Effect of pH and Temperature on Comparative Nonenzymatic Browning of Proteins Produced by Oxidized Lipids and Carbohydrates

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Bovine serum albumin (BSA) was incubated for 24 h in the presence of 10 mM ribose (RI), methyl linoleate hydroperoxides, or the secondary products of methyl linoleate oxidation (SP), at five temperatures (25, 37, 50, 80, and 120 °C) and different pHs (4, 7, and 10), to study the influence of these variables in the browning, fluorescence, amino acid losses, and pyrrolization of the modified proteins. All treated proteins exhibited similar colors and fluorescence spectra, and the spectra of their Ehrlich adducts were also analogous. However, at 25−50 °C the proteins treated with oxidized lipids exhibited higher color changes, amino acid losses, and pyrrolization than the BSA treated with RI, and these effects were much higher in proteins treated with RI at 80−120 °C. The effect of pH was similar in proteins treated with RI or SP. These results suggested a similarity for browned proteins obtained from both carbohydrates and oxidized lipids. In addition, both reactions seem to be complementary, because melanoidin formation derived from oxidized lipids can be produced under conditions different from those carbohydrate/protein reactions.

Keywords: *Oxidized lipid/protein reactions; Maillard reaction; nonenzymatic browning; fluorescence; protein damage; protein pyrrolization*

INTRODUCTION

The nonenzymatic browning of proteins is the consequence of a constellation of dendritic and chaotic processes, mediated by a range of carbohydrate, lipid, and amino acid reactants, in which small differences in metal ions, antioxidants, and reaction conditions may have profound effects on the chemistry of the reaction (Ledl and Schleicher, 1990; Baynes, 1996). These processes cause the deterioration of food during storage and processing, which has been attributed to destruction of essential amino acids, decrease in digestibility, inhibition of proteolytic and glycolytic enzymes, interaction with metal ions, and formation of antinutritional and toxic compounds (Friedman, 1996). On the other hand, beneficial consequences of these reactions have also been described, including production of antimutagens (Yen and Hsieh, 1994), antioxidants (Bedinghaus and Ockerman, 1995), antibiotics (Einarsson et al., 1983), and antiallergens (Oste at al., 1990).

Although nonenzymatic browning has been mostly related to carbohydrate/protein reactions, lipid oxidation products may also react with amino acid residues to produce brown pigments (Nawar, 1996), with analogous antioxidative properties (Alaiz et al., 1997a). But, because both carbohydrate/protein and oxidized lipid/protein processes are not favored under the same reaction conditions, both reactions might be produced to different extents under different conditions. The present investigation was undertaken to study the effect of pH and temperature on the color, fluorescence, and amino acid modification of proteins by both oxidized lipids and carbohydrates. To obtain comparable results, both types of modified proteins were produced by reaction of a carbohydrate or a lipid oxidation product with a protein under specific conditions, and, then, the modified protein was desalted, freeze-dried, and analyzed for color, fluorescence, and compositional changes. Bovine serum albumin (BSA) was selected as the model protein for a variety of reasons. It represents a major class of proteins, it is free of prosthetic groups and other complicating factors, and its primary, secondary, and tertiary structure has been quite well characterized in the literature (Peters, 1996). Ribose (RI) was selected as a model carbohydrate because pentoses have a high reactivity for the Maillard reaction (Namiki and Hagashi, 1983). Hydroperoxides (HP) and secondary products (SP) of methyl linoleate oxidation were used as model oxidized lipids because they are representative of the different lipid oxidation products that are produced in foods.

EXPERIMENTAL PROCEDURES

Materials. Chromatographically pure methyl linoleate was a gift from Prof. Eduardo Vioque. RI was purchased from Aldrich Chemical Co. (Miwaukee, WI). Essentially fatty acid free BSA and PD-10 columns packed with Sephadex G-25 medium were purchased from Sigma Chemicals Co. (St. Louis, MO). Other reagents and solvents used were analytical grade and were purchased from reliable commercial sources.

