Browning and Fluorescence Development during Microwave Irradiation of a Lysine/(*E*)-4,5-Epoxy-(*E*)-2-heptenal Model System

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A mixture of L-lysine and (*E*)-4,5-epoxy-(*E*)-2-heptenal was exposed to microwave irradiation for several periods of time, at different pHs and lipid/amino acid ratios, to study the contribution of oxidized lipid/amino acid reactions to the nonenzymatic browning of foods. CIELAB L^* and a^* values significantly decreased, and CIELAB b^* values and fluorescence intensity significantly increased as a function of time and the assayed conditions. These changes were produced faster in this model system than in an analogous glucose/lysine system, and the color and fluorescence reached after 100 s of irradiation were comparable for both systems. The studied system rapidly developed color and fluorescence at pH >6, and the concentration of epoxyaldehyde and, to a lesser extent, L-lysine greatly influenced the final color and fluorescence. All of these results suggest that oxidized lipid/amino acid reactions may play a role in the nonenzymatic browning reactions of foods produced during microwave heating.

INTRODUCTION

The application of microwave processing in both home and institutional meal preparation has grown considerably over the past 30 years, mainly because of its simplicity, speed, and convenience as compared to conventional cooking methods (Klein, 1982; Cremer and Richman, 1987; Yoshida et al., 1991; Yeo and Shibamoto, 1991c) and in spite of the lack of browning and desirable flavors showed by the foods prepared in this way (Yeo and Shibamoto, 1991a,b).

Nowadays, microwave ovens are considered among the most efficient types of oven and the most rapid method for reheating food items. However, the heating effect of microwave energy on various food components may differ significantly from that of conventional cooking. Various chemical reactions have been reported to be induced by microwave energy (Lie Ken Jie and Yan-kit, 1988; Hafez et al., 1989), and the rate enhancements observed, as compared with those of conventional heating, are supposed to be owed, at least to some extent, to superheating caused by absorption of microwaves (Bond et al., 1991).

When fried foods are reheated in a microwave oven after frying, further oxidation of fat-containing foods may occur and, also, the reaction between the oxidized lipids and other food components. Little is known about how microwave reheating affects the quality of the lipids of fried foods (Yoshida et al., 1990), and there are virtually no papers on the effect of microwaves on oxidizing lipid–protein interactions. This study was undertaken to examine the effect of microwaves on oxidized lipids and, particularly, on the interaction between oxidized lipids and proteins using a model system that consisted of (*E*)-4,5-epoxy-(*E*)-2-heptenal and L-lysine.

The oxidation of polyunsaturated fatty acids produces many compounds capable of interacting with amino groups or proteins and inducing changes in the properties of foods (Eskin, 1990; Hidalgo and Kinsella, 1989). (*E*)-4,5-Epoxy-(*E*)-2-heptenal [a product of the oxidation of $\omega - 3$ pentaenoic fatty acids (Swoboda and Peers, 1976)] was selected for this study because it is the major volatile compound formed during the oxidation of butterfat in a model system (Swoboda and Peers, 1978) and it has been shown to react with food proteins, producing changes in color and fluorescence (Hidalgo et al., 1992). Lysine was used because it is usually lost during the deterioration of foods produced by peroxidizing lipids (Karel, 1984; Hurrell and Finot, 1985). Color and fluorescence were measured to evaluate the contribution of oxidized lipid/amino acid interactions to the overall nonenzymatic browning reactions of foods during microwave heating.

EXPERIMENTAL PROCEDURES

Materials. (*E*)-4,5-Epoxy-(*E*)-2-heptenal was prepared from (*E*)-2-(*E*)-4-heptadienal as described by Swoboda and Peers (1978). (*E*)-2-(*E*)-4-Heptadienal and L-lysine were purchased from Aldrich Chemical Co. (Milwaukee, WI) and L-cysteine, D-glucose, and 3-chloroperoxybenzoic acid from Fluka Chemie AG (Buchs, Switzerland). All other chemicals used were of analytical grade and were purchased from reliable commercial sources.

