Fluorescence Kinetics of Soybean Flour Oxidation

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ABSTRACT: Changes in fluorescence and oxidation indices of soybean flour were studied during storage at 37, 50, and 60°C. The peroxide value and 2-thiobarbituric acid value did not increase in proportion to the extent of oxidation. The front-surface fluorescence of oxidized soybean flour had an excitation maximum around 350 nm and an emission maximum between 420 and 430 nm. The fluorescent compounds were soluble in the organic phase of chloroform/methanol (2:1, vol/vol) after addition of water, and the solution showed excitation and emission maxima around 360 and 440 nm, respectively. The fluorescent compounds might have been produced from interactions of oxidizing soybean oil with other constituents of soybean flour. The fluorescence intensity in the organic phase increased steadily throughout the storage period. Fluorescence kinetics of soybean flour oxidation showed a zero-order increase, and the rate followed an Arrhenius relationship with an activation energy of 47.8 kJ/mol. These results suggest that analysis of fluorescence intensity in the organic phase is a useful indicator for determination of the oxidative deterioration of soybean flour.

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KEY WORDS: Fluorescence, kinetics, oxidation, soybean flour.

The oxidation of lipids always leads to the development of off-flavors in fat-containing foods and renders these foods unacceptable or reduces their shelf life. The oxidation products can further react with some food constituents, especially proteins, and cause functional property changes (1). Peroxide value and 2-thiobarbituric acid (TBA) value are commonly used as indices of lipid peroxidation (2). The fluorescent products resulting from the interaction of oxidizing lipids and proteins also have been used for the assessment of oxidative deterioration in foods (3–5).

Fluorescence spectroscopy is a rapid, sensitive method for the characterization of molecular environments and events. Fluorescent compounds, chosen for their specific spectral properties, have been employed as empirical and practical tools for discerning food quality. Fluorescence measurements are usually carried out using transmission or reflectance techniques (6). The transmission technique, with a detector at right angles to the incident radiation, is employed almost exclusively in commercial instruments and is used for detecting solution fluorescence (7). The reflectance technique has been used primarily for examining front-surface fluorescence of turbid suspensions or solid materials (8,9).

Soybeans are important sources of proteins and vegetable oil. Soybean flour, soy protein concentrate, and soy protein isolate have different protein contents and are used in different applications (10). However, the oxidation of lipids during soy protein processing results in protein insolubility (11). Interactions of oxidizing soybean oil and soy proteins result in changes in intrinsic fluorescence and decrease in protein solubility, soluble protein hydrophobicity, and free amino groups of proteins (12).

In the present study, soybean flour was stored at 37, 50, and 60°C under air in order to monitor the changes in intrinsic fluorescence and oxidation indices. The kinetics and the temperature dependence of intrinsic fluorescence formation were examined. The feasibility of using fluorescence kinetics for the determination of oxidative deterioration in soybean flour was investigated.

MATERIALS AND METHODS

Materials. Soybean flour was obtained from Sunlight Foods (Taipei, Taiwan), and soybean oil was bought from a local market. The composition of soybean flour was (g/100 g) as follows: protein (41.0), crude fat (22.5), crude fiber (1.7), ash (5.1), and moisture (3.4). Defatted soybean flour was obtained from Sigma Chemical (St. Louis, MO). Ammonium thiocyanate and TBA were purchased from Merck (Darmstadt, Germany). Quinine sulfate dihydrate and anhydrous ferrous chloride were supplied by Fluka (Buchs, Switzerland). Chloroform, containing 0.6–1.0% ethanol as a stabilizer, and methanol were purchased from BDH Laboratory Supplies (Poole, England).

Storage and treatment of soybean flour. Soybean flour was stored under air in the dark at 37, 50, and 60°C. At various time intervals, 0.5 g of each of the samples was withdrawn and extracted with 10 mL chloroform/methanol (2:1, vol/vol), incubated at room temperature for 10 min, and filtered through Toyo # 1 filter paper (Toyo Roshi, Tokyo, Japan). The clear filtrate was adjusted to 10 mL with additional chloroform/methanol (CM). Aliquots of the CM extracts were subjected to assays of peroxide value, TBA value, and transmission fluorescence.

