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Contribution of Phospholipid Pyrrolization to the Color Reversion Produced during Deodorization of Poorly Degummed Vegetable Oils

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The Ehrlich reaction was optimized to determine the formation of pyrrolized phospholipids in edible oils in an attempt to understand the color reversion produced during the deodorization of poorly degummed edible oils. The procedure consisted of the treatment of the oil with *p*-(dimethylamino)-benzaldehyde in tetrahydrofuran/2-propanol at a controlled acidity and temperature and the spectrophotometric determination of adducts produced. The extinction coefficient of Ehrlich adducts was calculated by using 1-[1-(2-hydroxyethyl)-1*H*-pyrrol-2-yl]propan-1-ol (**1**) as a standard and was 15 300 M^{-1} cm^{-1}. The response was linear and reproducible within the range of 0.334−48.6 μM of compound **1**. When the assay was applied to a soybean oil treated with 100−1000 ppm of phosphatidylethanolamine and submitted to deodorization, the formation of pyrrolized phospholipids was observed at the same time that the disappearance of the phospholipid and the oil darkening were produced. The main changes were observed during the first steps of the deodorization process, when the oil was heated between 80 and 160 °C. During the initial heating of the oil until achieving 200 °C, oil darkening, phosphatidylethanolamine disappearance, and pyrrolized phospholipid formation were correlated, therefore suggesting a contribution of phospholipid pyrrolization to the oil darkening produced.

KEYWORDS: Carbonyl-amine reactions; nonenzymatic browning; oil degumming; oil deodorization; oil refining; phospholipid analysis; phospholipid modification; pyrrole derivatives; pyrrole polymerization

INTRODUCTION

The stability and final quality of edible vegetable oils and their byproducts are determined by the residual presence of certain minor compounds such as phospholipids, which provide good information about the proper oil processing and storage conditions (*1*−*3*). During the degumming process, phospholipids are eliminated by thermal treatment with water (hydratable phospholipids) and other degumming agents such as phosphoric acid, citric acid, or acid mixtures (nonhydratable phospholipids). The presence of these nonhydratable phospholipids in the oil may reduce the efficiency of degumming and cause neutral oil loss. In addition, oil residual phospholipids will contribute to off-flavors and colors in the refined oil (*4*). Although these consequences are usually avoided by proper oil processing, the mechanisms by which they are produced are not clearly understood at present.

The role of phospholipids, or other lipids, in nonenzymatic browning reactions is a consequence of their ability to be oxidized (*5*, *6*). Although lipid oxidation products may polymerize to produce brown-colored oxypolymers (*7*, *8*), in the presence of other compounds (e.g., proteins, antioxidants, etc.), oxidative reactions are usually finished by reactions with compounds other than those originating from oxidation of the lipid substrate, and this can influence reaction rates and produce significant consequences in the color, flavor, and texture of foods. Particularly, the reaction of lipid oxidation products with amino groups of amines, amino acids, and proteins has long been related to the browning observed in many fatty foods during processing and storage (*9*−*11*), and these reactions are also produced in amino phospholipids by reaction of lipid oxidation products with the primary amino group of phosphatidylethanolamine or phosphatidylserine. A recent study has found that the nonenzymatic browning produced during phospholipid oxidation is, at least partially, a consequence of amino-carbonyl reactions that produce hydroxyalkylpyrroles (**I**), among other pyrroles, in a first step. The later polymerization of these intermediates is responsible for the browning and fluorescence produced in these reactions (*12*). These reactions are summarized in **Scheme 1**.

Oil deodorization decomposes peroxides and some color pigments, removes the constituents responsible for off-odors and -flavors, and reduces the free fatty acid down to 0.02−0.03% (*13*). When phospholipids are present, lipid oxidation products