Sample Preparation. Typically, (*E*)-4,5-epoxy-(*E*)-2-heptenal (0.1 mmol) was suspended in 5 mL of 0.3 M sodium phosphate (pH 7.0) and sonicated until permanent emulsion with a Braun Labsonic U sonicator. Lysine (0.2 mmol) was then added and, for some experiments, also the corresponding amount of sodium chloride. Solutions were irradiated at the high setting of an 800-W Panasonic microwave oven (Model NN-8559) for different periods of time. At the end of the irradiation period, the samples were cooled and diluted for color and fluorescence measurements.

The influence of pH on browning and fluorescence development during microwave irradiation was studied by using the following buffers: 0.3 M sodium citrate for pH 3–6, 0.3 M sodium phosphate for pH 6–8, 0.3 M sodium borate for pH 8–10, and 0.3 M sodium phosphate for pH 11–12. Epoxyaldehyde/lysine ratio studies were carried out with 0.1 and 0.2 mmol of epoxyaldehyde and 0.1, 0.2 and 0.4 mmol of lysine. To study the influence of electrolytes, 0, 1.25, 2.5, or 5 mmol of sodium chloride was added to the reaction mixture before the irradiation.

The reaction between sugars and amino acids was carried out analogously. Briefly, glucose (0.1 mmol) was dissolved in 5 mL of 0.3 M sodium phosphate (pH 7.0), and 0.2 mmol of the amino acid (lysine or cysteine) was added. The resulting solution was irradiated for different periods of time in the same conditions as above.

Measurement of Color. Samples for color evaluation were prepared by diluting 200 μL of the reaction with 2.8 mL of deionized water. The weighted-ordinate method was used (Hunter, 1973). Tristimulus values (X, Y, Z) were calculated from the transmittances (T) obtained in a Hewlett-Packard 8450-A UV–vis spectrophotometer. Transmittances were recorded at constant intervals (10 nm) from 400 to 700 nm using

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Table I. CIELAB L^* a^* b^*, Hunter Color Difference, Yellowness Indices, and Fluorescence Development during Microwave Heating of Epoxyheptenal/Lysine (I), Glucose/Lysine (II), and Glucose/Cysteine (III) Mixtures[a]

