

## Photodegradation of Riboflavin in Milks Exposed to Fluorescent Light

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### ABSTRACT

Photodegradation of riboflavin in 50-ml samples of fluid milks exposed to fluorescent light (2690 lux) followed first-order reaction kinetics, and the reaction rate ( $s^{-1}$ ) averaged  $1.86 \times 10^{-5}$  in skim milk and  $1.47 \times 10^{-5}$  in whole milk. Additional evidence led us to conclude that photodegradation of riboflavin proceeds prior to the appearance of a light-induced off-flavor.

### INTRODUCTION

Increasing emphasis on the nutritional aspects of foods has renewed interest in photochemical reactions in milk from exposure to light. Hence, major emphasis has been placed on the photodegradation of riboflavin since milk contributes 40 to 50% of the total dietary riboflavin in the United States and many other western nations (5). The extent of riboflavin photodegradation in fluid milk depends on numerous factors including the wavelength of light, the intensity of light, exposure time, protective effect of the milk container, surface area of milk in relation to volume in the package, and temperature of the product (4, 8). Whereas factors influencing the extent of photodegradation have been investigated extensively (8), kinetics of the photochemical reaction in milk have had scant attention. Singh et al. (9) on limited data indicated that "riboflavin degradation in milk can be best described by assuming a first order reaction." From a practical standpoint their studies emphasized the utility of kinetic studies in selecting optimum storage conditions for preventing photodegradation of

riboflavin in milk. A recently developed procedure for quantitating riboflavin content in fluid milks lends itself readily to this type of investigation and forms the basis of this study. Objectives were 1) to determine if riboflavin is photodegraded prior to development of an off-flavor in whole milk exposed to light, 2) to compare reaction rates of whole and skim milk, and 3) to determine the reaction order of photodegradation of riboflavin in fluid milks.

### MATERIALS AND METHODS

Fifty milliliter samples of commercial milk in  $20 \times 2.5$  cm pyrex screw cap culture tubes were exposed to fluorescent light (2690 lx) for 1 to 32 h at 2.5 C in a walk-in cooler. The light source consisted of two 15 watt cool white fluorescent bulbs (Westinghouse F15T8/CW)<sup>2</sup> positioned 15.25 cm above the sample tubes which were placed in a horizontal position. Following exposure, the samples and a distilled water control were passed through (flow rate 2.5 ml/min) individual columns of Amberlite XAD-4 prepared in the following manner: 4.0 g of resin in redistilled methanol were added to a  $53 \times .8$  cm glass buret which was fitted with a teflon stopcock and plugged with glass wool. The resin was washed consecutively with 75-ml portions of methanol, acetone, and distilled water at a flow rate of 2.5 ml/min, with the solvent level kept above the resin at all times. A plug of glass wool was added to the top of the resin before the sample was introduced. Following passage of the sample through the column, the resin was washed free of unabsorbed sample with 75 ml of distilled water, and the absorbed constituents were eluted with 30 ml of methanol. The methanol effluent was evaporated to dryness in a  $8.5 \times 2.3$  cm screw cap vial.

### Silicic Acid Chromatography

Isolation of riboflavin from other XAS-4 absorbed materials, including lumichrome, was accomplished by silicic acid chromatography in

the following sequence of steps:

*Step 1.* 1.5 g of silicic acid (Mallinckrodt 100-mesh), previously washed (6) and vacuum oven dried (65 C and .064 atm for 16 h), was slurred in methylene chloride and added to a 15 × 1.0 cm chromatographic column plugged with glass wool. The silicic acid was washed with 15 ml of methylene chloride, and a small wad of glass wool was added to the top of the column.

*Step 2.* One-tenth milliliter of methanol was added to the sample vial followed by 9.9 ml of methylene chloride. The contents were mixed thoroughly and added to the silicic acid column. Following percolation of the sample, the silicic acid was eluted with 10 ml of 1% methanol in methylene chloride.

*Step 3.* The sample vial was rinsed with two 2.0-ml portions of 2% methanol in methylene chloride. The solution was added to the column, and the silicic acid was eluted with 25 ml of 2% methanol in methylene chloride.

*Step 4.* The sample vial was rinsed with two 2.0-ml portions of 7.5% methanol in methylene chloride. The rinses were added to the column and the silicic acid was eluted with 20 ml of 7.5% methanol in methylene chloride.

*Step 5.* The sample vial was rinsed with two 2.0-ml portions of 10% methanol in methylene chloride. The rinses were added to the column, and riboflavin was eluted with 40 ml of 10% methanol in methylene chloride.

#### Quantitative Determination

The riboflavin fraction was evaporated to dryness under a stream of nitrogen in an 8.5 × 2.3 cm screw cap vial. The dried sample was dissolved in 3 ml of distilled water by gentle

shaking and warming the solution in a water bath at 40 C. The sample was cooled to room temperature, and the amount of riboflavin was determined spectrophotometrically at 445 nm, with a molar absorptivity of 11,800. Preliminary studies on known concentrations of riboflavin showed an 80% recovery following XAD-4 extraction and silicic acid chromatography. Therefore, the concentrations determined were adjusted accordingly.