HP and SP were prepared and purified according to Frankel et al. (1989), as described previously (Alaiz et al., 1997b). The HP fraction consisted of a 1:1 mixture of methyl 9-hydroperoxy-10,12-octadecadienoate and methyl 13-hydroperoxy-9,11-octadecadienoate, which could be identified after reduction and silylation (Hidalgo and Zamora, 1995). The SP fraction was free of hydroperoxides and consisted of a very complex mixture of compounds. No attempts were taken to identify the compounds present in the mixture, although many short chain aldehydes could be easily detected. Nevertheless, it is impor-

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tant to point out the presence of 4,5-epoxy-2-decenal in the mixture, because epoxyalkenals are able to modify very rapidly protein residues producing brown pigments and fluorescence (Hidalgo and Zamora, 1993a). Specifically, the reaction of 4,5-epoxy-2-decenal with the ϵ-amino group of lysine converted this amino acid into ϵ-*N*-pyrrolylnorleucine (Zamora and Hidalgo, 1995).

Preparation of BSA Modified by Oxidized Lipids and Carbohydrates. BSA (2.5 mg) was dissolved in 2.5 mL of 0.3 M sodium phosphate buffer pH 7.4, sodium acetate pH 4.0, or sodium borate pH 10.0 and was untreated (control) or treated with 10 mM RI, HP, or SP (the average molecular weight used for SP was 200). The mixtures were incubated for 24 h at 25, 37, 50, 80, or 120 °C, and then, the solutions, which contained the control (COBSA) and the proteins modified by carbohydrates and oxidized lipids (RIBSA, HPBSA, and SPBSA), were desalted using PD-10 columns and freeze-dried. To confirm the reproducibility of the model, duplicates of the different modified proteins were prepared in different days. Analytical determinations were carried out in all the samples, and mean values are given in the Results section.

Analytical Measurements. Modified proteins were dissolved in deionized water, and protein concentration, measured according to Bradford's method using bovine serum albumin as standard (Bradford, 1976), was adjusted to 1 mg/mL for analytical determinations.

The color of the solutions was determined by using of the weighted-ordinate method (Hunter, 1973). Tristimulus values (*X*, *Y*, *Z*) were calculated from the transmittances (*T*) obtained in a Beckman spectrometer. Transmittances were recorded at constants intervals (10 nm) from 400 to 700 nm using 1 cm glass cells. These readings were then converted by means of a computer program into the corresponding tristimulus and CIELAB L*a*b* color values (CIE, 1978).

The difference of color (ΔE) between control and modified proteins was calculated, according to Hunter (1973), using the following equation:

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

Yellowness index (YI) was determined according to Francis and Clydesdale (1975):

$$YI = 142.86 b^*/L^* \quad (2)$$

Fluorescence spectra were recorded on a Perkin-Elmer LS-5 fluorescence spectrometer of 50 μL samples diluted with 3 mL of deionized water. A slit width of 5 nm was used, and the instrument was standardized with quinine sulfate (0.1 μM in 0.1 N H_2SO_4) to give a fluorescence intensity of 100 at 450 nm, when excitation was at 350 nm.

Amino acid analysis of protein samples was carried out after acid hydrolysis following a previously described procedure (Alaiz et al., 1992). Briefly, 100 μL of protein solutions was treated with 1 mL of 6 N HCl and incubated for 20 h at 110 °C. The resulting amino acids were, sequentially, taken to dryness, dissolved in 3 mL of 1 M sodium borate, pH 9, derivatized with diethyl (ethoxymethylene)malonate, and determined by reverse-phase high-performance liquid chromatography.

Formation of pyrrole amino acid residues in modified proteins was studied by determination of protein pyrrolization. Protein pyrrolization was analyzed by detection of pyrrolized proteins with *p*-(dimethylamino)benzaldehyde under acid conditions (Mattocks, 1968; Liddell et al., 1993) as described previously (Hidalgo et al., 1998). Briefly, protein solutions (400 μL) were diluted with 300 μL of 50 mM sodium phosphate, pH 7.4, and treated with 110 μL of a 2% solution of *p*-(dimethylamino)benzaldehyde in 3.5 N HCl/ethanol (4:1). The resulting solution was incubated at 45 °C for 30 min, and then, the protein was precipitated with 405 μL of 30% trichloroacetic acid at 0 °C for 1 h and centrifuged at 2250*g* for 15 min. Finally, the pellet was treated with 1 mL of 6 M guanidine HCl containing 20 mM potassium phosphate/trifluoroacetic acid, pH 2.3, for 30 min at 37 °C with vortexing, and the pyrrole content was determined by measuring the absorbance of the solution at the maximun produced at 575−585 nm. The results were calculated by using the extinction coefficient of the Ehrlich adduct of ϵ-*N*-pyrrolylnorleucine (35 000 M^{-1} cm^{-1}) (Hidalgo et al., 1998).