reaction	exposure time, s	L^*	a^*	b^*	ΔE	YIa	YIb	fluorescence 340/440	fluorescence 390/450
I	0	99.3 ± 0.4[b]	−0.14 ± 0.01[b]	0.5 ± 0.2[b]		1.2 ± 0.4[b]	0.8 ± 0.3[b]	3.7 ± 0.8[b]	4.0 ± 0.3[b]
	40	98.4 ± 0.2[b]	−1.3 ± 0.1[c]	5.8 ± 0.6[c]	5.5 ± 0.7[b]	10.1 ± 1.0[c]	8.5 ± 0.91[c]	137 ± 17[c]	69.1 ± 8.6[c]
	60	96.9 ± 0.3[c,d]	−1.8 ± 0.2[c]	10.4 ± 0.4[d]	10.3 ± 0.6[c]	17.9 ± 0.5[d]	15.3 ± 0.5[d]	236 ± 16[d]	129 ± 8[d]
	80	96.2 ± 0.1[d]	−1.8 ± 0.2[c]	12.2 ± 1.1[d]	12.1 ± 1.3[c]	21.0 ± 1.7[d]	18.0 ± 1.6[d]	308 ± 28[d,e]	211 ± 10[e]
	100	95.6 ± 0.1[d]	−1.8 ± 0.1[c]	13.1 ± 1.0[d]	13.2 ± 1.2[c]	22.8 ± 1.6[d]	19.6 ± 1.5[d]	362 ± 28[e]	262 ± 12[f]
II	0	99.9 ± 0.1[b]	−0.11 ± 0.01[b]	0.04 ± 0.01[b]		0.30 ± 0.02[b]	0.06 ± 0.01[b]	2.0 ± 0.1[b]	2.1 ± 0.1[b]
	40	100.0 ± 0.1[b]	−0.12 ± 0.01[b]	0.11 ± 0.01[b]	0.09 ± 0.01[b]	0.42 ± 0.01[b]	0.15 ± 0.01[b]	1.9 ± 0.1[b]	2.4 ± 0.2[b]
	60	99.9 ± 0.1[b]	−0.15 ± 0.01[b]	0.14 ± 0.01[b]	0.11 ± 0.02[b]	0.45 ± 0.02[b]	0.20 ± 0.02[b]	9.3 ± 0.9[b]	5.4 ± 0.5[b]
	80	98.1 ± 0.6[c]	−1.62 ± 0.60[c]	7.0 ± 3.2[c]	7.5 ± 3.1[c]	11.9 ± 5.2[c]	10.3 ± 4.7[c]	299 ± 52[c]	221 ± 44[c]
	100	96.6 ± 0.7[c]	−2.93 ± 0.30[d]	15.3 ± 2.4[d]	15.9 ± 2.5[d]	25.4 ± 3.9[d]	22.8 ± 3.7[d]	620 ± 136[d]	547 ± 110[d]
III	0	99.90 ± 0.01	−0.10 ± 0.01[b]	0.13 ± 0.01[b]		0.47 ± 0.02[b]	0.18 ± 0.02[b]	1.9 ± 0.1[b]	2.4 ± 0.1[b]
	40	99.90 ± 0.01	−0.13 ± 0.01[b]	0.09 ± 0.01[b]	0.05 ± 0.01[b]	0.38 ± 0.02[b]	0.13 ± 0.02[b]	1.9 ± 0.1[b]	2.7 ± 0.1[b]
	60	99.98 ± 0.01	−0.13 ± 0.01[b]	0.09 ± 0.01[b]	0.09 ± 0.01[b]	0.38 ± 0.02[b]	0.14 ± 0.01[b]	2.6 ± 0.3[b]	3.0 ± 0.2[b]
	80	99.99 ± 0.06	−0.16 ± 0.02[b]	0.21 ± 0.08[b]	0.30 ± 0.11[b,c]	0.57 ± 0.13[b]	0.30 ± 0.11[b]	15 ± 5[b,c]	4.9 ± 1.0[b]
	100	99.86 ± 0.03	−0.25 ± 0.03[c]	0.59 ± 0.11[c]	0.48 ± 0.10[c]	1.21 ± 0.18[c]	0.84 ± 0.15[c]	42 ± 8[c]	10 ± 2[c]

[a] Values are mean ± SEM for three determinations. Means in the same column for each reaction with different superscripts are significantly different ($p < 0.01$) as assessed by the method of Scheffé.

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 irradiated and nonirradiated (control) samples was determined by the following equation (Hunter, 1973):

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

Yellowness index (YIa) was expressed according to the method of Hunter (1942) as

$$\text{YIa} = [100(1.28X - 1.06Z)]/Y \quad (2)$$

A second yellowness index (YIb) was also calculated (Francis and Clydesdale, 1975) as

$$\text{YIb} = 142.86b^*/L^* \quad (3)$$

Measurement of Fluorescence. Spectra were recorded on a Perkin-Elmer LS-5 fluorescence spectrophotometer of 100-μL samples diluted with 2.9 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 50 at 450 nm, when excitation was done at 350 nm.

Statistical Analysis. Statistical comparisons between several groups were made using ANOVA. When significant F values were obtained, group differences were evaluated according to the method of Scheffé (Snedecor and Cochran, 1980).