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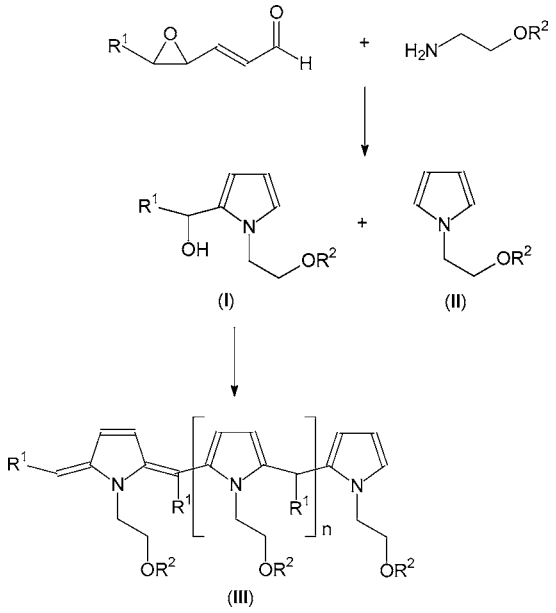
Scheme 1. Formation of Pyrrolized Phospholipids by Reaction of Amino Phospholipids with 4,5(*E*)-Epoxy-2(*E*)-heptenal[a]



[a] R^1 is an alkyl group, and R^2 is the phospholipid without the alcohol moiety. 1-[1-(2-Hydroxyethyl)-1*H*-pyrrol-2-yl]propan-1-ol (**1**) is **I** with R^1 = ethyl, R^2 = H.

formed during peroxide decomposition might react with a phospholipid amino group and contribute to the oil darkening observed. Because phospholipid darkening is nowadays believed to be a consequence, at least partially, of pyrrole formation and polymerization, the first objective of this study was to develop a procedure to quantitatively determine pyrrolized phospholipids in edible oils so that the formation of these derivatives may be determined along with the deodorization process.

Pyrroles have been traditionally determined in a qualitative or semiquantitative extent by the Ehrlich reagent, a procedure that consists of their treatment with *p*-(dimethylamino)benzaldehyde under acidic conditions. This procedure was optimized to quantitatively determine pyrrolized proteins produced as a consequence of lipid peroxidation and oxidative stress (*14*). However, this procedure is not applicable as such to pyrrolized phospholipids because of the differences in the solubilities of both phospholipids and proteins. The present study has reinvestigated the Ehrlich reaction to quantitatively determine the presence of pyrrolized phospholipids in edible oils. In addition, the developed procedure has been applied to the study of the deodorization of poorly degummed vegetable oils.

EXPERIMENTAL PROCEDURES

Materials. 1-[1-(2-Hydroxyethyl)-1*H*-pyrrol-2-yl]propan-1-ol (**1**) was prepared as described previously (*12*). Soybean phosphatidylethanolamine was isolated from soybean lecithin by column chromatography on silicic acid/Celite (2:1) using chloroform/methanol (3:1) as the eluent (*12*). Refined olive and soybean oils were obtained from the Institute's Pilot Plant (Instituto de la Grasa, CSIC, Seville, Spain) and further deodorized in our laboratory under vacuum (1 mm) at 250 °C for 4 h using a laboratory-scale apparatus described previously by Dobarganes et al. (*15*). A solution of 0.1 N HCl in 2-propanol was obtained from Merck KGaA (Darmstadt, Germany). Other reagents and solvents were of analytical grade and were purchased from reliable commercial sources.

Preparation of the Ehrlich Reagent. The reagent was prepared by dissolving 200 mg of *p*-(dimethylamino)benzaldehyde in 10 mL of 0.1 N HCl in 2-propanol. The resulting solution was stable at 4 °C for at least 1 month.

Compound 1 Determination with *p*-(Dimethylamino)benzaldehyde. Compound **1** was added to a solution of refined soybean or olive oil (15 g) in tetrahydrofuran (total volume 25 mL). This solution (370 μL) was introduced into a microreaction vessel and treated with 700 μL of 0.1 N HCl in 2-propanol and 170 μL of the Ehrlich reagent (the final concentration of compound **1** was 0.334−48.6 μM), the cap was rapidly replaced, and the solution was incubated at 60 °C for 30 min. The absorbance of the maximum at ∼512 nm was measured in the next 6 h.

Determination of Pyrrolized Phospholipids during Deodorization of Poorly Degummed Oils. Phosphatidylethanolamine was added to refined soybean oil at two concentrations (100 and 1000 ppm, which corresponded to 4.2 and 41.9 ppm of phosphorus, respectively) and deodorized under vacuum (1 mm) at 250 °C for 4 h. At different intervals of time, samples were withdrawn and analyzed for color, phosphatidylethanolamine, and pyrrole contents.

