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Effect of Tocopherols in the Antioxidative Activity of Oxidized Lipid–Amine Reaction Products

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Phosphatidylethanolamine (PE), phosphatidylcholine (PC), lysine (Lys), and mixtures of them were tested for antioxidative activity in a tocopherol-stripped olive oil (TSO) and the same oil after addition of 250 μg of α-tocopherol g of oil/(tocopherol-added olive oil, TAO) to evaluate the role of tocopherol in the antioxidant activity of oxidized lipid−amine products. Neither PE nor PC nor Lys protected TSO when tested alone, but both PE and Lys increased the induction period (IP) of TAO. On the contrary, PE/Lys and PC/Lys mixtures, but not PC/PE mixtures, protected both TSO and TAO. These results were a consequence of both the formation of oxidized lipid−amine products, which were determined by gas chromatography−mass spectrometry after their conversion into volatile derivatives, and a synergism between α-tocopherol and the produced compounds. These results were confirmed by analyzing the antioxidative activity of two of the produced carbonyl−amine products: 6-amino-2-(1*H*-pyrrol-1-yl)hexanoic acid (**1**) and 2,3-dipalmitoylpropyl 2-(1*H*-pyrrol-1-yl)ethyl phosphate (**2**). The hydrophilic compound **1** was more antioxidant than the analogous lipophilic compound **2**, and this antioxidative activity was observed in TAO and not in TSO. All these results suggested that antioxidative activity of carbonyl−amine products may be greatly increased with the addition of tocopherols, and those products derived from Lys are more antioxidant in bulk oils than those derived from PE.

KEYWORDS: Carbonyl−amine reactions; antioxidants; lipid oxidation; nonenzymatic browning; phospholipids; pyrrolized phospholipids; Rancimat

INTRODUCTION

Food is a mixture of numerous chemicals, including proteins, amino acids, carbohydrates, lipids, vitamins, and minerals. It is well-known that processing and/or storage may promote chemical reactions among food components. Among the many reactions occurring in processed foods, nonenzymatic browning reactions of reactive carbonyls with free amino groups play a very important role in the formation of many products, including toxic ones (*1−3*). The produced carbonyl−amine reaction products may be both volatile and nonvolatile and include a high variety of chemical structures. Thus, among the nonreversible covalent compounds with a heterocyclic structure, the formation of furans, pyrroles, dithianes, thiazoles, thiazolidines, imidazoles, and pyrazines, among other compounds, has been described (*4−6*). All these compounds play a significant role in many food characteristics, including its stability.

The antioxidative properties of carbonyl−amine reaction products were first observed in the early 1950s in an oil system (*7*) and have later been shown in many other food systems, including cookies (*8*), coffee (*9*), etc. However, the exact nature of the antioxidants formed is not yet well-known (*10*), and to our knowledge, the influence of other food components in the antioxidative activity exhibited by oxidized lipid−amine reaction products has not been yet investigated.

The objective of this study was to investigate the role of tocopherols in the antioxidative activity of the oxidized lipid−amine reaction products formed during the oxidation of aminophospholipids and lipid/amino acid mixtures. Formation of oxidized lipid−amine reaction products during the oxidation of aminophospholipids and lipid/amino acid mixtures has been the objective of different studies, and the antioxidative activity of these compounds have been shown (*11−14*). In addition, a recent study has found that these compounds can be produced in situ and their antioxidative activity can be evaluated at the same time that they are produced when they are formed in the Rancimat vessel (*15*). Thus, in the Rancimat, the oil is exposed to a stream of atmospheric oxygen at elevated temperature. The oil is then oxidized, and the volatile decomposition products formed are trapped in a measuring vessel with distilled water and continuously detected with a conductivity cell. When amino compounds are present, such as amino phospholipids or amino acids, the oxidized lipids produced during oil oxidation react with the amino compounds and produce in situ oxidized lipid−amine reaction products with antioxidative activities. As a continuation of that study, the present study describes the

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stability of a tocopherol-stripped olive oil (TSO), and the same oil after addition of 250 μg of α-tocopherol/g of oil (tocopherol-added olive oil, TAO), in the presence of phosphatidylethanolamine (PE), phosphatidylcholine (PC), lysine (Lys), and mixtures of them.