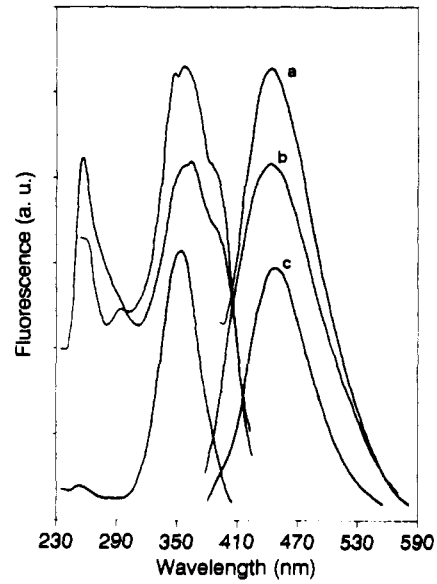

Figure 1. Fluorescence spectra of glucose/lysine (a), epoxyheptenal/lysine (b), and glucose/cysteine (c) mixtures after 100 s of microwave irradiation time.

RESULTS

When a mixture of lysine and (*E*)-4,5-epoxy-(*E*)-2-heptenal was irradiated in a microwave oven, brown color and fluorescence were developed. CIELAB $L^*a^*b^*$ values significantly changed with irradiation time (Table I). CIELAB L^* stayed unchanged for the first 30 s (data not shown) and then decreased significantly for the following irradiation period. Analogous behavior was observed for the chromaticity coordinates a^* and b^*. There was an initial reduced-rate phase (between 0 and 30 s) that was followed by an accelerated phase (a^* value decreased and b^* value increased in this phase). At the end of the irradiation period (between 60 and 100 s) a new reduced-rate phase was observed and changes in CIELAB L^*, a^* and b^* values were produced much more slowly. L^*, a^*, and b^* values were used for calculating color differences (ΔE) and yellowness indexes (YIa and YIb), which showed similar behavior as a function of time.

The brown color developed in the epoxyaldehyde/lysine system was similar to that developed in the glucose/lysine reaction (Table I), and both were more intense than that observed in the glucose/cysteine system (Table I). After 100 s of microwave irradiation, the only significant difference between the colors produced in the epoxyaldehyde/lysine and glucose/lysine reactions was in the a^* value. The glucose/lysine reaction reached an a^* value 63% lower than that produced in the epoxyaldehyde/lysine system. However, the kinetics of both reactions were clearly different. The epoxyaldehyde/lysine reaction quickly developed color and had only 30 s of reduced-rate phase with no significant differences. This reduced-rate phase lasted 80 s with glucose/lysine and 100 s or higher with glucose/cysteine.

Microwave irradiation of the three reactions also produced the development of fluorescence. The spectra of the samples (Figure 1) showed broad bands and shoulders for epoxyaldehyde/lysine and glucose/lysine reactions, suggesting the presence of several fluorophores. On the contrary, the glucose/cysteine reaction developed narrower bands. The intensities of two bands were measured: one at 440 nm, when the sample was excited at 340 nm, and the other at 450 nm, when excitation was done at 390 nm (Table I). Both measurements gave different values;

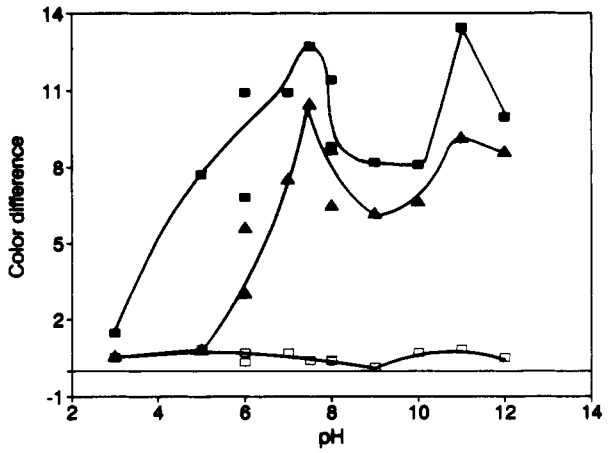

Figure 2. Effect of pH on color difference of an epoxyheptenal/lysine mixture after microwave irradiation for 0 (□), 50 (▲), and 100 s (■).