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Peroxide value. Peroxide values were determined by the ferric thiocyanate method (13). The CM extract (1.0 mL) was mixed with 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of 0.02 M ferrous chloride in 0.35% HCl, and then diluted with 3.8 mL CM. The absorbance of the mixture was determined at 500 nm after reacting for 3 min. The peroxide value was expressed as milliequivalents of peroxide per kilogram of sample.

TBA value. The TBA value was determined according to the method of McDonald and Hultin (14) with modifications. On the day of use, a solution was prepared consisting of 0.5% (wt/vol) TBA, 0.25% (wt/vol) trichloroacetic acid, and 0.06% (wt/vol) butylated hydroxytoluene in methanol. The CM extract (2.0 mL) was mixed with 4.0 mL of the solution and then heated to 70°C for 30 min. After cooling, the absorbance of the mixture was measured at 532 nm. The TBA value was expressed as nanomoles of malonaldehyde per gram of sample using a molar extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$ for malonaldehyde.

Front-surface fluorescence of soybean flour. The front-surface fluorescence of soybean flour was measured by dispersing the flour in fluorescence-free glycerin/water (1:1, vol/vol) (15). The spectra were obtained with a F-2000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) equipped with a front-surface sample cell holder. The excitation light, provided by a xenon lamp, was incident at an angle of 37 degrees to lower the reflected light. The excitation spectra of the samples were scanned from 220 to 400 nm with the emission wavelength fixed at 425 nm. The emission spectra were scanned from 400 to 600 nm with the excitation wavelength fixed at 350 nm. The spectra were measured under the following conditions: scan speed, 240 nm/min; response, 0.5 s; bandpass, 20 nm; photomultiplier voltage, 400 V.

Transmission fluorescence of the CM extracts. Six milliliters of the CM extract was transferred to a centrifuge tube, mixed well with 2 mL distilled water, and then centrifuged at $1000 \times g$ for 10 min. The organic and water layers were collected and used to obtain the fluorescence spectra and fluorescence intensity.

The spectra of the solution fluorescence were determined using a Hitachi F-2000 spectrofluorometer with a quartz cuvette (10 mm pathlength) in the conventional right-angle orientation. The excitation spectra of the solvent layers were scanned from 220 to 400 nm with the emission wavelength fixed at 440 nm. The emission spectra were scanned from 400 to 600 nm with the excitation wavelength fixed at 360 nm. The fluorescence intensity was determined at an excitation wavelength of 360 nm and emission wavelength of 440 nm. The intensity was expressed as a relative ratio for a standard solution of 0.1 ppm quinine sulfate in 0.1 N H_2SO_4 (16).

Effects of components on fluorescence formation. Defatted soybean flour (0.2 g) in each test tube was mixed with 0.010, 0.015, 0.025, 0.030, 0.035, 0.040, and 0.045 g of soybean oil, respectively. The mixtures were mixed well in CM to form a paste and the solvent was then removed at 40°C under reduced pressure.

The mixtures were oxidized at 60°C in air, in the dark. Bulk soybean oils of 0.010, 0.015, 0.025, 0.030, 0.035, 0.040, and 0.045 g, respectively, were also stored in the same condition. Samples were analyzed after 3, 6, and 9 d and at the beginning of storage. Each test tube withdrawn was extracted with 10 mL CM. Aliquots of the CM extracts were subjected to transmission fluorescence assays. The fluorescence intensity was determined at an excitation wavelength of 350 nm and emission wavelength of 440 nm. The intensity was expressed as a relative ratio for a standard solution of 0.01 ppm quinine sulfate in 0.1 N H_2SO_4. The rate of fluorescence formation in each mixture was calculated according to the slope of the increase in relative ratio over 9 d.

Statistical analysis. Experiments were performed twice, and all data were the average of at least three measurements. Kinetic data were statistically analyzed by linear regression of the appropriate function to get the rate constants using the method described by Labuza (17).