EXPERIMENTAL PROCEDURES

Materials. PE was isolated from soybean lecithin by column chromatography on silicic acid/Celite (2:1) with chloroform/methanol (3:1). PC was isolated from soybean lecithin according to Singleton et al. (*16*). The identity and purity of the obtained phospholipids were confirmed by HPLC and ^{1}H and ^{13}C NMR spectroscopy. Each phospholipid exhibited a single peak in HPLC (*12*) and the characteristic ^{1}H and ^{13}C signals of their polar heads (*17*). The fatty acid composition of PE was as follows: palmitic acid (19.8%), stearic acid (3.2%), oleic acid (7.6%), linoleic acid (61.6%), and linolenic acid (7.8%). The fatty acid composition of PC was as follows: palmitic acid (3.6%), stearic acid (2.3%), oleic acid (8.3%), linoleic acid (75.9%), and linolenic acid (9.9%).

6-Amino-2-(1*H*-pyrrol-1-yl)hexanoic acid (**1**) was isolated by semipreparative high-performance liquid chromatography from a 4,5-epoxy-2-heptenal/lysine mixture incubated for 16 h at 25 °C. Fractionation of the reaction mixture was carried out according to the previously described procedure (*18*), and the purity and identity of the compound were confirmed by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy and mass spectrometry. Chemical structure for this compound is given in **Figure 1**. Its NMR and MS data were described previously (*18*).

2,3-Dipalmitoylpropyl 2-(1*H*-pyrrol-1-yl)ethyl phosphate (**2**) was isolated by column chromatography from a 4,5-epoxy-2-heptenal/1,2-dipalmitoyl-*rac*-glycero-3-phosphoethanolamine mixture incubated at 37 °C. Fractionation of the reaction mixture was carried out according to the previously described procedure (*12*), and the purity and identity of the compound were confirmed by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy. Chemical structure for this compound is given in **Figure 1**. Its NMR and MS data were described previously (*12*).

Virgin olive oil was obtained from SOS Cuétara S. A. (Andujar, Jaén, Spain). Natural tocopherols and other polar components in the oils were removed according to the procedure of Frankel et al. (*19*), which was modified. Briefly, 500 g of oil diluted in 500 mL of hexane was mixed with 50 g of activated charcoal and shaken mechanically for 30 min. The suspension was filtered and the absorbent was washed thoroughly with hexane. The solvent was removed and the procedure was repeated twice to yield a tocopherol-free oil. Tocopherol content was determined directly in the oil by HPLC with a fluorescence detector (*20*). The obtained stripped oil was used directly (TSO) or after addition of 250 μg of α-tocopherol/g of oil (TAO). The quantity (250 μg of α-tocopherol/g of oil) was chosen because this is a common tocopherol content in olive oils (*21*).

Activated charcoal was purchased from Merck KGaA (Darmstadt, Germany), and α-tocopherol, Lys, 1,2-dipalmitoyl-*rac*-glycero-3-phosphoethanolamine, and BHT were obtained from Sigma Chemical Co. (St. Louis, MO). Other reagents and solvents were purchased from reliable commercial sources.

Measurement of Antioxidative Activity. Oxidative stability of TSO and TAO was compared with TSO and TAO samples containing PE, PC, Lys, BHT, **1**, **2**, or mixtures of PE/Lys, PC/Lys, or PC/PE added at concentrations of 100−400 μg/g of oil. Tested compounds (5 mg) were dissolved in 2 mL of water containing 1% Tween 20 and added to the oil at the concentration indicated. The total amount of solvent added to the oil was always 400 μL, included control oils.

Oil samples (2.5 g) were heated at 90 (TSO) or 110 °C (TAO) in a Metrohm Rancimat (Metrohm AG, Herisau, Switzerland). A continuous airstream (10 L/h) was passed through the heated sample, and the volatiles were absorbed in a conductivity cell. Conductivities were continuously monitored until a sudden rise signified the end of the induction period (IP).

Determination of Carbonyl−Amine Reaction Products. Some of the produced pyrrolic carbonyl−amine reaction products formed in the Rancimat vessel during oxidation process were determined after conversion of these nonvolatile derivatives into volatile pyrroles by the procedure described by Zamora and Hidalgo (*22*). Briefly, 500 mg of oil was treated successively with 2 mL of 0.3 M sodium citrate, pH 3; 1 mL of *p*-anisidine solution (1 mg/mL in 0.3 M sodium citrate, pH 3); and 50 μL of BHT solution (0.8% in acetic acid). The resulting mixture was stirred, bubbled with nitrogen, and heated at 110 °C for 20 h under an inert atmosphere. After that time, the resulting mixture was extracted three times with 2 mL of chloroform−methanol (3:2), and the organic layer was recovered and taken to dryness under nitrogen. The resulting residue was dissolved in 500 μL of chloroform and analyzed by GC−MS.