The color was determined spectrophotometrically employing a Shimadzu UV-2401 PC UV−vis spectrophotometer. Color differences (ΔE) between control (untreated oil) and deodorized oils at the different time periods were calculated from the determined CIELAB L^*, a^*, and b^* values according to Hunter (*16*):

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

The yellowness index (YI) was determined according to Francis and Clydesdale (*17*):

$$\mathrm{YI} = 142.86 b^*/L^*$$

Phosphatidylethanolamine was determined by high-performance liquid chromatography (HPLC) after solid phase extraction (SPE) of the phospholipids. The SPE methodology was described by Carelli et al. (*18*) and was slightly modified. The diol phase cartridge was conditioned prior to extraction with 2 mL of methanol, 2 mL of chloroform, and 4 mL of hexane. The oil (1 g), containing 0.5 mg of soybean phosphatidylcholine, which was added as an internal standard, was dissolved in 230 μL of chloroform and introduced in the column. A large percentage of the triacylglycerols was released from the sorbent bed by passing 2.5 mL of chloroform through it. The phospholipids were recovered by elution with 7 mL of methanol that contained 0.5 mL/100 mL of a 25% ammonia solution. The eluate was collected into a conical vial, evaporated to dryness under nitrogen, and dissolved in 200 μL of chloroform−methanol (2:1). This phospholipid fraction was analyzed by HPLC.

The HPLC system consisted of a 126 Programmable Delivery Module and a 168 Diode Array Detector Module (Beckman). Data acquisition and processing were effected with Software System Gold 6.0 version (Beckman). The separations were carried out on a LiChrospher 100 NH_2 (5 μm) 25 cm × 0.4 cm column using acetonitrile−methanol−water−acetic acid−ammonia (630:350:15:0.3:1.3) as the eluent. The flow rate was 1 mL/min, and the phospholipids were detected at 205 nm.

The pyrrolized phospholipids were determined directly by using up to 600 mg of the oil, which was diluted with tetrahydrofuran (maximum total volume 1 mL). This solution was treated with 700 μL of 0.1 N HCl in 2-propanol and 170 μL of the Ehrlich reagent and rapidly incubated at 60 °C for 30 min. The absorbance of the maximum at ∼512 nm was measured.

Statistical Analysis. All determinations are expressed as mean values ± standard deviations (SD) of 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 Student−Newman−Keuls test (*19*). All statistical procedures were carried out using Primer of Biostatistics: The Program (McGraw-Hill, Inc., New York). The significance level is $p < 0.05$ unless otherwise indicated.

RESULTS

Reaction of 1-[1-(2-Hydroxyethyl)-1*H*-pyrrol-2-yl]propan-1-ol (1) with *p*-(Dimethylamino)benzaldehyde. Although the

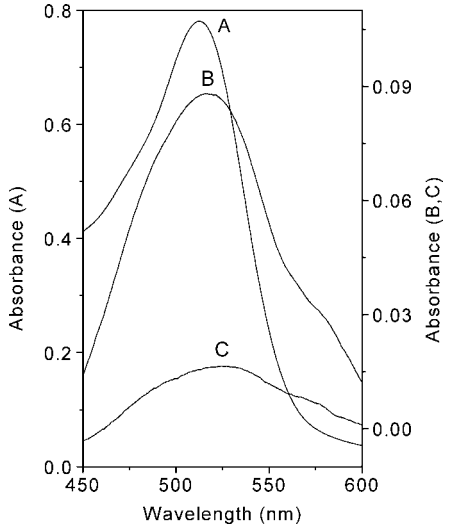


Figure 1. Absorbance spectra of Ehrlich adducts of (**A**) 1-[1-(2-hydroxyethyl)-1*H*-pyrrol-2-yl]propan-1-ol (**1**) in soybean oil, (**B**) a soybean oil treated with 1000 ppm of phosphatidylethanolamine and heated under vacuum at 160 °C, and (**C**) a soybean oil treated with 100 ppm of phosphatidylethanolamine and heated under vacuum at 160 °C.

