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Kinetics of fluorescence formation in whole milk powders during oxidation

Jer-Hour Liang *

Department of Food Health, Chia Nan University of Pharmacy and Science, Tainan, Taiwan 71710, ROC

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Abstract

Changes in the fluorescence and peroxide value of whole milk powders were studied during storage at 37, 50, 60 and 70°C. The fluorescence of oxidized whole milk powders showed an excitation maximum at 350 nm and an emission maximum at 440 nm. The fluorescent compounds were soluble in the organic phase of chloroform–methanol (2:1, v/v), and the intensity increased in proportion to both storage time and temperature. Fluorescence kinetics of whole milk powder oxidation showed a zero-order increase, and the rate followed an Arrhenius relationship with activation energies in the range of 50–70 kJ/mol. Experimental kinetic equations were derived to predict the fluorescence intensity in whole milk powders due to oxidation at different times and temperatures.

1. Introduction

Lipid oxidation in whole milk powders is a major cause of deterioration during processing and storage (Mc Cluskey et al., 1997). The reaction of unsaturated lipids with molecular oxygen results in the formation of hydroperoxides which then break down to off-flavour compounds. The oxidation products can further react with some food constituents and cause intrinsic fluorescence changes (Hasegawa, Endo & Fujimoto, 1992; Liang, 1999a).

Many methods have been used to measure lipid oxidation in dairy products, such as the peroxide value, the TBA test, and sensory assessment (Min, Lee, Lindamood, Chang & Reineccius, 1989; Chan, Gray, Gomaa, Harte, Kelly & Buckley, 1993; Shiratsuchi, Shimoda, Imayoshi, Noda & Osajima, 1994). However, each assay gives information about different stages of oxidation and has limitations in its use. For example, the peroxide value indicates the initial products of autoxidation, and it is only applicable for following peroxide formation in the early stage of oxidation. During the course of oxidation, the peroxide value may reach a peak and then decline. The TBA test at absorbance 530 nm, which indicates the concentration of malonaldehyde, is more sensitive when used with polyunsaturated lipids containing three or more double bonds (Frankel, 1993). The test is not suitable for the measurement of decomposition products of lipids containing mainly oleic acid and linoleic acids.

Solution fluorescence spectrophotometry has been used to study lipid oxidation in biological tissues. Fluorescent lipofuscin pigment, associated with aging in animal tissues, has been assumed to be derived from the reaction of lipid peroxides with amino compounds (Kikugawa, 1986). The fluorescent products resulting from the interaction of oxidizing lipids and proteins have also been used for the assessment of oxidative deterioration in foods (Kamarei & Karel, 1984; Liang 1996). However, little information is available on intrinsic fluorescence associated with lipid oxidation in whole milk powders. Bouzas, Kamarei and Karel (1985) reported that fluorescent compounds mainly due to lipid–protein interaction were formed during oxidation of milk fat globule membranes. The fluorescent compounds, with excitation maxima at 350 nm and emission maxima at 440 nm, are soluble in the organic layer of chloroform–methanol (2:1, v/v).

In this study, whole milk powders were oxidized at 37, 50, 60 and 70°C in air to investigate the changes in fluorescence and oxidation parameters during storage. The objective was to describe the kinetics of fluorescence formation and their use in studying whole milk powder oxidation.

* Fax: +886-6-266-6411.
E-mail address: iliangjh@mail.chna.edu.tw (J.-H. Liang).

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2. Material and methods

2.1. Materials

Four commercial whole milk powders (WMP) and one skimmed milk powder (SMP) were tested: (a) WMP1 (New Zealand Dairy Board, Wellington, New Zealand; 28.0% fat, 26.5% protein and 35.7% carbohydrate); (b) WMP2 (Nestle Australia Ltd., Sydney, Australia; 28.2% fat, 25.7% protein and 37.4% carbohydrate); (c) WMP3 (Nestle Australia Ltd., Sydney, Australia; 28.0% fat, 26.0% protein and 37.1% carbohydrate); (d) WMP4 (Wei-Chuan Foods Corporation, Taipei, Taiwan; 28.0% fat, 25.8% protein and 37.5% carbohydrate); and (e) SMP (New Zealand Dairy Board, Wellington, New Zealand; 0.8% fat, 37.6% protein and 49.8% carbohydrate). These milk powders were obtained from local markets. Ammonium thiocyanate was purchased from Merck (Darmstadt, Germany). Quinine sulfate dihydrate and anhydrous ferrous chloride were supplied by Fluka (Buchs, Switzerland). Other reagents and solvents used were of analytical grade and were purchased from reliable commercial sources.