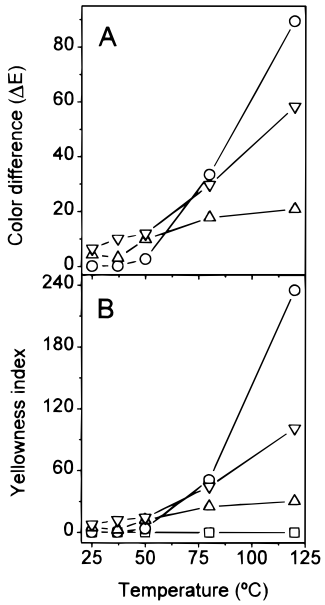

Figure 1. Effect of the temperature of incubation on ΔE (A) and YI (B) of COBSA (□), RIBSA (○), HPBSA (△), and SPBSA (▽). BSA was incubated at pH 7 for 24 h in the absence (control) or in the presence of 10 mM RI, HP, or SP.

RESULTS

Effect of Temperature on the Color and Fluorescence Changes Produced in BSA Incubated with Carbohydrates and Oxidized Lipids. The incubation of BSA with either a carbohydrate or an oxidized lipid increased its color and fluorescence. Figure 1A shows ΔE for RIBSA, HPBSA, and SPBSA as a function of the incubation temperature. ΔE increased either exponentially or linearly as a function of the temperature. Thus, ΔE increased exponentially for RIBSA ($r = 0.952$, $p = 0.013$), linearly for HPBSA ($r = 0.953$, $p = 0.012$), and exponentially for SPBSA ($r = 0.992$, $p = 0.00086$).

Kinetics analogous to those observed for ΔE were also observed for YI (Figure 1B). No changes were observed in the YI of COBSA as a function of the temperature. However, YI increased exponentially for RIBSA ($r = 0.972$, $p = 0.005$), linearly for HPBSA (0.957, $p = 0.011$), and exponentially for SPBSA ($r = 0.993$, $p = 0.00074$).

Changes in fluorescence were not correlated with color changes and were different for each one of the tested proteins. However, the spectra obtained for the three treated proteins were similar among them. Figure 2 shows the fluorescence spectra obtained for the four proteins (COBSA, RIBSA, HPBSA, and SPBSA) prepared at 50 °C and pH 7.

The fluorescence intensity exhibited by COBSA, RIBSA, HPBSA, and SPBSA as a function of the incubation temperature is shown in Figure 3. COBSA only exhibited small changes in fluorescence intensity as a function of incubation temperature. However, fluorescence of RIBSA increased as a function of the temperature in the 25−80 °C range and decreased afterward. HPBSA did not exhibit a clear dependence on the temperature, and a high fluorescence was always

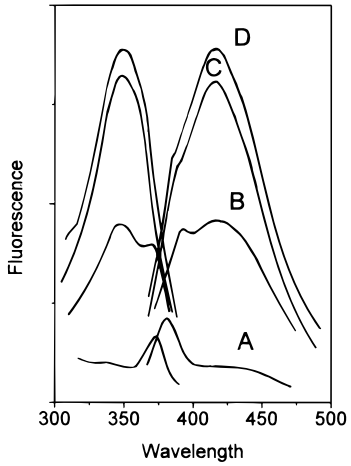

Figure 2. Fluorescence spectra of COBSA (A), RIBSA (B), HPBSA (C), and SPBSA (D). BSA was incubated at pH 7 and 50 °C for 24 h in the absence (control) or in the presence of 10 mM RI, HP or SP.

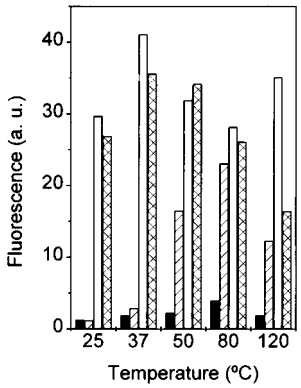

Figure 3. Effect of the temperature of incubation on the fluorescence of COBSA (solid bars), RIBSA (striped bars), HPBSA (open bars), and SPBSA (crosshatched bars). BSA was incubated at pH 7 for 24 h in the absence (control) or in the presence of 10 mM RI, HP, or SP.