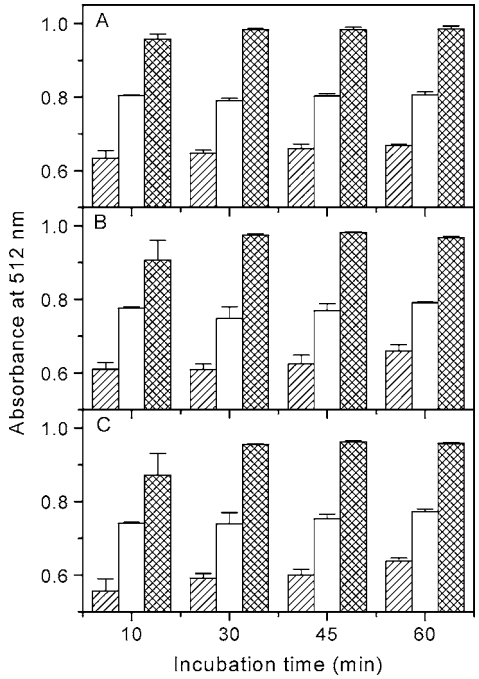


Figure 2. Effect of the temperature and the incubation time on the absorbance of the adduct produced with 1-[1-(2-hydroxyethyl)-1*H*-pyrrol-2-yl]propan-1-ol (**1**) in 0.07 M HCl. Temperatures tested as follows: 30 (stripped bars), 45 (open bars), and 60 (crosshatched bars) °C. (**A**) Samples measured after incubation, (**B**) incubated samples measured after 2 h at room temperature, and (**C**) incubated samples measured after 6 h at room temperature. Values are means ± SD for three experiments.

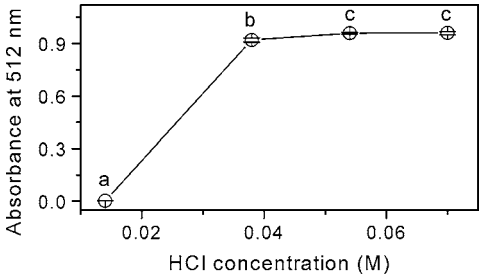


Figure 3. Effect of HCl concentration on the absorbance of the adduct produced with 1-[1-(2-hydroxyethyl)-1*H*-pyrrol-2-yl]propan-1-ol (**1**). The samples were incubated during 30 min at 60 °C. Values are means ± SD for three experiments. Means with different letters are significantly different ($p < 0.05$).

presence of a high amount of vegetable oil considerably complicated the quantification of the present pyrroles, it was possible to directly determine pyrrolized phospholipids in a quantitative way by employing the appropriate solvents and incubation conditions. Compound **1** was employed as a standard because it is the major product of the reaction between the ethanolamine and the lipid peroxidation product 4,5(*E*)-epoxy-2(*E*)-heptenal (*12*). Analogously to other pyrrole derivatives, compound **1** reacted with *p*-(dimethylamino)benzaldehyde under acidic conditions to produce a colored derivative (**Figure 1A**). A mixture of tetrahydrofuran and 2-propanol resulted in the most convenient solvent for obtaining homogeneous solutions with stable colors.

The wavelength of the obtained maximum as well as the stability of the color were dependent on the temperature and the pH. **Figure 2A** shows the absorbance at the maximum at ~512 nm obtained for the adduct of compound **1** with *p*-(dimethylamino)benzaldehyde in the presence of soybean oil at three temperatures (30, 45, and 60 °C) and as a function of the incubation time. As observed in the figure, an increase in the incubation temperature increased the intensity of the produced color. The maximum assayed temperature was 60 °C to avoid losses of the solvent. In addition, the intensity of the obtained colors increased with the incubation time until a stable color was obtained. Thus, the most intense colors were obtained after 60 min at 30 °C, 45 min at 45 °C, and 30 min at 60 °C.

Figure 2B,**C** shows the absorbances determined in the incubated samples after maintaining them for 2 and 6 h, respectively, at room temperature. The stability of the colors also depended on the incubation temperature. Thus, the intensity of the absorption maximum of the adduct formed at 30 °C for less than 60 min decreased about 12% after 6 h; the color of the adduct formed at 45 °C decreased about 5% after 6 h; and the intensity of the color produced at 60 °C after incubation for more than 10 min remained unchanged after 2 h and decreased only 2% after 6 h. Therefore, the most intense and stable colors were obtained by incubating the samples at 60 °C for at least 30 min.

