RESEARCH PAPERS

Effect of Fluorescent Light on Repartition of Riboflavin in Homogenized Milk

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ABSTRACT

This study was designed to determine the effect of fluorescent light on riboflavin in homogenized milk and in the resulting fat, casein, and acid whey isolated therefrom. The major proportion (82%) of riboflavin in homogenized milk was associated with the acid whey fraction, about 15% with the casein phase and about 3% with the fat phase. Riboflavin loss due to light exposure (2150 lx for 48 h at 7 C) was greatest in the whey fraction. Further separation of the whey proteins by gel filtration on Sephadex G-75 superfine indicated that the riboflavin associated with the high molecular weight fraction decreased following exposure. In the whey fraction, 95% of the riboflavin was in the free form and not associated with the major whey proteins. The free riboflavin was most labile to light exposure.

INTRODUCTION

The exposure of homogenized, pasteurized whole milk to fluorescent light destroys a portion of the riboflavin and affects the flavor of milk. Losses of up to 28% of riboflavin have been reported by Sattar and deMan (21), who subjected milk stored in a clear polyethylene pouch to 2150 lx of fluorescent light for 24 h at 4 C. Similarly, Dimick (7) reported 10 to 17% riboflavin destruction in milk held in glass and plastic containers exposed to 1075 lx for 72 h at 7 C. Hansen et al. (12) demonstrated losses of 21% in milk exposed to 2150 lx for 144 h at 3.3 C. Hedrick and Glass (13) exposed homogenized whole milk to 1612 lx for 24 h at 6 C in plastic containers and reported riboflavin losses of about 13%. Singh et al. (22) in a kinetic analysis of riboflavin destruction, found 11% loss in milk held in glass and blow-molded polyethylene containers exposed to 3230 lx of fluorescent light for 48 h at 4.4 C. Thus, riboflavin degradation can range from approximately 10 to 28% in milk stored in transparent containers illuminated at 1000 to 3000 lx of cool white fluorescent light for 24 to 72 h. Exposure of the milk to light also brings about a light-induced flavor which may influence acceptance of the milk by the consumer. Riboflavin has been implicated in the development of this off-flavor, which is attributed to the formation of methional (3-methyl-thiopropanal) through the photolysis of methionine photosensitized by the riboflavin (20). Recently methional has been identified positively in light exposed skim milk (1). Storgards and Lundquist (24) also believed that methionine was responsible for the off-flavor through the oxidation of methionine, without the amino acid being split off from the whey protein molecule. They reported that this seems possible "since some of the riboflavin appears to be linked to the serum proteins." Lipoproteins have been reportedly acted upon by riboflavin, resulting in a degradation of methionine, tryptophan, tyrosine, cystine, and lysine (10). Protein degradation is involved in the off-flavor (26) and more specifically the serum proteins and not the casein or free amino acids (5, 14, 24). The association of riboflavin with proteins has been reported (2, 9, 17, 18, 19) in the form of flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN) with free riboflavin amounting to 54% to 95%.

Other researchers working with model systems evaluated the binding effectiveness of the riboflavin to various milk proteins (15). Furthermore, Aurand et al. (4) indicated that milk proteins form a loosely bound complex with riboflavin. This complex is thought to depend on tryptophan in protein. This is correlated with the fact that, upon exposure to light under

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aerobic conditions, the amounts of tryptophan and riboflavin decrease. This decrease also had a direct relationship to the intensity of the flavor development (23).

The purpose of this study was to localize the riboflavin in homogenized milk and determine the effect of light on its concentration in the different phases of milk.

EXPERIMENTAL PROCEDURES

Mixed herd milk routinely supplied to the university creamery was standardized to 3.25% milk fat, homogenized (176 kg/cm^2), and pasteurized (74 C for 16 s). The milk samples, contained in sterilized 25 ml borosilicate vials (surface-to-volume ratio—1.4:1), were exposed to fluorescent light in a refrigerated (7 ± 1 C) sliding door display case. The light intensity was 2150 lx and the duration of exposure was 24 h or 48 h, during which time the samples were agitated periodically. Lighting consisted of 40 W cool white fluorescent lamps (F40CW) mounted parallel to the shelves and located 15 cm from the vials. The control samples were held in an unlighted refrigerator at the same temperature as the refrigerated display case.

Following light exposure, the milk was separated into cream, clear supernatant, and casein by ultracentrifugation with a fixed angle rotor in an International Preparative Ultracentrifuge, Model B-35 at a speed of 30,000 rpm for 105 min. The resulting cream was subjected to a total lipid extraction using the Folch procedure (11). The lower organic phase isolated in this procedure contained all the fat with no evidence of riboflavin, whereas the upper aqueous phase contained the water soluble materials, mainly proteins and riboflavin. The volume of the aqueous phase was measured and transferred into a low actinic flask for extraction and determination of the total riboflavin content. All procedures were in subdued light.

The clear supernatant from the homogenized milk was acidified to pH 4.67 with 1N HCl and recentrifuged at 27,000 rpm for 60 min. This resulted in an acid whey fraction and a residual casein fraction. The residual casein and the casein from the first ultracentrifugation were pooled, washed with a .1 M sodium acetate-acetic acid buffer (pH 4.67) and centrifuged at 3000 rpm for 20 min at room temperature in an International Centrifuge, Model K. The casein plug then was dissolved in a phosphate buffer (pH 6.90) by a Potter Elvehjem homogenizer. The amount of protein in the casein homogenate was measured by the dye binding method, using a Pro Milk (Foss Electric) Analyzer and amido black dye. The analyzer was calibrated for whole milk; thus, it slightly underestimated the amount of casein.

The acid whey was fractionated into its major protein fractions on a glass column (2.5 × 45 cm) of Sephadex G-75, superfine (Pharmacia, Piscataway, NJ) with an ascending acetate

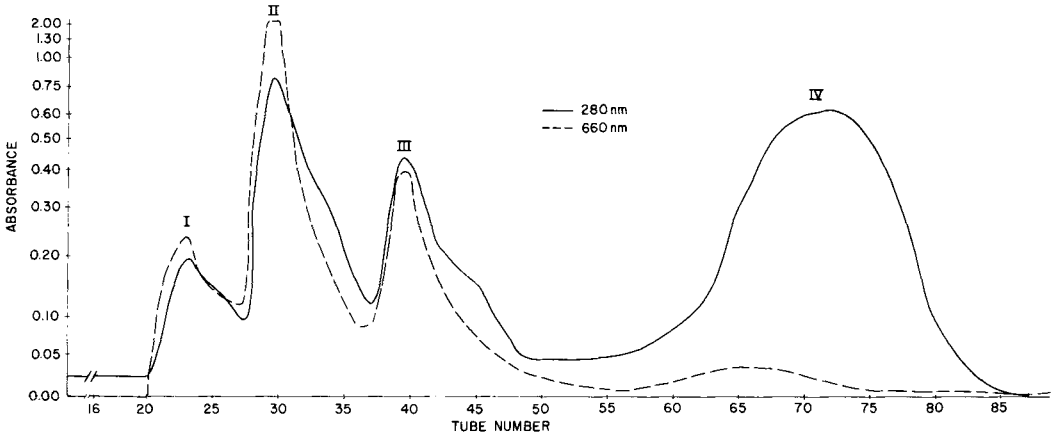

FIG. 1. Schematic representation of the absorbance of the acid whey protein fractions from homogenized milk eluted on Sephadex G-75. Absorbance at 660 nm represents the protein content by the Lowry procedure (16).