GC−MS analyses were conducted with a Hewlett-Packard 6890 GC Plus coupled with an Agilent 5973 MSD (mass-selective detector, quadrupole type). A fused-silica HP5-MS capillary column (30 × 0.25 mm i.d.; coating thickness 0.25 μm) was used. Working conditions were as follows: carrier gas helium (1 mL/min at constant flow); injector 250 °C; oven temperature from 70 (1 min) to 240 °C at 5 °C/min and then to 325 °C at 10 °C/min; transfer line to MSD 280 °C; ionization EI 70 eV. Identification of 1-(4-methoxyphenyl)-1*H*-pyrrole (**3**), 1-(4-methoxyphenyl)-2-pentyl-1*H*-pyrrole (**4**), and 2-pentylfuran (**5**) was carried out by comparison of their retention indices and mass spectra with those of authentic compounds. Retention index and mass spectra of compounds **3** and **4** were described previously (*23*).

Statistical Analysis. IPs are expressed as mean values ± standard deviations (SD) of, at least, two independent experiments. Statistical comparisons among different groups were made by analysis of variance. When significant F values were obtained, group differences were evaluated by the Student−Newman−Keuls test (*24*). All statistical procedures were carried out with Primer of Biostatistics: The Program (McGraw-Hill, Inc., New York). The significance level is $p < 0.05$ unless otherwise indicated.

RESULTS

Antioxidative Activities of PE, PC, Lys, and BHT in TSO and TAO As Determined by the Rancimat Method. When TSO was treated with 0−400 μg of PE, PC, or Lys/g of oil, the IP of the oil did not change significantly (**Figure 2A**). A protective effect was observed, however, when 100−400 μg of BHT/g of oil was added. In fact, TSO stability increased with the amount of BHT added, and a correlation was observed between the amount of BHT added and the IP of TSO in the range 100−400 μg of BHT/g of oil ($r = 0.93$, $p = 0.07$).

This behavior was different when the different compounds were tested in TAO (**Figure 2B**). Thus, the IP of TAO increased with the quantity of PE added in the range 200−400 μg/g of oil. In fact, there was a linear increase in the IP of TAO as a function of PE concentration ($r = 0.98$, $p = 0.004$). A similar effect was observed when Lys was added, which also exhibited a linear relationship between the IP of TAO and the amount of amino acid added ($r = 0.97$, $p = 0.007$); and also, with BHT, the antioxidative activity of which was also a function of BHT concentration ($r = 0.94$, $p = 0.017$). Differently than PE, Lys, or BHT, PC did not protect TAO.

The IPs obtained for TAO treated with phospholipids, Lys, or BHT were lower than those previously described for a refined olive oil treated with the same amounts of these compounds (*15*). This is likely a consequence of the presence in the refined olive oil of components, other than tocopherols, that also contributed to oil stability and which were not present in TAO after the treatment with activated charcoal. However, the increases observed in the IPs of TAO treated with PE, Lys, and BHT were higher than those previously described for the refined olive oil. In addition, there was a correlation ($r = 0.96$, $p < 0.0001$) between the increases of IPs produced in the TAO after addition of PE, PC, and Lys and those produced in the refined olive oil after addition of the same compounds at the same concentrations (**Figure 3**), therefore suggesting that tocopherols are the main minor components that play a role in the antioxidative activity