The pH of the incubation media was also very important to obtain intense and stable colors. **Figure 3** shows the effect of pH on the intensity of the absorption maximum. A HCl concentration of 0.054−0.07 mM produced the highest intense colors. These colors decreased slightly in the range of 0.038−0.054 mM (the intensity of the color obtained using 0.038 mM HCl was 4% lower than that obtained with 0.07 mM) and almost disappeared at lower HCl concentrations. Therefore, the incubation should be done at a HCl concentration near to 0.07 mM, although it could be lower if a high amount of oil has to be introduced in the assay. Thus, by using 600 mg of the oil diluted to 1 mL with tetrahydrofuran and adding 700 μL of 0.1 N HCl in 2-propanol and 170 μL of the Ehrlich reagent, the HCl concentration employed is 0.047 mM. By using these reaction conditions, the intensity of the color of the obtained adducts was only 2% lower than those obtained when employing 0.07 mM HCl. A higher amount of oil would decrease the HCl concentration too much in the assay and should be avoided.

Assay Validation. The calibration curve of compound **1** was obtained from 12 independent experiments carried out on four

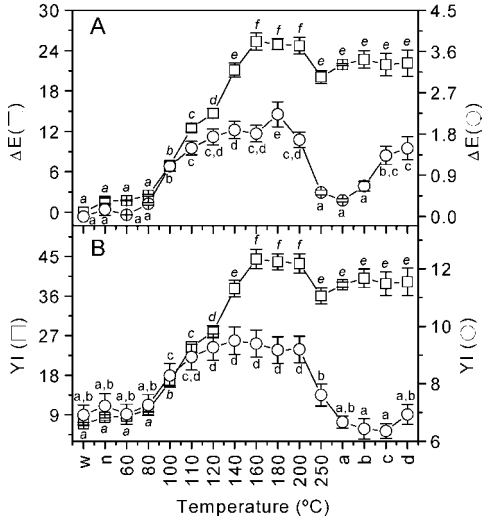


Figure 4. Color changes produced in a soybean oil with 100 (○) and 1000 (□) ppm of phosphatidylethanolamine added as a function of the deodorization temperature. The oil was heated under vacuum until achieving 250 °C and then heated for (a) 1, (b) 2, (c) 3, and (d) 4 h at 250 °C. The color of a control oil with no phosphatidylethanolamine added is marked as w. The nonheated oil with the phospholipid added is marked as n. Both (**A**) color difference (Δ*E*) with the control oil without phospholipid added and (**B**) YI were determined. Values are means ± SD for three experiments. Means with different letters are significantly different ($p < 0.05$). Letters in italics correspond to the deodorization with 1000 ppm of the phospholipid.

different days. The absorbance at the maximum at ~512 nm was directly proportional to the concentration of compound **1** ($r \geq 0.999$, $p < 0.0001$) in the range of 0.334−48.6 μM, and the extinction coefficient obtained for the adduct of compound **1** was 15 300 M^{-1} cm^{-1}. The coefficients of variation (cv) obtained within this range were lower than 12%. Although compound **1** could be detected at lower concentrations than 0.334 μM, the variability in absorbance was considered unacceptable (cv > 15%), and a concentration of 0.3 μM was considered the practical lower limit of quantification.

Determination of Pyrrolized Phospholipids during Deodorization of Poorly Degummed Oils. When a soybean oil containing phosphatidylethanolamine was submitted to deodorization, the oil became darker at the same time that the disappearance of phosphatidylethanolamine and the appearance of pyrrolized phospholipids were observed. **Figure 4A** shows the Δ*E* observed in the oil during the deodorization at the two concentrations of phosphatidylethanolamine assayed (100 and 1000 ppm). The color remained unchanged if the oil was heated under vacuum at a temperature below 80 °C. However, when the temperature was increased over 80 °C, Δ*E* increased as a function of the temperature until achieving the maximum at about 140−200 °C, when phosphatidylethanolamine was added at 100 ppm, and 160−200 °C when phosphatidylethanolamine was added at 1000 ppm. A further increase in the deodorization temperature or time did not produce a higher Δ*E*. On the contrary, Δ*E* values decreased afterward and only a slight increase was observed at the end of the deodorization process.

