (▷). The addition of the effects of methylguanidine and butylamine is also shown (◇).

Fig. 3. Effect of the concentration of a second amino compound in the formation of: A, 2-phenylethylamine (PEA); and B, phenylacetaldehyde (PAC), by phenylalanine (Phe) degradation in the presence of 2,4-decadienal. The assayed amino compounds were lysine (△), cysteine (□), and butylamine (▷). The effect of the double concentration of butylamine is also shown (▶).

Fig. 4. Arrhenius plot for the formation of: A, 2-phenylethylamine; and B, phenylacetaldehyde, by phenylalanine degradation in the presence of 2,4-decadienal and a second amino acid. The assayed amino acids were arginine (○), lysine (△), and cysteine (□). Plots also include the results obtained in the absence of the second amino acid (▽).

Table 1

2-Phenylethylamine and phenylacetaldehyde formation in ternary mixtures of phenylalanine, 2,4-decadienal and a second amino acids

Second amino acid	2-Phenylethylamine		Phenylacetaldehyde	
	Nitrogen	Air	Nitrogen	Air
None	21.9 ± 3.6	10.6 ± 2.4	21.1 ± 3.4	129.7 ± 4.6
Glycine	50.6 ± 2.0 ***	26.1 ± 2.1 ***	10.7 ± 0.5 ***	119.3 ± 3.0 **
Alanine	30.5 ± 0.7 **	23.8 ± 6.9 ***	14.0 ± 0.3 ***	103.9 ± 9.9 **
Valine	40.4 ± 5.3 ***	16.7 ± 0.6 **	11.6 ± 3.2 ***	57.0 ± 7.8 ***
Leucine	35.4 ± 8.1 ***	19.5 ± 1.5 ***	7.4 ± 3.9 ***	45.2 ± 13.0 ***
Isoleucine	48.8 ± 1.4 ***	21.2 ± 1.6 ***	6.3 ± 1.8 ***	42.7 ± 9.9 ***
Proline	45.1 ± 10.4 ***	18.1 ± 2.0 ***	5.2 ± 2.5 ***	32.3 ± 1.9 ***
Tryptophan	33.2 ± 0.8 ***	17.0 ± 2.6 **	2.4 ± 0.7 ***	13.3 ± 1.3 ***
Methionine	24.6 ± 1.2	12.9 ± 0.2	9.4 ± 1.7 ***	46.9 ± 7.1 ***
Serine	43.5 ± 1.7 ***	16.6 ± 5.0 *	24.7 ± 3.1	123.4 ± 5.1
Threonine	24.5 ± 1.7	15.1 ± 1.0 *	15.4 ± 0.1 *	79.4 ± 10.7 ***
Cysteine	63.9 ± 5.8 ***	24.3 ± 11.8 **	3.4 ± 0.7 ***	25.5 ± 10.9 ***
Tyrosine	31.9 ± 6.4 **	13.7 ± 0.4	15.8 ± 1.1 **	65.9 ± 6.6 ***
Asparagine	35.4 ± 5.3 ***	11.3 ± 2.4	14.5 ± 0.6 **	76.2 ± 14.5 ***
Glutamine	31.4 ± 4.4 **	9.2 ± 2.1	14.3 ± 1.2 **	73.9 ± 9.3 ***
Aspartic acid	38.3 ± 0.8 ***	16.9 ± 6.8 *	18.1 ± 4.8	77.7 ± 14.5 ***
Glutamic acid	39.6 ± 4.3 ***	16.6 ± 3.6 *	23.2 ± 0.6	111.3 ± 21.8 **
Lysine	62.3 ± 11.6 ***	26.6 ± 7.7 ***	15.4 ± 1.1 ***	61.4 ± 9.8 ***
Arginine	89.6 ± 7.2 ***	24.1 ± 3.3 ***	18.6 ± 3.3	99.5 ± 23.2 **
Histidine	44.9 ± 1.0 ***	15.0 ± 1.5 *	14.8 ± 2.1 **	35.4 ± 7.1 ***

Values are mean ± SD (in μmol/mmol of phenylalanine) for, at least, three independent experiments. Means in the same column with an asterisk are significantly different from its control: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