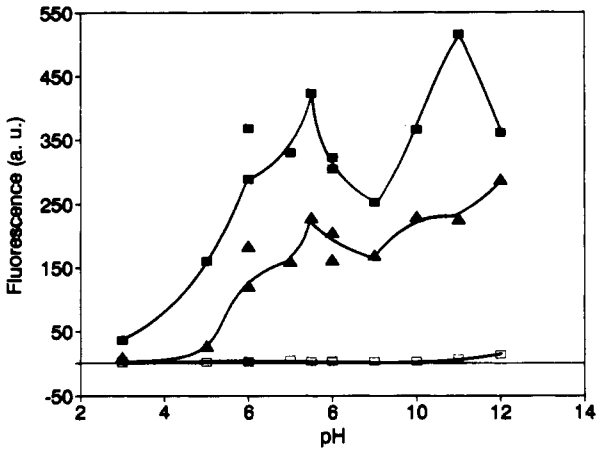

Figure 3. Effect of pH on fluorescence intensity of an epoxyheptenal/lysine mixture after microwave irradiation for 0 (□), 50 (▲), and 100 s (■).

however, the intensity of fluorescence of both maxima followed a similar behavior as a function of time. They were significantly correlated (r = 0.988, $p < 0.001$) for epoxyaldehyde/lysine and, also, for glucose/lysine (r = 0.997, $p < 0.001$) and glucose/cysteine (r = 0.997, $p <$ 0.001). Yellowness index and intensity of fluorescence and color difference and intensity of fluorescence were also correlated for epoxyaldehyde/lysine (r = 0.97, $p <$ 0.001, and r = 0.97, $p < 0.001$, respectively), glucose/lysine (r = 0.999, $p < 0.001$, and r = 0.999, $p < 0.001$, respectively), and glucose/cysteine (r = 0.98, $p < 0.001$, and r = 0.93, p < 0.001, respectively).

The pH effect on color and fluorescence development during microwave irradiation of the amino acid/epoxyaldehyde model system is shown in Figures 2 and 3, respectively. Nonirradiated (control) samples and 50- and 100-s irradiated samples showed initial, middle, and final states of the sample. Both color and fluorescence developments depended on the pH. The reaction was inhibited at strongly acid pHs and reached maximum color and fluorescence at pH around 7 and 11. Color and fluorescence formation kinetics (Figures 4 and 5, respectively) was similar to that described above with an initial slow browning–fluorescence development phase, followed by a browning–fluorescence acceleration phase and finally a new slow browning–fluorescence development phase (this last phase was not observed at acid pHs). The cross point of the two first phases, considered the browning–fluorescence acceleration irradiation time, was clearly dependent

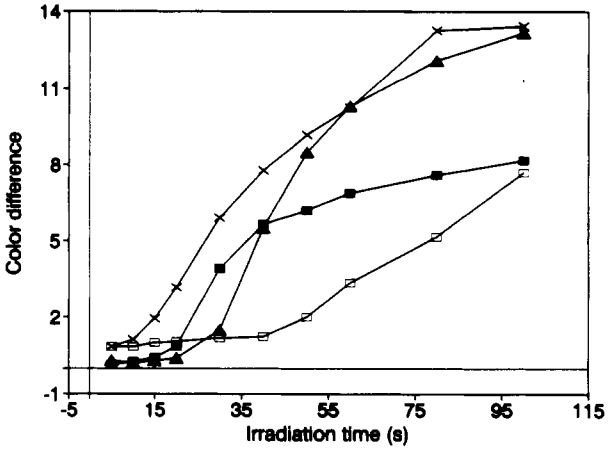

Figure 4. Color difference of the epoxyheptenal/lysine model system as a function of irradiation times for samples at pH 5 (□), 7 (▲), 9 (■), and 11 (×).