These color differences were a consequence of the browning of the oil, which could be easily followed by determining its YI. **Figure 4B** shows the YI of the oil during the deodorization process for the oil treated with 100 and 1000 ppm of phosphatidylethanolamine. The YI followed a behavior very similar to Δ*E*; in fact, both determinations were correlated ($r = 0.75$, $p = 0.00077$ and $r = 0.9998$, $p < 0.0001$, for 100 and 1000 ppm of phosphatidylethanolamine, respectively), and these correlations were higher ($r = 0.98$, $p < 0.0001$ and $r = 0.9998$, $p < 0.0001$, for 100 and 1000 ppm of phosphatidylethanolamine, respectively) if only the values determined during the heating until 200 °C were considered.

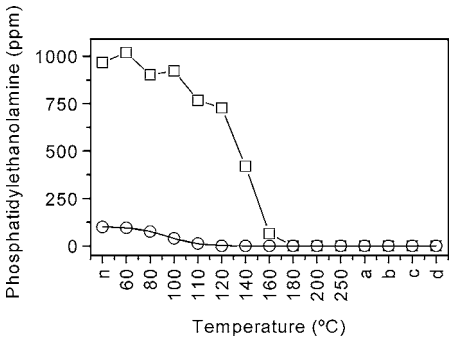


Figure 5. Phosphatidylethanolamine content in a soybean oil with 100 (○) and 1000 (□) ppm of phosphatidylethanolamine added as a function of the deodorization temperature. The oil was heated under vacuum until achieving 250 °C and then heated for (a) 1, (b) 2, (c) 3, and (d) 4 h at 250 °C. The nonheated oil with the phospholipid added is marked as n.

Both Δ*E* and YI data indicated that the main color changes were produced at the beginning of the deodorization process when the oil was heated under vacuum until achieving its deodorization temperature. Present peroxides are likely to be decomposed in this step; therefore, their decomposition products might react with the free amino groups of phospholipids to produce the brown pigments. A further heating of the oils usually produced clearer oils, which could become darker after several hours of deodorization.

Oil darkening was parallel to the disappearance of the added phosphatidylethanolamine. Phosphatidylethanolamine was converted during deodorization into other derivatives, which were detected by HPLC but not yet identified (data not shown). **Figure 5** shows the phosphatidylethanolamine content in the oil along with the deodorization process at the two concentrations assayed: 100 and 1000 ppm. The phosphatidylethanolamine content remained unchanged at a temperature lower than 80−100 °C and then decreased rapidly until disappearing at about 160 °C. This disappearance was correlated with the color changes produced during the heating of the oil until 200 °C ($r = -0.98$, $p < 0.0001$ and $r = -0.99$, $p < 0.0001$, for Δ*E* and YI, respectively, in an oil treated with 100 ppm of phosphatidylethanolamine, and $r = -0.95$, $p < 0.0001$ and $r = -0.95$, $p < 0.0001$, for Δ*E* and YI, respectively, in an oil treated with 1000 ppm of phosphatidylethanolamine).

Both oil darkening and phosphatidylethanolamine disappearance were also parallel to the appearance of pyrroles in the oil. **Figure 1B**,**C** shows the spectra of the Ehrlich adducts produced in an oil treated with 1000 and 100 ppm, respectively, of phosphatidylethanolamine and heated at 160 °C under vacuum. These spectra were analogous to the spectrum of the adduct of compound **1** (**Figure 1A**). **Figure 6** shows the temperature−course of pyrrole formation in the oil. Analogously to oil darkening and phosphatidylethanolamine disappearance, pyrrole formation also occurred during the first steps of the heating process of the oil, and there was a correlation between pyrrolization, oil darkening and phosphatidylethanolamine content during the heating of the oil until 200 °C ($r = 0.67$, $p = 0.032$; $r = 0.66$, $p = 0.038$; and $r = -0.66$, $p = 0.054$, for Δ*E*, YI, and phosphatidylethanolamine content, respectively, in an oil treated with 100 ppm of phosphatidylethanolamine, and $r = 0.93$, $p = 0.00018$; $r = 0.93$, $p = 0.00013$; and $r = -0.97$,