All experimental procedures for the isolation and quantitation of riboflavin from exposed and unexposed samples were without direct lighting.

#### RESULTS AND DISCUSSION

The nanamoles of riboflavin remaining in 50-ml samples of whole and skim milks exposed to fluorescent light for 1 to 32 h under the conditions of this study are in Table 1. Photodegradation was kinetically of a first-order type and could be expressed mathematically by  $kt = \ln a^0/a$ , where  $k$  is the rate constant,  $a^0$  the initial concentration, and  $a$  equals the nm of riboflavin remaining at time  $t$ . By this equation the slope of a straight line, by least squares, corresponding to the reaction rate was obtained. Figure 1 illustrates the conformity of the experimental data of whole milk sample 1 ( $r = .99$ ) and skim milk sample 1 ( $r = .98$ ) to the equation. The reaction rates for all samples are in Table 2.

The role of riboflavin in the photochemical reactions leading to off-flavors in fluid milk is well established (8). Storgards and Ljungren had concluded (10) that skim milk was more readily susceptible than high fat fluid products to off-flavors. It was not unexpected, therefore,

TABLE 1. Effect of time on riboflavin concentration in fluid milks exposed to 2690 lx of fluorescent light.

Milk sample	nM Riboflavin/50 ml fluid milk							
	Exposure time (h)							
	0	1	2	4	8	16	24	32
Whole 1	160.6	149.5	146.3	135.4	113.0	65.7	....	29.3
2	149.7	144.9	144.9	132.2	104.5	74.7	....	35.6
3	159.0	150.3	143.9	118.1	105.9	59.0	....	29.8
4	156.7	149.4	133.5	108.3	92.3	49.7	35.4	25.8
5	131.4	122.6	117.8	92.6	67.0	44.9	30.6	22.9
Skim 1	138.3	123.1	118.4	97.9	58.3	35.9	20.21	15.4
2	137.2	126.0	124.5	99.2	75.5	58.0	....	18.1
3	166.2	163.1	155.1	136.1	61.1	43.7	....	19.9

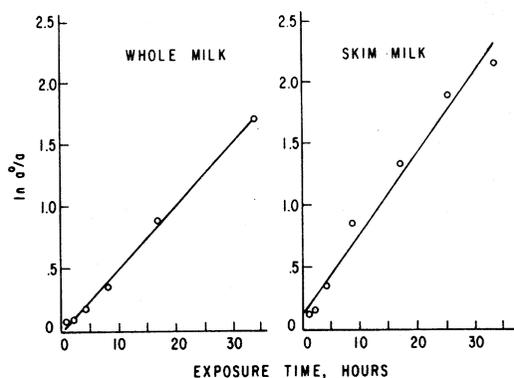


Figure 1. First order reaction plot of riboflavin in whole and skim milk exposed to 2690 lux of fluorescent light.

that the reaction rates for the photodegradation of riboflavin in the skim milk samples were greater. The lower reaction rate on whole milk sample 2, although not readily explained, may reflect differences in processing conditions (10), length of storage prior to exposure (2), and/or a higher fat content relative to the other whole milk samples.

Reaction rates do not reflect reaction rates obtainable on representative samples of milk exposed under similar light conditions in commercial milk containers. Previous studies (1) demonstrated that light of wavelength 436 nm penetrates whole and skim milk to an effective depth of 1.0 and 1.2 cm. Hence, the photodegradation of riboflavin in undisturbed milk samples would be confined to that portion of the milk in relatively close proximity to the light source. The experimental results on representative samples of milk exposed to light in commercial milk containers, nevertheless, should fit the equation for first-order kinetics. In this respect, an analysis of the data in Hansen et al. (3) on representative samples of whole milk exposed to 2152 lx of fluorescent light in .95-liter polyethylene containers revealed that the photodegradation of riboflavin in that study conformed to first-order kinetics and  $k(s^{-1}) = 5.0 \times 10^{-7}$ .

Hansen et al. (3) suggested that riboflavin was not photodegraded prior to the onset of off-flavors in fluid whole milk because they detected no changes in the riboflavin content of fluid milks at the time of flavor development. Flavor studies on the whole milk samples

TABLE 2. Specific reaction rates of riboflavin photodegradation in fluid milks.

Milk sample	Reaction rate $k (s^{-1})$
Whole 1	$1.50 \times 10^{-5}$
2	$1.28 \times 10^{-5}$
3	$1.47 \times 10^{-5}$
4	$1.58 \times 10^{-5}$
5	$1.52 \times 10^{-5}$
Skim 1	$1.90 \times 10^{-5}$
2	$1.75 \times 10^{-5}$
3	$1.92 \times 10^{-5}$

revealed that whereas off-flavors were not obvious prior to 4 h of exposure, a decrease in the riboflavin content and qualitative detection of lumichrome (7) was determined readily following 2 h of exposure to fluorescent light. These observations, in addition to the evidence showing that the photodegradation of riboflavin conforms to first-order reaction kinetics, without an apparent induction period, led us to conclude that riboflavin is photodegraded prior to appearance of an off-flavor.

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