Table 1. Effect of Temperature on Lysine Recovered after Acid Hydrolysis in BSA Incubated with Carbohydrates and Oxidized Lipids at pH 7

	μmol/g of protein			
temp (°C)	COBSA	RIBSA	HPBSA	SPBSA
25	897	887	865	872
37	896	836	762	782
50	893	728	667	667
80	882	392	556	498
120	887	219	515	324

observed for the five assayed temperatures. SPBSA exhibited a behavior that was similar to the behavior observed for RIBSA, but the maximum of fluorescence was attained at a lower temperature. Thus, fluorescence for SPBSA increased in the range 25−37 °C and decreased afterward.

Effect of Temperature on Amino Acid Losses Produced in BSA Incubated with Carbohydrates and Oxidized Lipids. The incubation of BSA with either carbohydrates or oxidized lipids produced amino acid losses in the amino acids recovered after acid hydrolysis. According to the expected (Alaiz et al., 1997b), lysine and arginine were the amino acids which were mainly lost. Table 1 collects the data obtained for the lysine recovered in COBSA, RIBSA, HPBSA, and SPBSA after acid hydrolysis as a function of the incubation temperature. The incubation of BSA in the range 25−120 °C did not produced any losses in the lysine recovered after acid hydrolysis. However, when the BSA was incubated with either carbohydrates or oxidized lipids, high losses were produced in this amino acid, which were a function of the incubation temperature. RIBSA only decreased 1% at 25 °C, and these losses increased by 7% at 37 °C. However, temperatures higher than 37 °C produced very high losses in the lysine recovered. Thus, a decrease of 18% was observed at 50 °C, and these losses increased at 56% at 80 °C and at 75% at 120 °C. Differently from RIBSA, both HPBSA and SPBSA exhibited higher losses than RIBSA at lower temperatures, but these losses increased with temperature much more slowly than the observed for RIBSA. Thus, HPBSA lost the 4% of lysine residues at 25 °C, and these losses increased at 15, 25, 37, and 42% at 37, 50, 80, and 120 °C, respectively. Analogous behavior was observed for lysine recovered in SPBSA after acid hydrolysis. Thus, lysine losses were 3, 13, 25, 44, and 63% at 25, 37, 50, 80, and 120 °C, respectively. Lysine recovered after acid hydrolysis was correlated exponentially with the temperature for RIBSA ($r = -0.992$, $p = 0.0008$), HPBSA ($r = -0.951$, $p = 0.013$), and SPBSA ($r = -0.9997$, $p < 0.0001$).

Table 2. Effect of Temperature on Arginine Recovered after Acid Hydrolysis in BSA Incubated with Carbohydrates and Oxidized Lipids at pH 7

	μmol/g of protein			
temp (°C)	COBSA	RIBSA	HPBSA	SPBSA
25	341	337	326	322
37	338	317	322	314
50	335	249	298	290
80	336	185	268	240
120	336	78	248	136

Losses analogous to those obtained for lysine residues were also obtained for arginine residues after acid hydrolysis. These data have been collected in Table 2. The incubation of COBSA at 25−120 °C did not produce any changes in the arginine recovered. However, the incubation with RI decreased arginine content in the protein by 1, 7, 26, 45, and 77% when incubated at 25, 37, 50, 80, and 120 °C, respectively. Arginine decreased in HPBSA by 4, 5, 11, 20, and 26%, respectively, at the five assayed temperatures. Higher losses than those observed for HPBSA, but lower than those observed for RIBSA, were observed for SPBSA. Thus, arginine recovered after acid hydrolysis decreased in SPBSA by 6, 7, 13, 29, and 60%, respectively, at the five assayed temperatures. Arginine recovered after acid hydrolysis correlated linearly with the temperature for RIBSA ($r = -0.994$, $p = 0.00057$), HPBSA ($r = -0.978$, $p = 0.0039$), and SPBSA ($r = -0.989$, $p = 0.00136$).