2.2. Storage and treatment of milk powders

Whole milk powders were stored in air in the dark at 37, 50, 60 or 70°C. At various time intervals (3–7 days), 0.5 g of each of the samples was withdrawn and extracted with 10 ml chloroform–methanol (2:1, v/v), incubated at room temperature for 10 min, and filtered through Toyo No. 1 filter paper (Toyo Roshi, Tokyo, Japan). The clear filtrate (the CM extract) was adjusted to 10 ml with additional chloroform–methanol. Aliquots of the CM extracts were subjected to peroxide value and solution fluorescence assays. Experiments were performed twice and all data were duplicate measurements.

2.3. Peroxide value

Peroxide values were determined by the ferric thiocyanate method (Mitsuda, Yasumoto, & Iwami, 1966). Each 0.5 ml of the CM extract 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 3.3 ml chloroform–methanol. 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 by comparison with standards of ferric ions.

2.4. Solution fluorescence of the CM extracts

Six millilitres of the CM extract were transferred to a centrifuge tube, mixed well with 2 ml distilled water, and subsequently 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 by a Hitachi F-2000 spectrofluorometer (Hitachi, Tokyo, Japan) with a quartz cuvette (10 mm pathlength) in the conventional right-angle orientation. The excitation spectra 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 350 nm. 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 (Gillespie, 1985). The spectra were measured with conditions as follows: scan speed, 240 nm/min; response, 0.5 s; bandpass, 20 nm; photomultiplier voltage, 700 V.

2.5. Statistical analyses

Kinetic data was statistically analyzed by linear regression of the appropriate function to get the rate constants using the method described by Labuza (1984). All statistical procedures were performed using the Statistical Software Package for Windows (SPSS Inc., Chicago, IL, 1994).

3. Results and discussion

3.1. Fluorescence correlated with whole milk powder oxidation

Whole milk powders and skimmed milk powder were extracted with chloroform–methanol (CM), and the organic layers of the CM extracts were assayed by transmission spectrofluorometry (Fig. 1). In the excitation spectra, the fresh whole milk powder showed a single

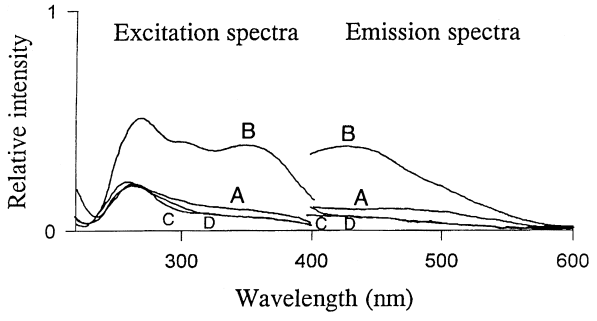

Fig. 1. Fluorescence excitation and emission spectra in the organic phase of chloroform–methanol extracts of milk powders: A, fresh whole milk powder (WMP3); B, whole milk powder (WMP3) stored at 37°C in air for 4 months; C, fresh skimmed milk powder (SMP); D, skimmed milk powder (SMP) stored at 37°C in air for 4 months.

excitation peak with a maximum wavelength of around 270 nm; the oxidized sample had two excitation peaks with wavelength maxima at 270 and 350 nm. In the emission spectra, the oxidized sample showed an intensive and broad peak with an emission maximum of around 440 nm. The fluorescence intensity at 350 nm excitation and 440 nm emission increased significantly during oxidation. On the other hand, the spectra of skimmed milk powder did not shift in wavelength during storage, and the change of fluorescence intensity at 350 nm excitation and 440 nm emission was still negligible for up to four months of storage. This result suggests that lipids in whole milk powders are the critical components contributing to the formation of the intrinsic fluorescence. However, it should be taken into consideration that the fluorescence spectra are not related to a single fluorescence compound, as the fluorescence spectrum of oxidized whole milk powder is quite broad. These fluorescent substances showed the same fluorescence spectra as those formed, for example, in oxidized milk fat globule membranes (Bouzas, Kamarei & Karel, 1985) and in the reaction between linoleic acid 13-hydroperoxide and β-lactoglobulin B (Hidalgo & Kinsella, 1989). The excitation and emission maxima were also similar to those of fluorescent compounds derived from the reaction of oxidized fatty acids and primary amines (Kikugawa & Ido, 1984). The fluorescent compounds should mainly derive from interactions of oxidizing lipids with other constituents of whole milk powder.

