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The Kinetics of Lumichrome in Skim Milk Using Nonlinear Regression Analysis*

S. L. Palanuk & J. J. Warthesen†

University of Minnesota, Department of Food Science and Nutrition, 1334 Eckles Avenue, St. Paul, MN 55108, USA

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ABSTRACT

The kinetics of the formation and subsequent degradation of lumichrome, a photodegradation product of riboflavin, were studied and correlated to the degradation of riboflavin in skim milk. High performance liquid chromatography was used to monitor the riboflavin and lumichrome concentrations. Lumichrome concentration in skim milk increased, then leveled off with continued light exposure. A nonlinear regression model was used to estimate the rates of lumichrome formation and degradation. A yield factor representing the amount of riboflavin that converts to lumichrome was estimated at 23% for the experimental conditions used in this study.

INTRODUCTION

Numerous studies have shown that riboflavin oxidizes in milk when exposed to light (Holmes & Jones, 1944; Stull, 1953; Parks & Allen, 1977). Degradation products of this irreversible photolysis reaction differ, depending on experimental conditions. When exposed to light under alkali conditions, riboflavin loses the ribityl side chain to form lumiflavin. In acidic or neutral solutions, riboflavin is photochemically converted to lumichrome.

Limited information about the irreversible conversion of riboflavin to

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† To whom all correspondence should be addressed.

lumichrome in food systems has been reported in the literature. Parks & Allen (1977) studied riboflavin photodegradation in milk and determined that lumichrome is the major degradation product. Woodcock *et al.* (1982) suggested that lumichrome is not the only or final riboflavin degradation product in enriched pasta but with continued light exposure, lumichrome may undergo further degradation. Furuya *et al.* (1984) found that when a riboflavin phosphate buffer solution is exposed to a light intensity of 1614 lux at 4°C, the content of lumichrome increases. However, as riboflavin continued to degrade, the lumichrome content leveled over time and did not account for all the riboflavin that was degraded. Because of the photoinstability and interdependence of these compounds, unique approaches are needed to study the kinetics of riboflavin and lumichrome reactions in milk.

The objective of this study was to determine the rate of lumichrome formation and degradation relative to riboflavin photodegradation in skim milk. By utilizing a differential model equation derived with specific assumptions and restrictions, we could estimate parameters for riboflavin and lumichrome that otherwise could not be easily determined.

MATERIALS AND METHODS

Light exposure conditions and sample preparation

Custom-built light chambers were maintained at a constant light intensity of 1614 lux and 4°C. Constant light intensity was provided by standard fluorescent lights (General Electric, cool white, No. F1518-CW). Light intensity was monitored with a General Electric type 214 light meter.

Commercial skim milk used in the study was purchased in paperboard containers to minimize the chances of prior light exposure. For each experiment the required quantity of milk was pooled and mixed prior to filling test containers.

Pyrex glass test-tubes (16 mm × 125 mm) were filled completely with skim milk, capped and placed on their sides within the light chamber. Triplicate samples were drawn randomly at selected time intervals up to 120 h. Riboflavin and lumichrome data from three separate experiments were combined and analyzed.

Extraction and chromatography conditions

The extraction method of Rashid & Potts (1980) was used for riboflavin and lumichrome in milk samples. A high performance liquid chromatography

method similar to that of Furuya *et al.* (1984) was used. The HPLC system consisted of a Model 6000A pump (Waters Associates, Inc.), Model 7120 Rheodyne injector with a 10 μl loop, Model FS 950 Fluoromat Fluorometer (Kratos, Inc.), and a Hewlett Packard Model 3380A recorder–integrator. The excitation and emission filters used for the analysis of riboflavin were 7–59, 3–70 and for lumichrome were FSA403 and FSA426. A mobile phase consisting of 50% deionized water, 49% methanol, and 1% glacial acetic acid was pumped through a Lichrosorb C18 column (25 cm × 4·6 mm, i.d.; 10 μm particles, Altex) at a flow rate of 1·5 ml/min. Riboflavin and lumichrome were quantified by comparing peak heights of standards with known concentrations to those of samples.

Data analysis

A degradation rate constant for riboflavin data was determined using linear regression (e.g. Weisberg, 1985). Nonlinear regression (Ralston, 1979) was used to estimate the following variables from the lumichrome data: (1) lumichrome formation and degradation rates, (2) riboflavin degradation rate and (3) a yield factor.

RESULTS AND DISCUSSION

Riboflavin photodegradation in milk follows first order kinetics (Sattar *et al.*, 1977; Allen & Parks, 1979). In this experiment the first order rate constant $\pm$ stardard error for riboflavin photodegradation as calculated using the riboflavin data is equal to $0{\cdot}0253 \pm 0{\cdot}0005\ h^{-1}$.

A plot of lumichrome concentration versus time is presented in Fig. 1. Lumichrome was not detected in unexposed skim milk samples. Lumichrome concentration in light exposed skim milk appears to follow a two-phase rate of increase. These results are similar to those of Woodcock *et al.* (1982) and Furuya *et al.* (1984). They found that the amount of lumichrome increases as riboflavin degrades, then levels off with continued light exposure. The leveling off of lumichrome concentration is due to a combination of lumichrome photodegradation and a decrease in the precursor riboflavin. Data scattering at extended exposure times reflect experimental variability in lumichrome formation and degradation.