RESULTS AND DISCUSSION

Peroxide and TBA values. The increase in peroxide and TBA values of soybean flour is shown in Figure 1. The peroxide values, representing the accumulation of hydroperoxide products, did not increase until the storage had proceeded at 37, 50, or 60°C for at least 15 d. After the initiation period, increase in peroxide value at 50°C was higher than that at 60°C. This result suggests that the hydroperoxides are unstable at 60°C. Thus, the amount of peroxides present at a given time during lipid peroxidation depends not only on the rate of initiation of peroxidation but also on the conversion of peroxides to other products. The TBA value is one of the most widely used tests for evaluating the extent of lipid oxidation (2). However, TBA value analysis in soybean flour showed poor correlations with storage time and temperature in the present study. These observations suggest that peroxide and TBA values are not appropriate for determining oxidative deteriora-

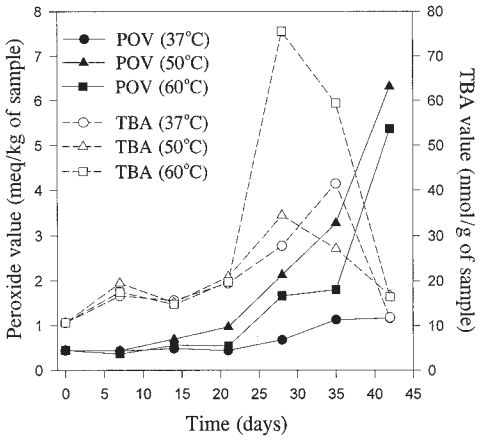

FIG. 1. Time courses of peroxide values (POV) and thiobarbituric acid (TBA) values of soybean flour during storage at 37, 50, and 60°C, respectively.

tion in soybean flour. Previous studies also have suggested that peroxide and TBA values cannot always be correlated quantitatively with reaction extent, as the oxidation products can further react with some food constituents and cause intrinsic fluorescence changes (4,18,19).

Correlation of fluorescence with soybean flour oxidation. The intensity of front-surface fluorescence around 425 nm emission increased significantly in oxidized soybean flour with 350 nm excitation (Fig. 2A). When the emission wavelength was set at 425 nm, the oxidized soybean flour had a broad peak near an excitation maximum of 350 nm, but fresh flour displayed only one peak with a wavelength of around 280 nm. The formation of fluorescence at 350 nm excitation and 425 nm emission was temperature dependent. Increases in temperature increased the fluorescence intensity. These fluorescence spectra are similar to those resulting from the interaction between oxidizing soybean oil and soy proteins (12).

The fluorescence compounds were found to be soluble in the organic phase of CM after addition of water. As shown in Figure 2B, the fluorescence intensity in the organic phase increased significantly in oxidized samples. At 37°C, the fluorescent compounds had an excitation maximum at 360 nm and an emission maximum at 440 nm. Increases in oxidation temperature shifted the excitation and emission maxima to longer wavelengths. In contrast, the fluorescence in the water phase of the CM extracts was relatively low and did not exhibit significant differences. The results demonstrate that the intrinsic fluorescence compounds due to oxidation of soybean flour are more lipophilic. On the other hand, that the front-surface fluorescence intensities of defatted oxidized flours were found to be higher than those of defatted fresh flour (Fig. 2C) implies that some fluorescent oxidation products are insoluble in CM.

Effects of oils on fluorescence formation. The effect of soybean flour components on the formation of intrinsic fluorescence was examined. As shown in Figure 3, the increase in fluorescence intensity was in proportion to the content of soybean oil in the defatted soybean flour–soybean oil system; however, bulk soybean oil produced no significant fluorescence under the same conditions. The fluorescence compounds might have been produced from the interaction of oxidizing soybean oil and other constituents of soybean flour. It has been reported in an earlier study that the spectra of fluorescence products formed as a result of interaction of oxidizing soybean oil and soy proteins were similar to those of Schiff base structures and were produced from the interaction of TBA-reactive substances and free-amino groups of proteins (12).