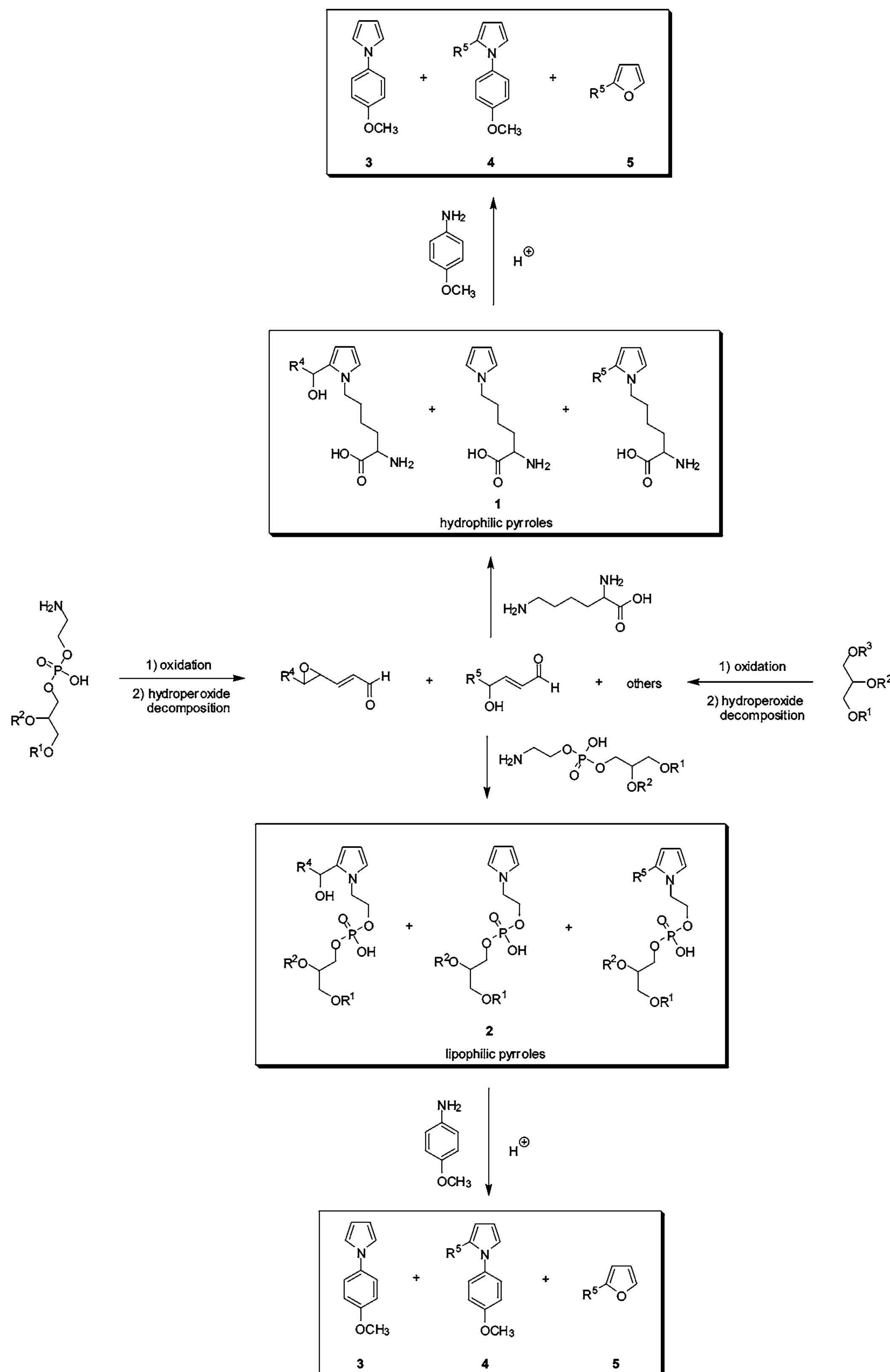

Figure 1. Pyrrole production by carbonyl–amine reactions and their later conversion into volatile pyrroles. The scheme includes only the phospholipid PE because PC cannot produce pyrrolized phospholipids, although PC also contributes to reactive carbonyls production. Although only the production of pyrroles in the ϵ-amino group of lysine is shown, analogous pyrroles are also produced in the α-amino group of lysine. R^1, R^2, and R^3 are fatty acid chains; R^4 and R^5 are alkyl chains. Compound **1** is the analogous derivative produced in the α-amino group of lysine. In compound **2**, $R^1 = R^2 = CH_3(CH_2)_{14}CO$. In compounds **4** and **5**, $R^5 = CH_3(CH_2)_4$.

of the carbonyl−amine reaction products that are produced in the Rancimat vessel. On the other hand, the increases of IPs produced by BHT in TAO and refined olive oil were different and appeared far from the straight line in **Figure 3**.