Table 2

2-Phenylethylamine and phenylacetaldehyde formation in ternary mixtures of phenylalanine, arginine, and lipid oxidation products

Lipid oxidation product	2-Phenylethylamine	Phenylacetaldehyde
None	3.9 ± 0.8 k,l	0.1 ± 0.2 h,i
13-Hydroperoxyoctadeca-9,11-dienoic acid	64.1 ± 0.3 c,d	9.2 ± 0.3 c,d,g,h
Methyl 13-hydroperoxyoctadeca-9,11-dienoate	70.3 ± 4.6 b,d	17.6 ± 2.2 b,d,e,j
Methyl 13-hydroperoxyoctadeca-9,11,15-trienoate	84.4 ± 5.6 a,b	46.9 ± 4.3 a
2-Pentenal	13.5 ± 3.9 j,l	7.3 ± 2.6 c,g,i,j
2-Octenal	60.8 ± 6.2 c,d,e	4.9 ± 1.3 d,i,k
2,4-Hexadienal	47.0 ± 4.3 e,f,g	16.5 ± 1.9 c,e,f
2,4-Heptadienal	72.8 ± 3.5 b,c	19.4 ± 3.4 b,c
2,4-Decadienal	89.6 ± 7.2 a	18.6 ± 3.3 b,c
4-Oxo-2-hexenal	20.2 ± 0.5 h,i,j	15.9 ± 1.9 b,f,g,k
4-Oxo-2-nonenal	31.7 ± 4.3 g,i	29.4 ± 1.2 b
4,5-Epoxy-2-heptenal	31.9 ± 4.9 g,h	20.7 ± 0.5 b,c
4,5-Epoxy-2-decenal	57.9 ± 4.8 d,f	46.7 ± 9.2 a
4-Hydroxy-2-nonenal	14.3 ± 0.8 j,k	0.3 ± 0.1 h,i

Values are mean ± SD (μmol/mmol of phenylalanine) for, at least, three independent experiments. Means in the same column with a different letter are significantly different ($p < 0.05$).

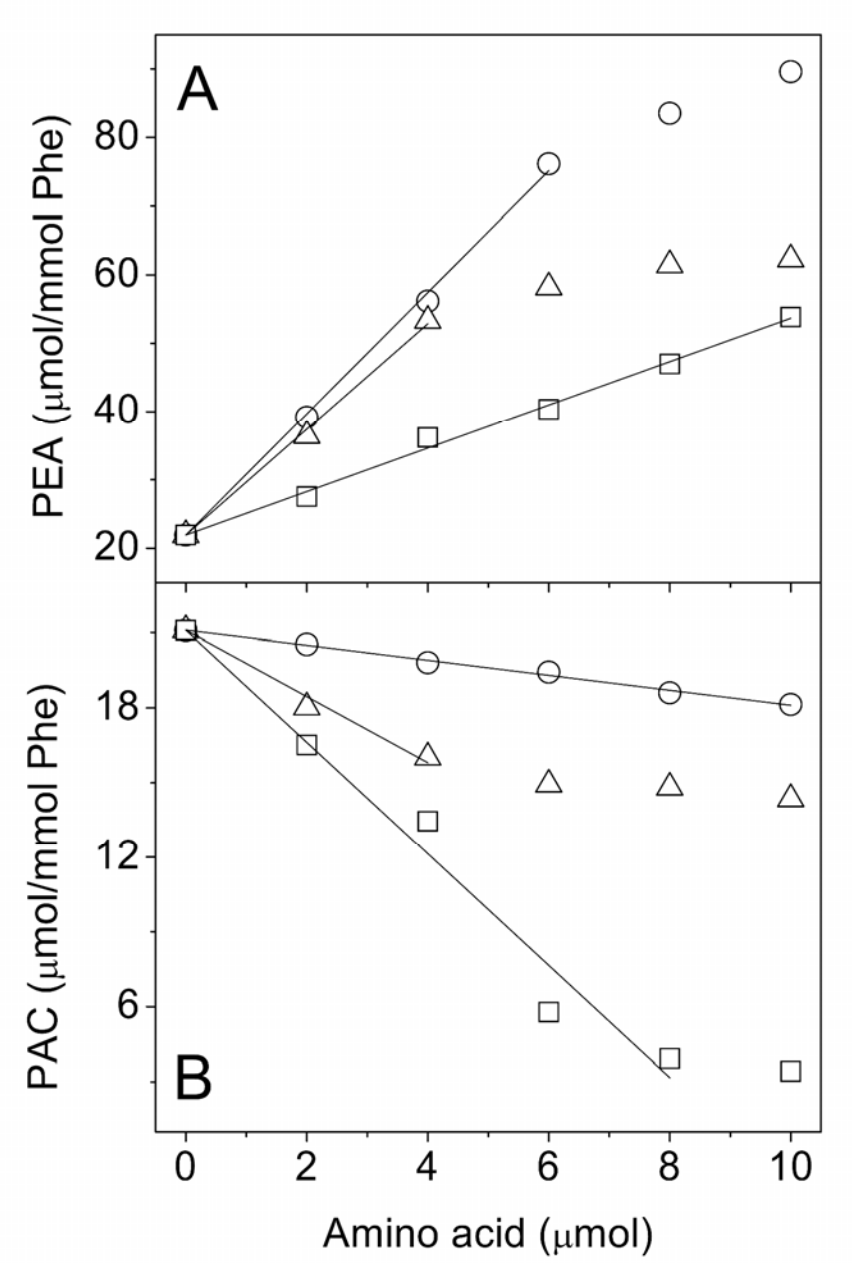
A
B
PEA (μmol/mmol Phe)
PAC (μmol/mmol Phe)
Amino acid (μmol)
20
40
60
80
6
12
18
0
2
4
6
8
10