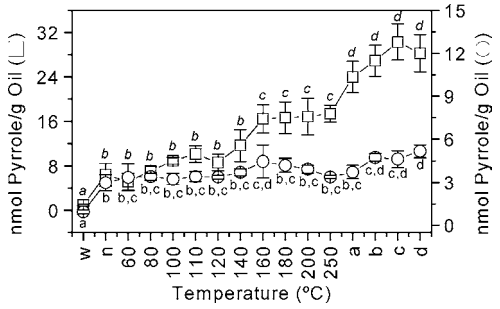


Figure 6. Pyrrolized phospholipid content in a soybean oil with 100 (○) and 1000 (□) ppm of phosphatidylethanolamine added as a function of the deodorization temperature. The oil was heated under vacuum until achieving 250 °C and then heated for (a) 1, (b) 2, (c) 3, and (d) 4 h at 250 °C. The pyrrolized phospholipid content of a control oil with no phosphatidylethanolamine added is marked as w. The nonheated oil with the phospholipid added is marked as n. Values are means ± SD for three experiments. Means with different letters are significantly different ($p < 0.05$). Letters in italics correspond to the deodorization with 1000 ppm of the phospholipid.

$p < 0.0001$, for ΔE, YI, and phosphatidylethanolamine content, respectively, in an oil treated with 1000 ppm of phosphatidylethanolamine). A further heating of the oil increased the pyrrole content determined, but this did not produce an increase in the oil color.

DISCUSSION

The importance of degumming for an appropriate oil refining has long been known (*3*). However, the mechanisms by which phospholipids are implicated in oil darkening are still poorly understood. The above results point out to nonenzymatic browning reactions as responsible, at least partially, for this oil darkening.

When a poorly degummed edible oil was heated under vacuum, the disappearance of amino phospholipids was rapidly observed at the same time that the browning of the oil was produced. Both oil darkening and phosphatidylethanolamine disappearance occurred when the oil was heated between 80 and 160 °C, and both were parallel to the pyrrolization of the phospholipids present in the oil. In fact, these three determinations were correlated during the heating of the oil previous to its deodorization. Because nonenzymatic browning in oxidized lipid/protein reactions has been related to the formation and polymerization of pyrrole rings produced in the reaction of protein amino groups with oxidized lipids (*20*−*22*), the formation of analogous pyrroles with the phospholipid amino group should also contribute to the oil darkening.

Although there was a clear correlation among oil darkening, phosphatidylethanolamine disappearance, and pyrrole formation during the first heating of the oil, this correlation was not observed when the oil was further heated under vacuum at 250 °C for 4 h. This heating produced a decrease in oil darkening and an increase in pyrrole content (**Figures 4** and **6**, respectively). Both opposite effects might be a consequence of the nonenzymatic browning produced as a consequence of the pyrrole formation and polymerization mechanism, which is still poorly understood in the last steps. Although this mechanism has been shown to take place at high temperature (*23*), it is not known how temperature may affect the polymerization reaction in addition to produce its acceleration. Thus, the high temperature might convert some pyrrole polymers into others that were less colored but that produced adducts with *p*-(dimethylamino)-benzaldehyde having a higher extinction coefficient. In this context, dehydrogenations, which extend the conjugation and therefore play a role in the color, have been described during the polymerization process at room temperature (*24*), but it is not yet known if the temperature may modify these processes.

Additional studies are also needed to analyze the presence of pyrrolized phospholipids in crude oils. Pyrrolized phospholipids can be produced during the processing or storage of the seeds, and these compounds might affect the efficiency of the degumming process. The procedure described in the present paper may be useful in these studies.

LITERATURE CITED

(1) Pan, L. G.; Campana, A.; Tomás, M. C.; Añón, M. C. A kinetic study of phospholipid extraction by degumming process in sunflower seed oil. *J. Am. Oil Chem. Soc.* **2000**, *77*, 1273−1276.

(2) List, G. R.; Mounts, T. L.; Lanser, A. C. Factors promoting the formation of nonhydratable soybean phosphatides. *J. Am. Oil Chem. Soc.* **1992**, *69*, 443−446.

(3) Beal, R. E.; Lancaster, E. B.; Brekke, O. L. The phosphorus content of refined soybean oil as a criterion of quality. *J. Am. Oil Chem. Soc.* **1956**, *33*, 619−624.

(4) O'Brien, R. D. *Fats and Oils. Formulating and Processing for Applications*; Technomic Publishing Co., Inc.: Lancaster, Pennsylvania, 1998; p 594.

(5) Hidalgo, F. J.; Zamora, R. The role of lipids in nonenzymatic browning. *Grasas Aceites* **2000**, *51*, 35−49.