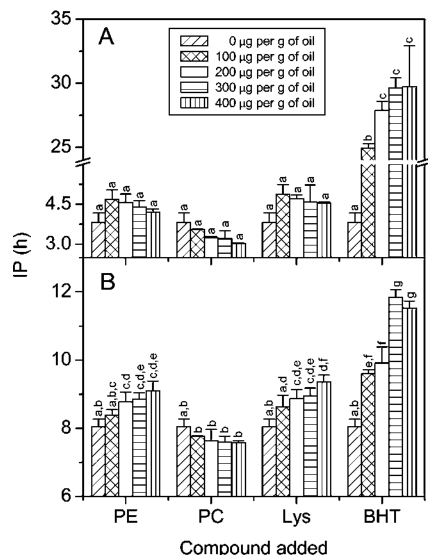

Figure 2. Rancimat IPs of (**A**) tocopherol-stripped olive oil (TSO) and (**B**) tocopherol-added olive oil (TAO), treated with 0 (control oil, slashed bars), 100 (crosshatched bars), 200 (open bars), 300 (horizontally striped bars), or 400 μg (vertically striped bars) of phosphatidylethanolamine (PE), phosphatidylcholine (PC), lysine (Lys), or BHT per gram of oil. Values are means ± SD for, at least, two experiments. Means with different letters are significantly different ($p < 0.05$).

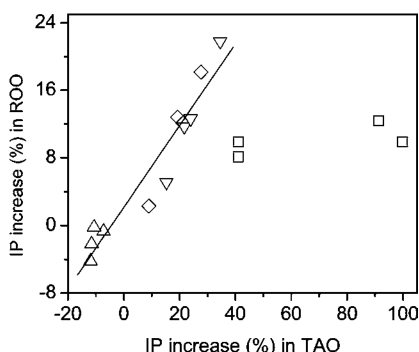

Figure 3. Plot of Rancimat IP increases observed in tocopherol-added olive oil (TAO) after addition of 100–400 μg of PE (◇), PC (△), Lys (▽), or BHT (□) per gram of oil, against the Rancimat IP increases obtained in a refined olive oil (ROO) after addition of the same compounds at the same concentrations.

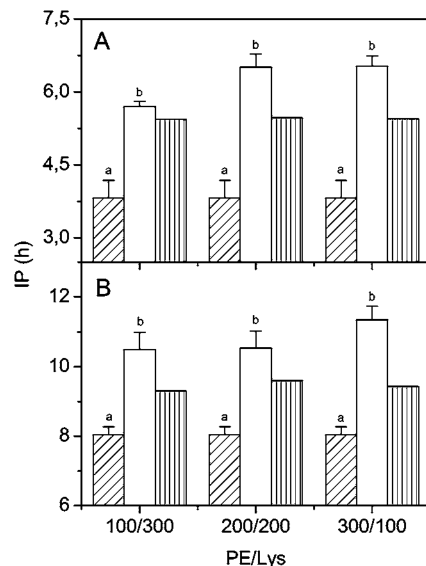

Figure 4. Rancimat IPs of (**A**) tocopherol-stripped olive oil (TSO) and (**B**) tocopherol-added olive oil (TAO), treated with nothing (control oil, slashed bars) or 100/300, 200/200, or 300/100 μg of PE/Lys mixtures/g of oil (open bars). The theoretical additive IP of the mixture has also been included for comparison (vertically striped bars). Experimental IP values are means ± SD for, at least, two experiments. Means with different letters are significantly different ($p < 0.05$).

Table 1. IP Increases and Synergism Observed in PE/Lys, PC/Lys, and PC/PE Mixtures

	IP increase,[a] h (%)		synergism,[b] h (%)	
mixture	TSO	TAO	TSO	TAO
PE/Lys (100/300)	1.88 (49)	2.46 (31)	0.26 (16)	1.20 (96)
PE/Lys (200/200)	2.69 (71)	2.50 (31)	1.04 (63)	0.94 (60)
PE/Lys (300/100)	2.71 (71)	3.31 (41)	1.08 (66)	1.92 (138)
PC/Lys (100/300)	1.04 (27)	2.38 (30)	0.53 (105)	1.74 (271)
PC/Lys (200/200)	1.52 (40)	2.51 (31)	1.19 (365)	2.09 (494)
PC/Lys (300/100)	1.82 (47)	2.61 (33)	1.37 (311)	2.47 (1732)
PC/PE (100/300)	0.21 (6)	0.94 (12)	−0.12	0.40
PC/PE (200/200)	0.25 (7)	0.26 (3)	0.06	−0.07
PC/PE (300/100)	0.05 (1)	−0.36 (−4)	−0.20	−0.26

[a] IP increase is the difference (in hours) between the IP of the treated oil and the IP of the control oil. This increase is also given as a percentage (in parentheses). [b] Synergism is the difference (in hours) between the IP of the treated oil and the IP calculated by adding the effects observed for the two components of the mixtures when added alone at the tested concentration. When observed, the synergism is also given as a percentage (in parentheses).