Figure 1

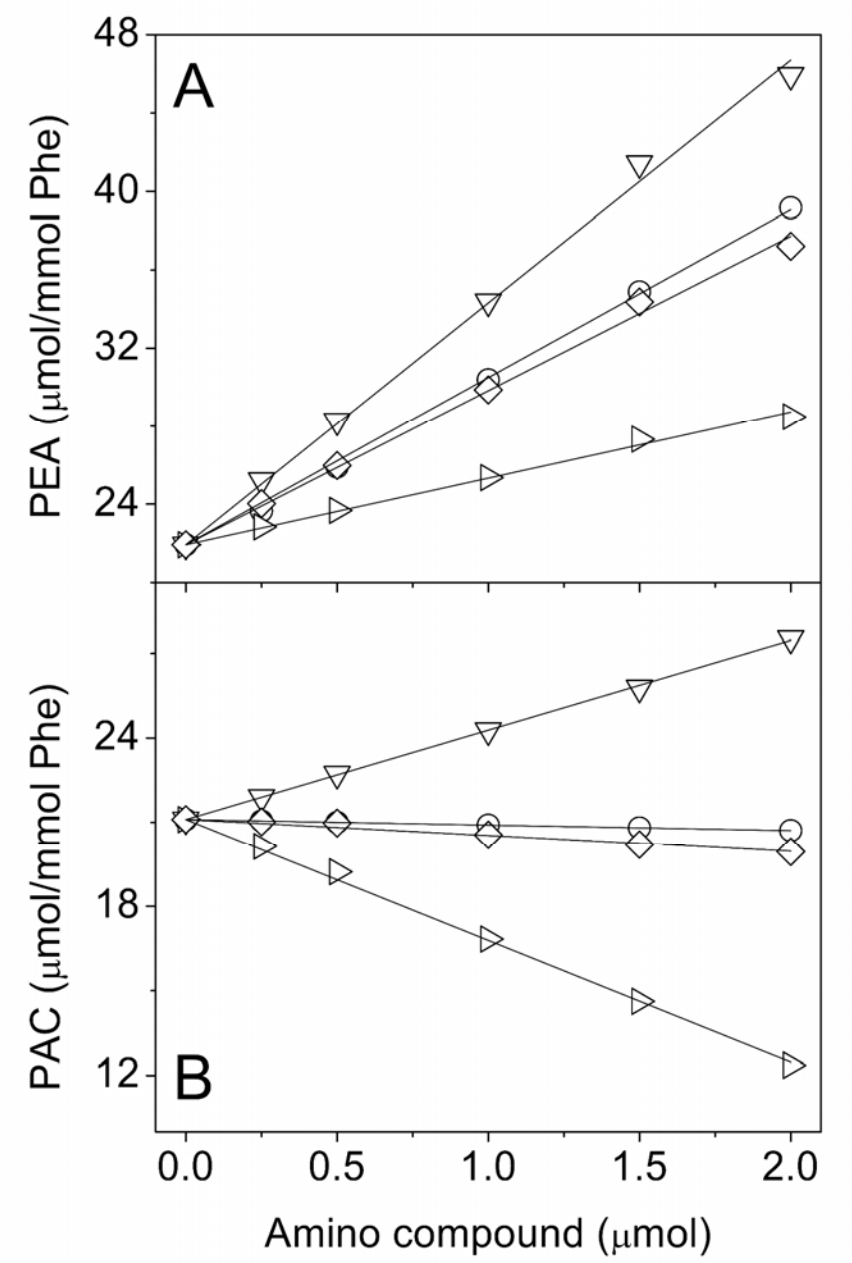
A
B
PEA (μmol/mmol Phe)
PAC (μmol/mmol Phe)
48
40
32
24
24
18
12
0.0
0.5
1.0
1.5
2.0
Amino compound (μmol)