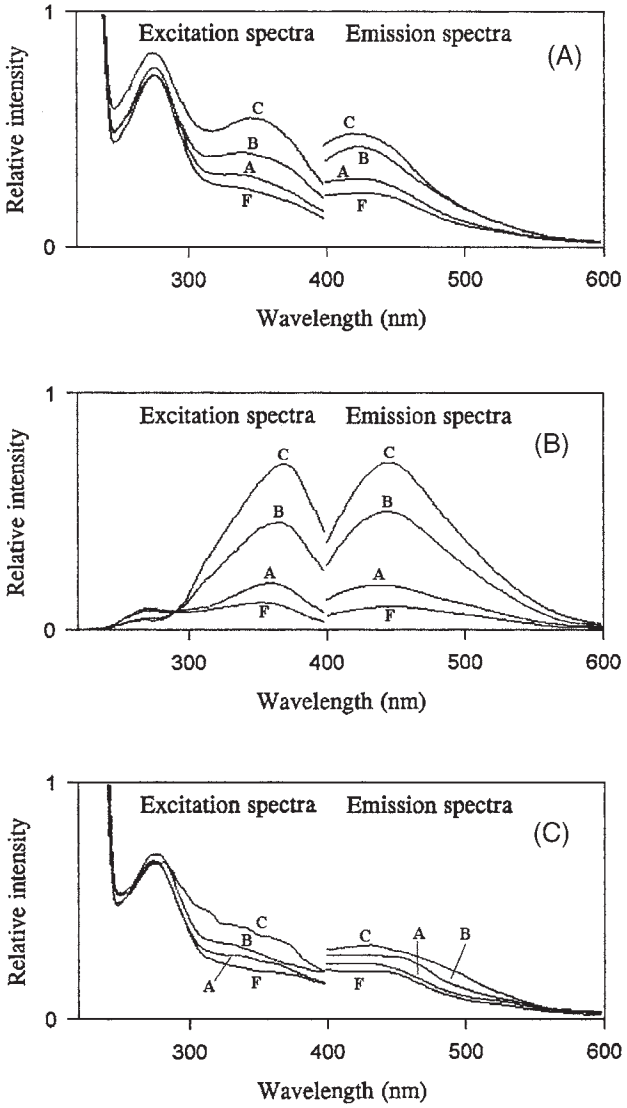

FIG. 2. (A) Front-surface fluorescence spectra of soybean flour. (B) Transmission fluorescence spectra in the organic phase of chloroform/methanol extractions. (C) Front-surface fluorescence spectra of soybean flour after extraction with chloroform/methanol. F, fresh sample; A, stored at 37°C in air for 2 mon; B, stored at 50°C in air for 2 mon; C, stored at 60°C in air for 2 mon.

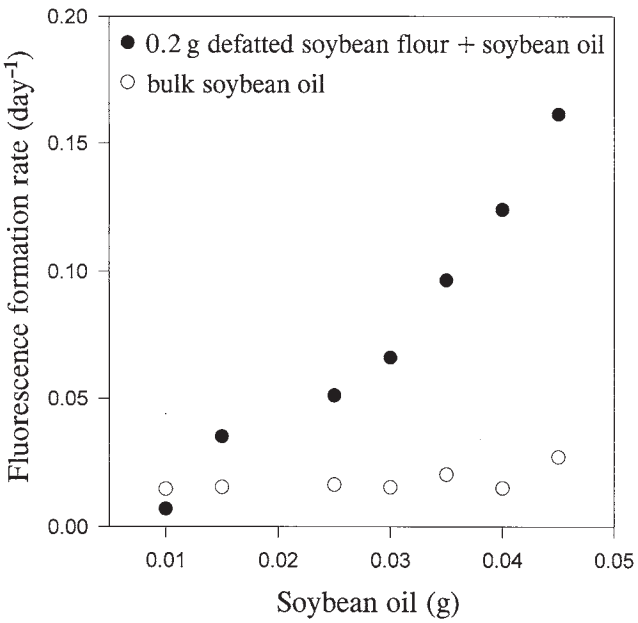

FIG. 3. Effects of oil levels on fluorescence formation rate during storage of bulk soybean oil and defatted soybean flour–soybean oil systems.