Antioxidative Activities of PE/Lys Mixtures in TSO and TAO As Determined by the Rancimat Method. When three mixtures of PE/Lys (100/300, 200/200, and 300/100 μg/g of oil) were added to both TSO and TAO, the stability of the treated oils increased significantly (**Figure 4**). This protective effect was observed also in TSO, which did not exhibit any change in stability when treated with PE or Lys individually.

Oil protection depended on the presence of tocopherol. Thus, although the absolute IP increases (in hours) exhibited by PE/Lys mixtures were usually higher in TAO than in TSO, the relative increases (percentage) were lower in TAO than in TSO (**Table 1**). Thus, the IP of TSO increased by 49−71% when treated with the three assayed mixtures of PE/Lys, and the IP of TAO increased by 31−41% when treated with the same mixtures of PE/Lys.

The three assayed PE/Lys mixtures exhibited synergism. Therefore, the protection exhibited for each PE/Lys mixture was higher than the additive protection of the two components at the concentrations added. This synergism was usually higher in TAO than in TSO (**Table 1**). Thus, the synergism exhibited was 16−66% for the PE/Lys mixtures assayed in TSO and 60−138% for the mixtures assayed in TAO.

Antioxidative Activities of PC/Lys Mixtures in TSO and TAO As Determined by the Rancimat Method. When PE was substituted by PC, the effects observed in the oil stability were very similar to those described for PE/Lys mixtures. Thus, the addition of PC/Lys mixtures to both TSO and TAO increased significantly the stability of both oils (**Figure 5**). Therefore, the protective effect of PC/Lys mixtures was observed also in TSO that did not exhibit any change in stability when treated with PC or Lys individually.

Analogously to the observed in PE/Lys mixtures, the absolute IP increases (in hours) exhibited by PC/Lys mixtures were higher in TAO than in TSO, but the relative IP increases (percentage) were usually lower in TAO than in TSO (**Table 1**).

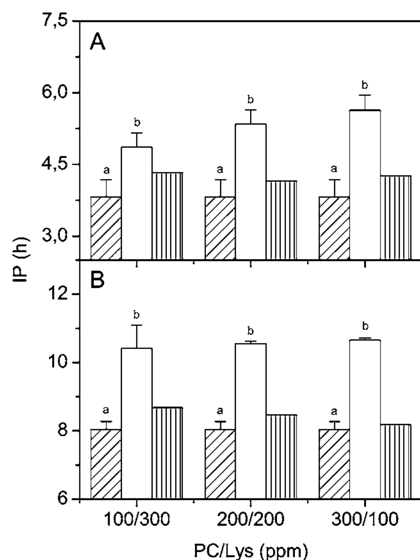

Figure 5. Rancimat IPs of (**A**) tocopherol-stripped olive oil (TSO) and (**B**) tocopherol-added olive oil (TAO), treated with nothing (control oil, slashed bars) or 100/300, 200/200, or 300/100 μg of PC/Lys mixtures/g of oil (open bars). The theoretical additive IP of the mixture has also been included for comparison (vertically striped bars). Experimental IP values are means ± SD for, at least, two experiments. Means with different letters are significantly different ($p < 0.05$).