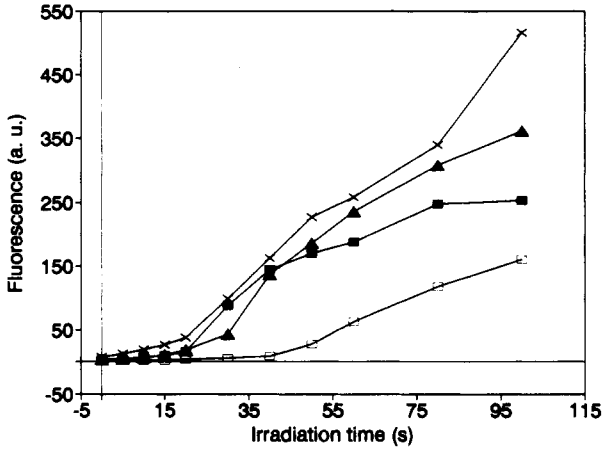

Figure 5. Fluorescence intensity of the epoxyheptenal/lysine model system as a function of irradiation times for samples at pH 5 (□), 7 (▲), 9 (■), and 11 (×).

Table II. Microwave Irradiation Time (in Seconds) Needed To Produce Changes in Color and Fluorescence of a Epoxyheptenal/Lysine Mixture[a]

							fluorescence	
pH	L^*	a^*	b^*	ΔE	YIa	YIb	340/440	390/450
3	100	32	34	100	32	64	40	51
5	29	40	39	34	38	44	38	41
6c[b]	26	28	23	24	23	25	22	26
6p[c]	29	27	23	22	25	29	25	33
7	18	20	18	17	18	17	17	21
7.5	18	19	19	19	21	23	25	24
8p[c]	16	18	18	19	17	19	18	18
8b[d]	13	14	13	15	12	15	16	16
9	13	14	14	16	13	16	17	17
10	9	7	5	7	4	8	9	8
11	3	12	8	8	8	12	14	5
12	0	3	0	3	0	3	7	0

[a] Calculated according to the method of Tsai et al. (1991). [b] Sodium citrate buffer. [c] Sodium phosphate buffer. [d] Sodium borate buffer.

on the pH and was smaller at higher pHs (Table II). However, final color and fluorescence intensity were not correlated with the irradiation time of the browning–fluorescence acceleration. Both color and fluorescence intensity reached maximum values at pH 7.5 and 11, respectively.

The influence of aldehyde/amino acid ratio on color and fluorescence development is shown in Figures 6 and 7, respectively. Color difference, and yellowness indices (data not shown), clearly depended on the oxidized lipid/

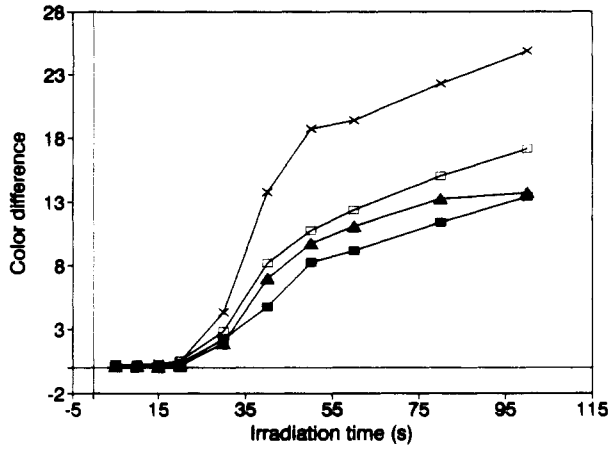

Figure 6. Effect of epoxyheptenal/lysine ratio [1:4 (□), 1:2 (▲), 1:1 (■), and 2:1 (×)] on color difference as a function of irradiation time.