Figure 2

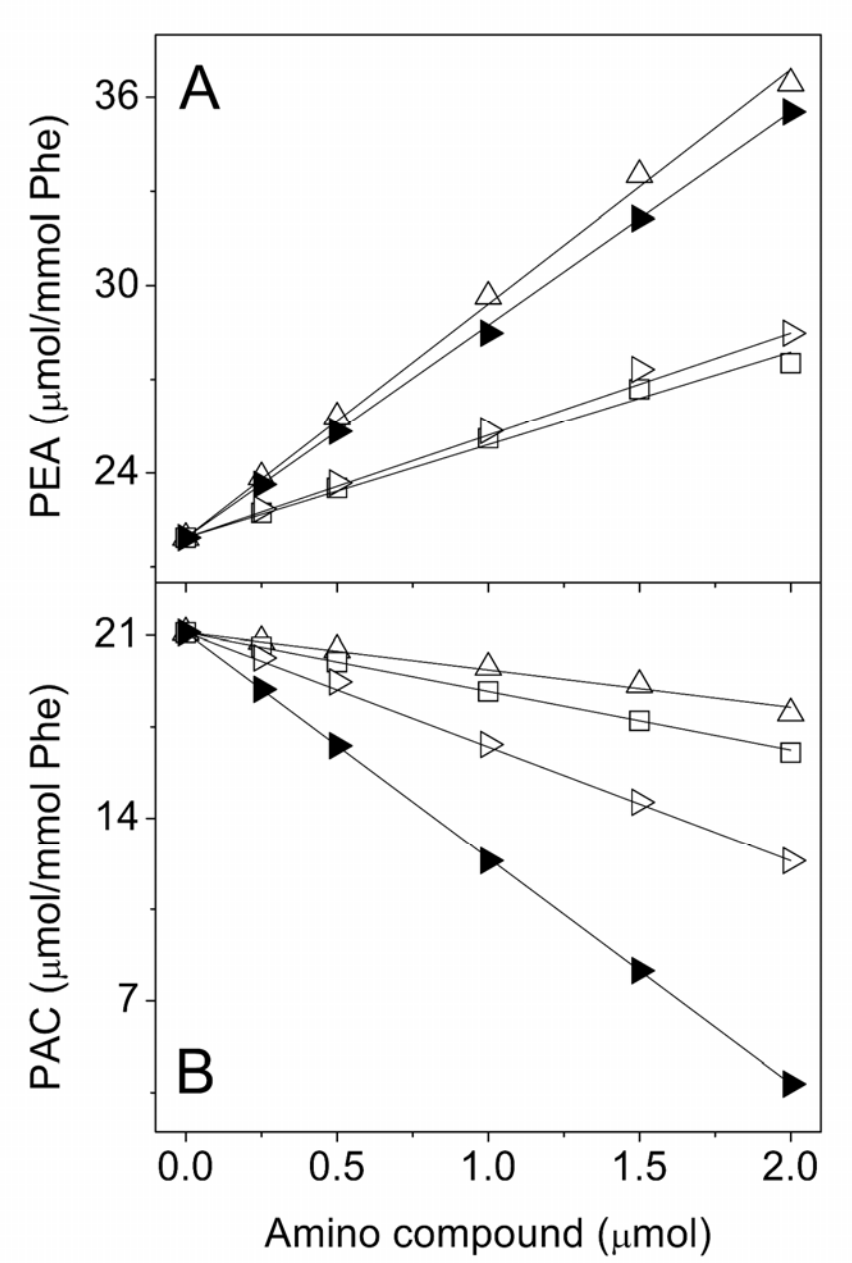
A
B
PEA (μmol/mmol Phe)
PAC (μmol/mmol Phe)
36
30
24
21
14
7
0.0
0.5
1.0
1.5
2.0
Amino compound (μmol)