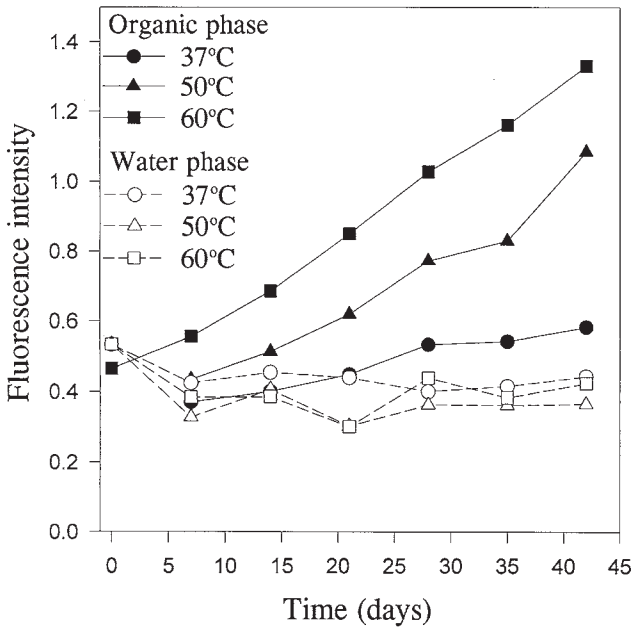

FIG. 4. Changes of fluorescence intensity with time in the organic and water phases of chloroform/methanol extractions after addition of water.

Fluorescence kinetics of soybean flour oxidation. Figure 4 shows the fluorescence intensities in the organic and water phases of CM extracts for soybean flour stored at different temperatures. The intensity in the organic phase increased in proportion to both temperature and storage time. The linear relationship shows that fluorescence formation can be described by zero-order kinetics. In contrast, the water phase did not build up significant fluorescence during storage. Table 1 shows the rate constants for fluorescence formation at 37, 50, and 60°C. The logarithm of the rate constant was calculated as a function of the reciprocal of the absolute temperature, which enabled calculation of the activation energy using the Arrhenius equation:

$$k = A \exp(-E_a/RT) \quad [1]$$

where k is the zero-order rate constant, A is the frequency factor, R is the gas constant (8.314 J mol^{-1} K^{-1}), T is the absolute temperature, and E_a is the apparent activation energy.

The rate of fluorescence formation in soybean flour followed an Arrhenius relationship with an E_a of 47.8 kJ/mol (Table 1). When soybean flour was oxidized at temperatures of 37–60°C, the formation rate of fluorescence could be derived from the relationship. The activation energy for the fluorescence compounds in this study is similar to that for hydroperoxides obtained in soybean oil oxidation (20). Furthermore, the E_a calculated in this study is similiar to those for TBA-reactive substances obtained in other foods under oxidation processes (21,22). However, it should be taken into consideration that E_a is not related to a single fluorescence compound, as the fluorescence spectrum of oxidized soybean flour is quite broad.

TABLE 1
Fluorescence Formation Kinetics in Soybean Flour Oxidation

Temperature	Fluorescence formation		
(°C)	Time (d)[a]	k (d^{-1})[b]	r^{2c}
37	0–42	0.0064	0.964
50	0–42	0.0178	0.961
60	0–42	0.0224	0.998
Activation energy		47.8 kJ/mol	0.935

[a]Data corresponding to the indicated time were employed to calculate zero-order kinetics.
[b]k is rate constant value of zero-order kinetics.
[c]r^2 is coefficient of determination.

In this investigation, E_a falls within the range of activation energies (40–100 kJ/mol) given for lipid oxidation reaction in other foods (23). The fluorescence accumulation in soybean flour is clearly dependent on the time and temperature of the oxidation process. These results suggest that the determination of fluorescence intensity in the organic phase of CM extract is a feasible index for the assessment of oxidative deterioration of soybean flour.

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