3.2. Fluorescence intensity and peroxide value

The change in fluorescence intensity in the organic phase of CM extracts with storage time was very distinct. The fluorescence intensity of the powders stored at higher temperatures increased more rapidly than those of powders stored at lower temperatures. As shown in Fig. 2, the fluorescence intensity of WMP1 increased with time throughout the storage period. The results with the other whole milk powders were similar to the results shown in Fig. 2 except that fluorescence formation proceeded at a faster or a slower rate. An earlier study showed that the fluorescence intensity is more reliable than the peroxide value and TBA value in evaluating oxidative deterioration in whole milk powders during storage at 37°C (Liang, 1999b). In this study, the peroxide values had a tendency to reach a maximum value and then level off (data not shown), indicating that hydroperoxides were unstable and tended to degrade again. In addition, the increase in peroxide value was not proportional to temperature, implying that the hydroperoxides were unstable at higher temperatures. Hence, 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 how quickly the peroxides decompose to give other products. The above results suggest that fluorescence intensity is a better index of whole milk powder oxidation than the peroxide value.

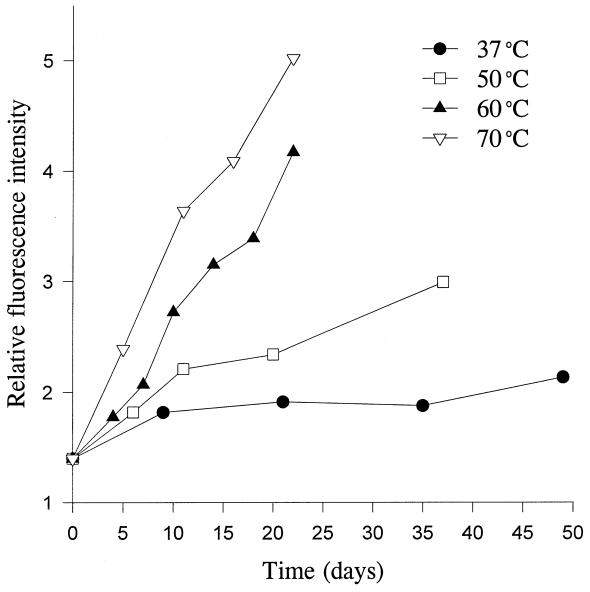

Fig. 2. Changes of fluorescence intensity with time in whole milk powder (WMP1) during oxidation at 37, 50, 60 and 70°C.

3.3. Fluorescence kinetics of whole milk powder oxidation

The fluorescence intensity in the organic phases of the CM extracts increased in proportion to both temperature and storage time. To check if the accumulation of fluorescence compounds could be predicted during the oxidation of whole milk powders, the corresponding kinetic equations were calculated. Statistical analysis of the coefficients of determination (r^2) showed that there was no significant difference ($P > 0.05$) between the zero-order rate and the first-order rate for each of the whole milk powders studied. Table 1 shows the rate constant from each relation for zero- and first-order reactions for fluorescence formation studied in WMP1. Labuza (1984) reviewed the application of chemical kinetics to deterioration of food. When the loss of food quality, as a function of time, was plotted, it was hard to distinguish between zero- and first-order for up to 50% loss of food quality. Thus, the simpler zero-order model was chosen in this study, which implied that the rate of increase of the fluorescence compounds was constant throughout the storage time studied.

To describe the influence of temperature on fluorescence formation, the linear regression method was employed, taking the Arrhenius equation as the mathematical model (Fig. 3). The temperature dependence of the rate constant value can be calculated according to the Arrhenius equation as follows:

$$\ln k = \ln k_0 - E_a/RT \quad (1)$$

where k (day^{-1}) is the rate constant at temperature T, k_0 is the collision factor, E_a (kJ/mol) is the apparent activation energy, R is the ideal gas constant (1.987 J mol^{-1} K^{-1}), and T is the absolute temperature (K). Table 2 shows the activation energy (E_a), the collision factor (ln k_0), and the coefficient of determination (r^2) obtained from Eq. (1) for each of the whole milk powders studied. The activation energies fall within the range of activation energies (40–100 kJ/mol) given for lipid oxidation reactions in food (Karel, 1985), but they were lower than that of the browning reaction in heated milk (100–150 kJ/mol) (Kessler & Fink, 1986; Pagliarini, Vernile & Peri, 1990). This equation enables us to predict the rate constant at any temperature between 37 and 70°C for each whole milk powder. With the rate constant, we can calculate the fluorescence intensity following zero-order kinetics by the following equation:

$$FI = FI_0 + kt \quad (2)$$

where FI and FI_0 represent the final and the initial fluorescence intensity, k is the rate constant and t is the duration of storage (day). The final intensity of fluorescence compounds due to oxidation in whole milk powders, produced after different durations and at different temperatures, can be calculated from Eq. (3), which is obtained by inserting Eq. (1) into Eq. (2).