Figure 3

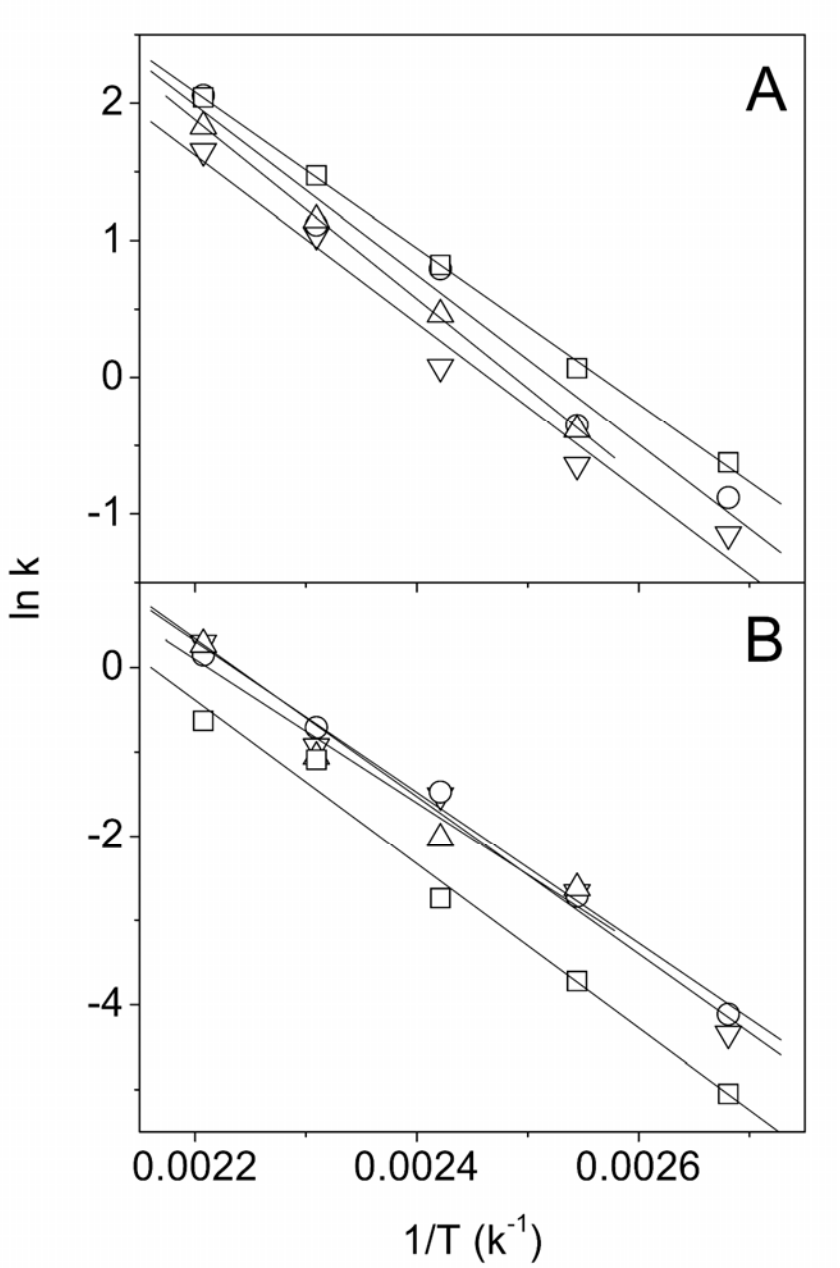

Figure 4