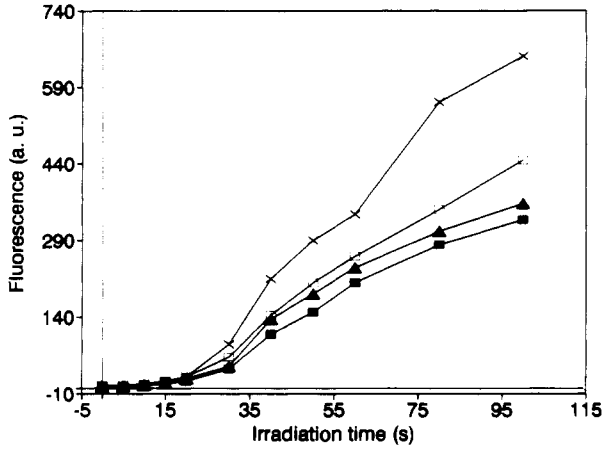

Figure 7. Effect of epoxyheptenal/lysine ratio [1:4 (□), 1:2 (▲), 1:1 (■), and 2:1 (×)] on the development of fluorescence as a function of irradiation time.

amino acid ratio. The final color was directly proportional to the aldehyde concentration and was also related to the lysine concentration. In the assayed conditions, the observed values of color differences and yellowness indexes were doubled when the epoxyaldehyde concentration was multiplied by two. Similar results were observed when the fluorescence development was studied. The fluorescence intensity clearly depended on the lysine/epoxyaldehyde ratio. Both fluorescence intensity and color differences were correlated for 1:4 epoxyaldehyde/lysine ratio ($r = 0.985$, $p < 0.001$), 1:2 ratio ($r = 0.986$, $p < 0.001$), 1:1 ratio ($r = 0.991$, $p < 0.001$), and 2:1 ratio ($r = 0.959$, $p < 0.001$).

Finally, the effect of electrolytes during microwave irradiation was studied by adding various amounts of sodium chloride. No clear effect of sodium chloride on color or fluorescence was observed (data not shown).

DISCUSSION

Nonenzymatic browning reactions are one of the major chemical reactions occurring in foods (Karel, 1984), and although it has been commonly associated with the interaction between reducing sugars and proteins (Maillard reaction), the interaction between oxidized lipids and proteins has been suggested to play a role into the above process (Eriksson, 1987; Hidalgo et al., 1990). The results obtained in this study show a great similarity between color and fluorescence developed after 100 s of microwave irradiation in both epoxyaldehyde/lysine and glucose/lysine systems and, therefore, suggest a contribution of oxidized lipid/amino acid reactions to the final color and fluorescence developed in foods during microwave heating. This contribution may be especially important at very short irradiation times when classic Maillard reactions have not been initiated but oxidized lipid/amino acid reactions produce color and fluorescence (Table I). The similarity in color and fluorescence produced by epoxyaldehyde/lysine and glucose/lysine systems suggests a certain relation between both reactions and also the possibility that both reactions evolve analogous melanoidin-type products.

The kinetics of both browning and fluorescence development in the epoxyaldehyde/lysine reaction depended on the pH and the oxidized lipid/amino acid ratio. Although final color and fluorescence were similar at pH around 7 and 11, the microwave irradiation time needed to produce changes in color and fluorescence was shorter at higher pHs. These results suggest the presence of several mechanisms for browning and fluorescence development in this reaction that may play different roles at different pHs. These hypothetical mechanisms were dependent on oxidized lipid/amino acid ratio, and final color and fluorescence were mainly due to the epoxyaldehyde concentration.

Finally, a very good correlation was always observed between color and fluorescence, suggesting that analogous mechanisms may be used for producing both color and fluorescence.

In conclusion, the results obtained in this study suggest that the reaction between oxidized lipids and amino acids may contribute to the browning of foods and that microwaves are able to accelerate these reactions, which consequences may be observed after very short irradiation times. Further studies are necessary to understand if reaction products produced during microwave irradiation are similar to those produced during conventional heating.

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Registry No. Lysine, 56-87-1; (*E*)-4,5-epoxy-(*E*)-2-heptenal, 69511-21-3; glucose, 50-99-7; cysteine, 52-90-4.