$$FI = FI_0 + k_0\, e^{-Ea/RT} t \quad (3)$$

By using these equations, it is possible to estimate the content of fluorescence compounds after a period of storage (t) and at a certain temperature (T) without previously having to calculate the rate constant at a specific temperature. Table 3 shows the experimental values and the predictive values (obtained with the above equations) of fluorescence intensity for whole milk powders during storage at 60°C. The experimental

Table 1
Coefficients of determination (r^2) and rate constant (k) for zero- and first-order reactions for fluorescence formation in whole milk powders

Sample	Temperature (°C)	Time[a] (day)	Zero-order analysis[b]		First-order analysis[b]	
			k (day^{-1})	r^2	k (day^{-1})	r^2
WMP1	37	0–49	0.0151	0.87	0.0077	0.85
	50	0–37	0.0345	0.84	0.0163	0.81
	60	0–22	0.1246	0.98	0.0489	0.97
	70	0–22	0.1731	0.98	0.0674	0.94

[a] Data corresponding to the indicated time were employed to calculate rate constant.
[b] N = 5–7.

Table 2
Kinetic parameters for formation of fluorescence compounds in whole milk powders during oxidation

Sample	Zero-order analysis		
	E_a (kJ/mol)	ln k_0	r^2
WMP1	69.6	22.8	0.962
WMP2	50.4	15.5	0.967
WMP3	65.0	21.1	0.930
WMP4	65.2	20.5	0.966

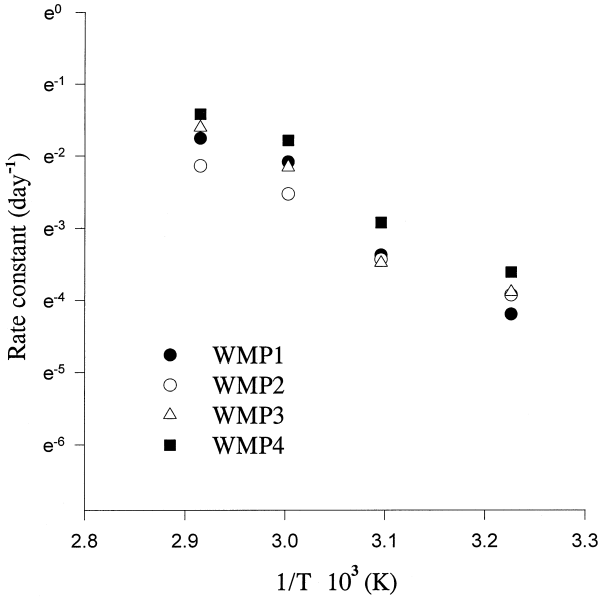

Fig. 3. Arrhenius plot for the formation of fluorescence compounds in whole milk powders.

Table 3
Experimental and predictive values of fluorescence intensity in whole milk powders during storage at 60°C

Time (days)	WMP1 exp./pred.	WMP2 exp./pred.	WMP3 exp./pred.	WMP4 exp./pred.
0	1.40[a]	0.99	1.56	1.50
7	2.07/2.06 (0.63)[b]	1.36/1.48 (8.75)	1.85/2.24 (20.9)	2.08/2.44 (17.3)
14	3.15/2.71 (13.8)	2.22/1.97 (11.4)	2.94/2.92 (0.85)	3.94/3.38 (14.2)
22	4.17/3.47 (16.9)	2.71/2.53 (6.79)	4.02/3.69 (8.23)	4.85/4.45 (8.16)

[a] The initial fluorescence intensity (FI_0).
[b] %error: |experimental−predicted|/experimental×100.

values and the predictive values are close with no statistical difference between them ($P > 0.05$).

In conclusion, fluorescence accumulation in whole milk powder is clearly dependent on the time and temperature of the oxidation process. These results suggest that the measure of fluorescence intensity should be taken into account as an oxidation index in whole milk powders. Obviously, more research should be done to separate and characterize the specific fluorescence compounds correlated with oxidation in whole milk powders.

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