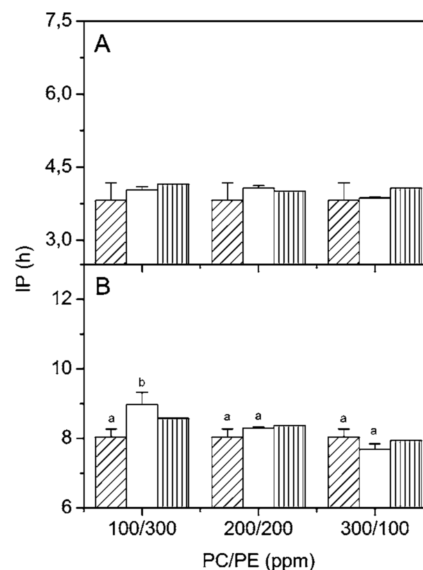

Figure 6. Rancimat IPs of (**A**) tocopherol-stripped olive oil (TSO) and (**B**) tocopherol-added olive oil (TAO), treated with nothing (control oil, slashed bars) or 100/300, 200/200, or 300/100 μg of PC/PE mixtures/g of oil (open bars). The theoretical additive IP of the mixture has also been included for comparison (vertically striped bars). Experimental IP values are means ± SD for, at least, two experiments. Means with different letters are significantly different ($p < 0.05$).

Thus, the addition of PC/Lys mixtures increased the IPs of TSO by 27−47% and the IPs of TAO by 30−33%.

PC/Lys mixtures also exhibited synergism, which was higher in TAO than in TSO (**Table 1**). Thus, the protection exhibited for each PC/Lys mixture was higher than the additive protection of the two components at the concentrations added. This synergism was 105−365% when tested in TSO and 271−1732% when tested in TAO.

Antioxidative Activities of PC/PE Mixtures in TSO and TAO As Determined by the Rancimat Method. Differently than the above-described PE/Lys and PC/Lys mixtures, the mixtures of PC and PE did not exhibit any synergism and the IP obtained was mostly the additive effect of the two phospholipids added individually (**Figure 6**). Thus, the treatment with PC/PE mixtures increased TSO IPs by 1−7% and TAO IPs from −4 to 12% (**Table 1**). Only the IP of TAO treated with 100 μg of PC/g of oil and 300 μg of PE/g of oil was significantly higher than the IP of the control TAO. These values were very close to the additive protection calculated considering the effect of each phospholipid individually. Therefore, the synergism was mostly null or negative (**Table 1**).

Determination of Carbonyl−Amine Reaction Products in the Oil Samples Oxidized in the Rancimat. When oils are heated under air in the Rancimat, a complex cascade of reactions is produced and some of the compounds formed play an essential role in the IPs obtained (*15*). Some of these reactions are given in **Figure 1**. The oxidation of oil triacylglycerols produces reactive carbonyls in addition to other compounds. When phospholipids are present, these compounds are also easily oxidized. The result of this oxidation is also the formation of new reactive carbonyls. All these carbonyls react with the amino compounds present in the oil to form pyrrole derivatives, among other carbonyl−amine reaction products. If this reaction takes place with the amino group of PE, the pyrroles produced are lipophilic, but if the reaction takes place with the amino group of Lys, the pyrroles produced are hydrophilic. Both types of pyrroles produced may be converted to volatile pyrroles after overnight heating in acid medium and in the presence of an aromatic amine (*22*). This reaction produces three main heterocyclic derivatives: 1-(4-methoxyphenyl)-1*H*-pyrrole (**3**), 1-(4-methoxyphenyl)-2-pentyl-1*H*-pyrrole (**4**), and 2-pentylfuran (**5**). Compounds **3**−**5** could be unambiguously identified by GC−MS in the oils treated with PE, Lys, and the different mixtures assayed at the end of the analysis in both TSO and TAO (data not shown), therefore confirming the formation of carbonyl−amine reaction products in both TSO and TAO, analogously to those observed in refined olive oils (*15*).

Antioxidative Activities of 6-Amino-2-(1*H*-pyrrol-1-yl)-hexanoic acid (1) and 2,3-Dipalmitoylpropyl 2-(1*H*-pyrrol-1-yl)ethyl phosphate (2) in TSO and TAO As Determined by the Rancimat Method. As an additional confirmation that the produced carbonyl−amine reaction products were effectively contributing to the antioxidative activity of the oils, two derivatives (one from lysine and the other from phosphatidylethanolamine) were synthesized and their antioxidative activities were tested under the same conditions employed in the study of antioxidative activities of phospholipids, Lys, and their mixtures.

When the TSO was treated with 0−400 μg of compound **1** or **2**/g of oil, the IP of the oil did not change significantly (**Figure 7A**). However, when these compounds were tested in TAO, compound **1** protected the oil when added at 200−400 μg/g of oil, and this protection was similar to that exhibited by BHT (**Figure 7B**). In addition, the addition of compound **2** to TAO also seemed to increase the IP of the oil as a function of the amount of compound added. However, this increase was not significant.