Effect of Temperature on Protein Pyrrolization Produced in BSA Incubated with Carbohydrates and Oxidized Lipids. Parallel to lysine losses, the transformation of some lysine residues into pyrrole amino acids is also produced by both carbohydrates (Ledl and Schleicher, 1990) and oxidized lipids (Hidalgo and Zamora, 1993b). Therefore, protein pyrrolization was produced when the BSA was incubated in the presence of RI, HP, or SP. Protein pyrrolization was determined by the reaction with the Ehrlich reagent (Hidalgo at al., 1998). This procedure has the advantage that it can be broadly applied because is not related to only one pyrrole derivative. On the other hand, extensively damaged proteins produced Ehrlich adducts that

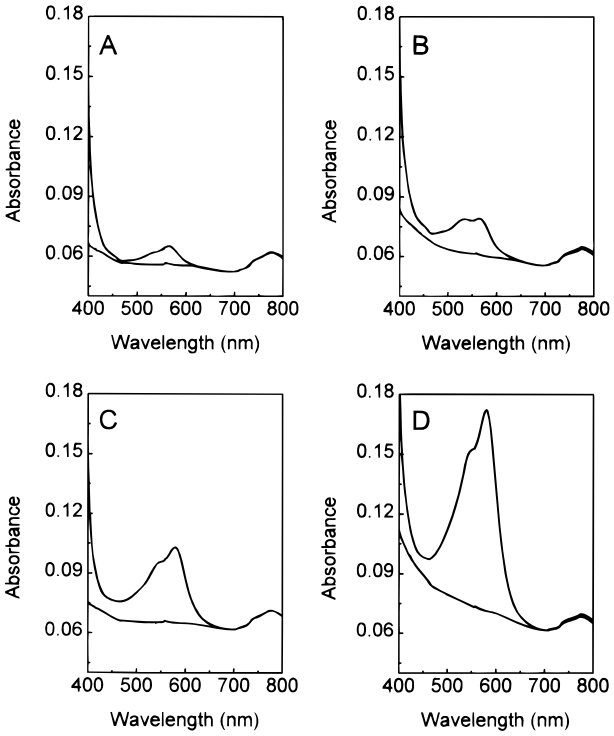

Figure 4. Absorbance spectra of Ehrlich adducts prepared in the reaction of COBSA (A), RIBSA (B), HPBSA (C), and SPBSA (D), with *p*-(dimethylamino)benzaldehyde. BSA was incubated at pH 7 and 50 °C for 24 h in the absence (control) or in the presence of 10 mM RI, HP, or SP.

Table 3. Effect of Temperature on Protein Pyrrolization Produced in BSA Incubated with Carbohydrates and Oxidized Lipids at pH 7

	μmol/g of protein			
temp (°C)	COBSA	RIBSA	HPBSA	SPBSA
25	0.46	0.41	4.43	2.58
37	0.46	0.75	5.50	4.24
50	0.53	0.99	*a*	7.55

[a] The Ehrlich adduct was only partially soluble.

were not soluble and, therefore, these samples could not be quantified spectrophotometrically. Thus, COBSA produced soluble adducts at all the assayed temperatures, but Ehrlich adducts of RIBSA, HPBSA, and SPBSA only were soluble when the modified proteins were obtained at 25, 37, and 50 °C. Figure 4 shows the UV spectra of the Ehrlich adducts obtained for COBSA, RIBSA, HPBSA, and SPBSA, which were prepared at 50 °C. All the spectra were similar suggesting that analogous pyrrole derivatives were produced with both carbohydrates and oxidized lipids. Table 3 collects the protein pyrrolization produced in all these proteins at 25−50 °C. These data are referred to the extinction coefficient of the ϵ-*N*-pyrrolylnorleucine (Hidalgo et al., 1998). According to these results HPBSA was the highest pyrrolized protein at 25−37 °C, followed by SPBSA, which exhibited a much higher dependence on the temperature than HPBSA and, finally, RIBSA.