A traditional, linear regression analysis of lumichrome was not appropriate due to the instability of lumichrome and incomplete molar conversion of riboflavin to lumichrome. Nonlinear regression analysis of lumichrome data can estimate formation and degradation rates. A set of nonlinear equations (Fig. 2) was derived as a solution to a series of first order

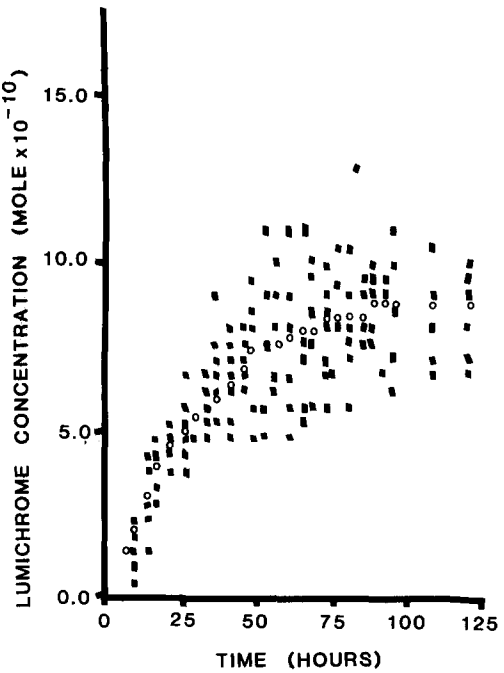

Fig. 1. Plot of lumichrome concentration in skim milk (■) exposed to light (1614 lux) for 120 h at 4°C with an overlay of points (○) as estimated with the kinetic model from the differential equation using nonlinear regression.

$$L_t = (k_3)(k_2)(R_0)\frac{\exp(-k_2\theta) - \exp(-k_1\theta)}{k_1 - k_2}$$

$$k_3 = (k_2)(YF)\frac{\text{Molecular Weight of Riboflavin}}{\text{Molecular Weight of Lumichrome}}$$

YF = Yield factor
k_1 = Lumichrome degradation rate
k_2 = Riboflavin degradation rate
k_3 = Lumichrome formation rate
θ = Time of exposure (h)
R_0 = Initial riboflavin concentration
L_t = Lumichrome concentration at θ

Fig. 2. A solution to a series of differential equations used to describe the kinetic parameters of lumichrome and riboflavin photodegradation.

differential equations that describe the kinetics of riboflavin and lumichrome. This equation was used to estimate lumichrome degradation rate (k_1), lumichrome formation rate (k_3), riboflavin degradation rate (estimated from lumichrome data) (k_2), and a yield factor (YF). The yield factor gives an indication of the amount of degraded riboflavin that forms lumichrome.

Use of the equation requires the following assumptions and restrictions:

1. The lumichrome photodegradation rate is slower than the lumichrome formation rate. If this were not true, lumichrome would not be detected.
2. Lumichrome follows a first-order rate of formation. This is not unreasonable since lumichrome is structurally similar and a direct breakdown product of riboflavin (a compound that follows first-order photodegradation).
3. The estimated riboflavin degradation rate (using lumichrome data) is restricted to a value between 0·0258 and 0·0248 h^{-1}. This is within two standard errors of the calculated degradation rate (riboflavin data).
4. The yield factor (amount of riboflavin that converts to lumichrome) is restricted to a value between 0% and 100%.

The function estimated by the nonlinear model equation (o) is presented in Fig. 1. The nonlinear function appears to overestimate the amount of lumichrome concentration at early exposure times. This could be because early data points are close to the detection limit of lumichrome and at low levels, the measurement error is larger. The model equation is weighted to estimate the kinetic rates and yield factor within the region of least data variability. While the data scattering increases with time, the model predicts a level of lumichrome that is consistent with the actual points. For practical reasons related to storage stability of skim milk, light exposure times of less that 50 h would be the most important under actual milk distribution conditions.

The estimated rates from the model equation are given in Table 1. The estimated rate constant of riboflavin degradation is 2·8 times greater than the rate constant of lumichrome formation, and the lumichrome formation rate constant is 6·3 times greater than the lumichrome degradation rate constant. The combined effect of both estimated rates results in the observed initial increase in lumichrome content followed by leveling off. The model predicts that 23·4% of the riboflavin is degraded to lumichrome. This low yield factor may be due to the formation of intermediate or other additional degradation products of riboflavin or lumichrome may bind to other milk components and not be detected by the analysis techniques.

TABLE 1
Kinetic Rates and Yield Factor as Estimated by Nonlinear Regression

Variable	*Estimated value*
k_1 (Lumichrome degradation rate)	0·001 4 h^{-1}
k_2 (Riboflavin degradation rate)	0·024 8[a] h^{-1}
k_3 (Lumichrome formation rate)	0·008 8 h^{-1}
YF (Yield factor)	23·4%

[a] Riboflavin degradation rate as determined from the lumichrome data.

Nonlinear regression enables the kinetic analysis of unstable compounds such as lumichrome by observing their formation and apparent photodegradation. Manipulation of lumichrome data using nonlinear regression provides a direct (quantitative) indicator of riboflavin loss and insight into the photodegradation mechanism. The riboflavin photodegradation rate and yield factor provide information regarding product nutrient and quality loss. The nonlinear model equation described in this paper can provide useful rate estimates for photodegradation reactions of riboflavin and lumichrome not easily obtained in food systems such as milk.

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