DISCUSSION

Carbonyl−amine reaction products are produced as a last step in the lipid oxidation process when lipid oxidation takes place in the presence of amino compounds (*25*). These products are formed by different reactions and they contribute to produce both positive and negative changes in food quality. Among positive consequences, the formation of compounds with

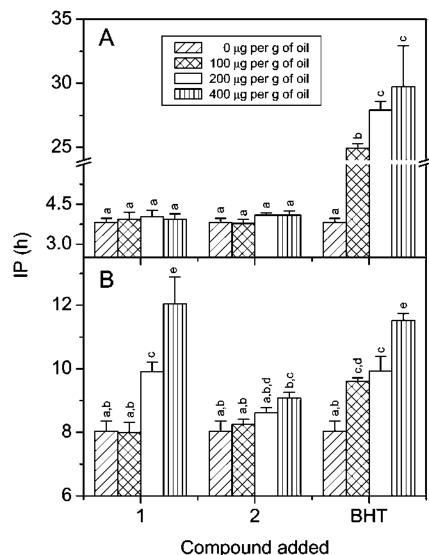

Figure 7. Rancimat IPs of (**A**) tocopherol-stripped olive oil (TSO) and (**B**) tocopherol-added olive oil (TAO), treated with 0 (control oil, slashed bars), 100 (crosshatched bars), 200 (open bars), or 400 μg (vertically striped bars) of 6-amino-2-(1*H*-pyrrol-1-yl)hexanoic acid (**1**), 2,3-dipalmitoylpropyl 2-(1*H*-pyrrol-1-yl)ethyl phosphate (**2**), or BHT/g of oil. Values are means ± SD for, at least, two experiments. Means with different letters are significantly different ($p < 0.05$). Structures for compounds **1** and **2** are given in **Figure 1**.

antioxidant properties has been described (*26*, *27*). However, most of these studies were carried out with isolated compounds and carbonyl−amine reaction products were not usually tested as a whole. The recent introduction of Rancimat to study the antioxidative activity of these compounds has allowed us to study both their formation in situ and their contribution to the oxidative stability of the tested oil at the same time that they were produced (*15*).

Analogously to the results observed in refined olive oils (*15*), the addition of either PE or Lys to both TSO and TAO always produced the formation of the corresponding carbonyl−amine reaction products, either in the polar head of the phospholipid or in one of the amino groups of lysine. However, the protection of the oil was not always observed when carbonyl−amine reaction products were produced.

Neither PE nor PC nor Lys protected TSO when added at 100−400 μg/g of oil. However, the stability of this oil increased when PE/Lys or PC/Lys mixtures, but not PC/PE mixtures, were added. On the other hand, both PE and Lys, and PE/Lys and PC/Lys mixtures, but not PC/PE mixtures, protected TAO. These results suggested a major contribution of tocopherols in the antioxidative activity of the carbonyl−amine products formed. Differently than the addition of PE, Lys, and their mixtures to TAO, only the addition of PE/Lys or PC/Lys mixtures increased the stability of TSO. This may be a consequence of the formation in these mixtures of Lys-based antioxidants that should be mostly hydrophilic and, therefore, with a high activity in the bulk oil assayed (*28*). The formation of phospholipid-based antioxidants, which were produced with the addition of PE or PC/PE mixtures, did not exhibit any protective effect. In addition, the addition of Lys to TSO did not protect the oil, most likely because, in the absence of the phospholipids, the formation of carbonyl−amino products takes place later than in the presence of highly unsaturated phospholipids (*15*).

A confirmation of these conclusions was obtained when single carbonyl−amine products were isolated and tested under the same conditions. Thus, the hydrophilic compound **1** protected the TAO and exhibited a higher antioxidative activity than the analogous lipophilic pyrrole **2** derived from PE. On the other hand, they did not protect TSO. Therefore, the protection exhibited by PE/Lys and PC/Lys in TSO should be due to other Lys-based carbonyl−amine products different than compound **1**.

All these results suggest that antioxidative activity of carbonyl−amine reaction products may be greatly increased with the addition of tocopherols, and the carbonyl−amine reaction products derived from Lys are more antioxidant in bulk oils than those derived from PE.

ABBREVIATIONS

BHT, butylated hydroxytoluene; IP, induction period; Lys, lysine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TAO, tocopherol-added olive oil; TSO, tocopherol-stripped olive oil.

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