Effect of pH on the Color and Fluorescence Changes Produced in BSA Incubated with Carbohydrates and Oxidized Lipids. The incubation of BSA with both lipids and carbohydrates developed browning and fluorescence in the protein. Analogously to the effect observed for the temperature, this browning and fluorescence were also dependent on the pH of the

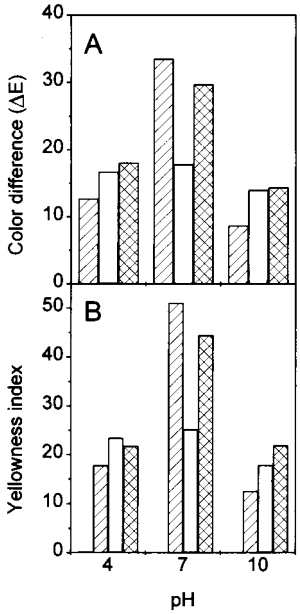

Figure 5. Effect of pH on ΔE (A) and YI (B) of COBSA (solid bars), RIBSA (striped bars), HPBSA (open bars), and SPBSA (crosshatched bars). BSA was incubated at 80 °C for 24 h in the absence (control) or in the presence of 10 mM RI, HP or SP.

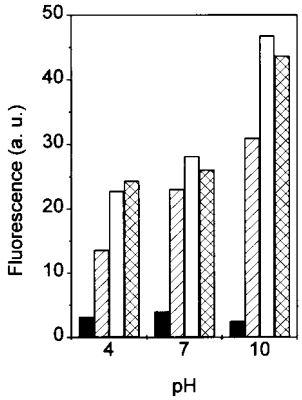

Figure 6. Effect of pH on fluorescence of COBSA (solid bars), RIBSA (striped bars), HPBSA (open bars), and SPBSA (crosshatched bars). BSA was incubated at 80 °C for 24 h in the absence (control) or in the presence of 10 mM RI, HP, or SP.

incubation media. A temperature of 80 °C was selected to study the effect of pH because at this temperature the reaction between RI and BSA was produced to a high extent. Figure 5A shows the effect of pH on ΔE of modified proteins produced at this temperature. Both RIBSA and SPBSA exhibited the highest ΔE at neutral pH and decreased at both acid and basic pHs, where ΔE values were similar. On the contrary, ΔE of HPBSA did not exhibit a clear relation with the pH of the reaction.

Results analogous to those of ΔE were also observed for the YI (Figure 5B). Thus, the highest YI were observed for RIBSA and SPBSA at neutral pH, and these values decreased at acid or basic pHs. On the contrary, few changes in YI were observed for HPBSA.

The effect of pH on the fluorescence is shown in Figure 6. Differently from the browning, the intensity of fluorescence increased with the pH for RIBSA, HPBSA, and SPBSA but not for COBSA. This increase was linear for RIBSA (r = 0.999, p = 0.034) and

Table 4. Effect of pH on Lysine Recovered after Acid Hydrolysis in BSA Incubated with Carbohydrates and Oxidized Lipids at 80 °C

	μmol/g of protein			
pH	COBSA	RIBSA	HPBSA	SPBSA
4	849	725	702	674
7	882	392	556	498
10	803	575	616	568

Table 5. Effect of pH on Arginine Recovered after Acid Hydrolysis in BSA Incubated with Carbohydrates and Oxidized Lipids at 80 °C

	μmol/g of protein			
pH	COBSA	RIBSA	HPBSA	SPBSA
4	347	316	314	303
7	336	185	268	240
10	334	259	257	234

exponential for HPBSA and SPBSA ($r = 0.973$, $p = 0.148$, and $r = 0.914$, $p = 0.266$, respectively).

Effect of pH on Amino Acid Losses Produced in BSA Incubated with Carbohydrates and Oxidized Lipids. Analogously to the effect of the temperature, the incubation of BSA with either carbohydrates or oxidized lipids at different pHs always produced amino acid losses in the amino acids recovered after acid hydrolysis. The lysine recovered after acid hydrolysis is shown in Table 4. For the three treated proteins (RIBSA, HPBSA, and SPBSA) the highest losses were produced at pH 7, followed by pH 10 and pH 4.

The results obtained for the amino acid arginine were not the same that the obtained for the amino acid lysine, although the treatment with either RI, HP, or SP always produced arginine losses (Table 5). The highest arginine losses for RIBSA were observed at neutral pH, followed by pH 10 and pH 4. However, both HPBSA and SPBSA exhibited the highest arginine losses at pH 10, followed by pH 7 and, finally, pH 4.