(6) Zamora, R.; Hidalgo, F. J. Coordinate contribution of lipid oxidation and Maillard reaction to the nonenzymatic food browning. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, in press.

(7) Venolia, A. W.; Tappel, A. L. Brown-colored oxypolymers of unsaturated fats. *J. Am. Oil Chem. Soc.* **1958**, *35*, 135−138.

(8) Buttkus, H. A. Fluorescent lipid autoxidation products. *J. Agric. Food Chem.* **1975**, *23*, 823−825.

(9) Stansby, M. E. Oxidative deterioration in fish and fishery products. I. Introduction. *Comp. Fish. Rev.* **1957**, *19*, 24−26.

(10) Pokorny, J.; Kolakowska, A. Lipid−protein and lipid-saccharide interactions. In *Chemical and Functional Properties of Food Lipids*; Sikorski, Z., Kolakowska, A., Eds.; CRC Press: Boca Raton, FL, 2002; pp 345−362.

(11) Zamora, R.; Alaiz, M.; Hidalgo, F. J. Contribution of pyrrole formation and polymerization to the nonenzymatic browning produced by amino-carbonyl reactions. *J. Agric. Food Chem.* **2000**, *48*, 3152−3158.

(12) Zamora, R.; Hidalgo, F. J. Phosphatidylethanolamine modification by oxidative stress product 4,5(*E*)-epoxy-2(*E*)-heptenal. *Chem. Res. Toxicol.* **2003**, *16*, 1632−1641.

(13) Carlson, K. F. Deodorization. In *Bailey's Industrial Oil & Fat Products*, 5th ed.; Huy, Y. H., Ed.; John Wiley & Sons: New York, 1996; Vol. 4, pp 339−390.

(14) Hidalgo, F. J.; Alaiz, M.; Zamora, R. A spectrophotometric method for the determination of proteins damaged by oxidized lipids. *Anal. Biochem.* **1998**, *262*, 129−136.

(15) Dobarganes, M. C.; Pérez-Camino, M. C.; Márquez-Ruiz, G.; Ruiz-Méndez, M. V. New analytical possibilities in quality evaluation of refined oils. In *Edible Fats and Oils Processing: Basic Principles and Modern Practices*; Eriksson, D. R., Ed.; American Oil Chemists' Society: Champaign, Illinois, 1990; pp 427−429.

(16) Hunter, R. S. *The Measurement of Appearance*; Hunter Associates Laboratory: Fairfax, VA, 1973.

(17) Francis, F. J.; Clydesdale, F. H. *Food Colorimetry: Theory and Applications*; AVI Publishing: Westport, CT, 1975.

(18) Carelli, A. A.; Brevedan, M. I. V.; Crapiste, G. H. Quantitative determination of phospholipids in sunflower oil. *J. Am. Oil Chem. Soc.* **1997**, *74*, 511−514.

(19) Snedecor, G. W.; Cochran, W. G. *Statistical Methods*, 7th ed.; Iowa State University Press: Ames, IA, 1980.

(20) Hidalgo, F. J.; Zamora, R. Fluorescent pyrrole products from carbonyl-amine reactions. *J. Biol. Chem.* **1993**, *268*, 16190−16197.
(21) Zamora, R.; Hidalgo, F. J. Comparative methyl linoleate and methyl linolenate oxidation in the presence of bovine serum albumin at several lipid/protein ratios. *J. Agric. Food Chem.* **2003**, *51*, 4661−4667.
(22) Hidalgo, F. J.; Nogales, F.; Zamora, R. Effect of the pyrrole polymerization mechanism on the antioxidative activity of nonenzymatic browning reactions. *J. Agric. Food Chem.* **2003**, *51*, 5703−5708.
(23) Hidalgo, F. J.; Zamora, R. Characterization of the products formed during microwave irradiation of the nonenzymatic browning lysine/(*E*)-4,5-epoxy-(*E*)-2-heptenal model system. *J. Agric. Food Chem.* **1995**, *43*, 1023−1028.
(24) Tressl, R.; Wondrak, G. T.; Krüger, R. P. New melanoidin-like Maillard polymers from 2-deoxypentoses. *J. Agric. Food Chem.* **1998**, *46*, 104−110.

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