Although no data are shown because Ehrlich adducts were not soluble at the assayed temperature (80 °C), the destruction of amino acids was parallel to the pyrrolation of the treated proteins. RIBSA, HPBSA, and SPBSA at the three assayed pHs always gave strongly colored Ehrlich adducts.

DISCUSSION

The reaction of BSA with both carbohydrates (RI) and oxidized lipids (HP and SP) produced the nonenzymatic browning of the protein and the development of fluorescence, together with the destruction of some amino acids and the transformation of some lysine residues into pyrrole derivatives. These processes produced analogous colors and fluorescence spectra in the three modified proteins studied and also exhibited similar spectra of the Ehrlich adducts produced between the modified protein and *p*-(dimethylamino)benzaldehyde. However, all these changes were not produced to the same extent for RIBSA, HPBSA, or SPBSA at the different temperatures and pH assayed. Thus, nonenzymatic browning, amino acid losses, and protein pyrrolization were lower in RIBSA than in both HPBSA and SPBSA in the 25−50 °C range. However, at higher temperatures, the highest color and amino acid changes were produced in RIBSA. In addition, ΔE was inversely correlated ($r = -0.9344$, $p = 0.02$; $r = -0.9824$, $p = 0.0028$; $r = -0.9523$, $p = 0.012$) with the lysine recovered after acid hydrolysis in RIBSA, HPBSA, and SPBSA, respectively.

Differently from the browning of the protein, the development of the fluorescence was not clearly related with the temperature, and BSA treated with oxidized lipids always produced more fluorescence than BSA treated with RI.

The effect of pH was different to the effect of temperature, and RIBSA exhibited a behavior very similar to SPBSA in relation to the browning, which was higher at neutral pH. On the contrary, HPBSA did not exhibit a clear dependence on the pH of the media. These results were not correlated with the lysine losses, which were mainly produced for the three proteins at pH 7, followed by pH 10 and pH 4. The browning was also not correlated with the fluorescence of the modified proteins, which exhibited the highest fluorescence when they were prepared at pH 10, followed by pH 7 and pH 4.

Carbonyl−amine or nonenzymatic browning reactions of proteins have been mostly related to the Maillard reaction between carbohydrates and the amino groups of proteins. However, some oxidized lipids are also able to produce melanoidin-like pigments by reaction with protein amino acid residues, which are analogous to Maillard melanoidins, and therefore, they are also contributing to the color and fluorescence produced in foods during processing and storage. This analogy between both types of melanoidin polymers, which are in agreement with the similarity of the absorbance and fluorescence spectra obtained for the different modified proteins and also with the similarity of the absorbance spectra produced by reaction of these proteins with *p*-(dimethylamino)benzaldehyde, might be a consequence of the similarity between their structures. In fact, a polypyrrole structure for melanoidin-like pigments produced in the reaction between oxidized lipids and amino groups has been proposed (Hidalgo and Zamora, 1993b). This polymeric structure is produced when polymerizes the *N*-alkyl-2-(hydroxyalkyl)pyrroles that are produced in a first step in the reaction between oxidized lipids and amines, amino acids, and proteins (Zamora and Hidalgo, 1995). Surprisingly, analogous *N*-alkyl-2-(hydroxyalkyl)pyrroles are also produced in the reaction of 2-deoxypentoses with amines, and these compounds are very reactive producing polymers that are very similar to the derived from oxidized lipids (Tressl et al., 1998). Because 2-deoxypentoses may be generated from hexoses (Tressl et al., 1993), this last mechanism might be a general route for production of melanoidins between carbohydrates and amino compounds, and therefore, these types of melanoidins would have analogous chemical structures when starting from both carbohydrates or oxidized lipids.

The results obtained in the present study are in agreement with the similarity expected for browned proteins obtained from both carbohydrates and oxidized lipids. However, because carbohydrate/protein and oxidized lipid/protein reactions are not favored under the same temperature and pH conditions, both reactions are, in some way, complementary, and melanoidin formation derived from oxidized lipids should be expected under conditions where carbohydrate/protein reactions should not be produced.

ABBREVIATIONS USED

BSA, bovine serum albumin; COBSA, control BSA; HP, hydroperoxides of methyl linoleate; HPBSA, BSA

modified by HP; RI, ribose; RIBSA, BSA modified by RI; SP, secondary products of methyl linoleate oxidation; SPBSA, BSA modified by SP; YI, yellowness index.

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