Effects of Ascorbic Acid on the Light-Induced Riboflavin Degradation and Color Changes in Milks

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Effects of ascorbic acid (0.025, 0.05, and 0.1%, w/v) on the light-induced riboflavin degradation and color changes in whole and skim milks were studied during 10 h fluorescent light illumination at 7 ± 2 °C. As the time of light illumination increased, the riboflavin content in milks greatly decreased, resulting in 30.1 and 59.1% reduction of riboflavin in whole milk and skim milk after 10 h exposure to fluorescent light, respectively. The ascorbic acid treatment effectively protected the photodegradation of riboflavin in both whole milk and skim milk, and its effectiveness is concentration dependent. The 0.1% ascorbic acid treatments resulted in 50.0 and 25.5% inhibition of riboflavin reduction in whole milk and skim milk after 10 h fluorescent light illumination, respectively. The color parameters of lightness (L), greenness ($-a$), and yellowness (b) in both whole milk and skim milk decreased after light illumination. Ascorbic acid treatment also protected effectively the changes in greenness and yellowness in both milks during the light illumination.

Keywords: *Riboflavin; color change; fluorescent light; ascorbic acid; skim milk*

INTRODUCTION

Light exposure leads to adverse effects on riboflavin contents and color in milks. Milk is the most important source of riboflavin in the diets in the United States and many other nations. The light-induced degradation of riboflavin in milk reportedly followed first-order reaction kinetics (Singh et al., 1975; Allen and Parks, 1979). The extent of riboflavin photodegradation in fluid milk depends on numerous factors such as the wavelength of light, light intensity, exposure time, types of the milk container, surface area of milk in relation to volume and temperature (Dunkley et al., 1962; Hedrick and Glass, 1975; Herreid et al., 1952; Maniere and Dimick, 1976). Since riboflavin gives yellowish green color to milk, color changes in milks by light exposure is, to at least some extent, related to the photodecomposition of the riboflavin in the milks. But the light-induced riboflavin destruction may not be the sole reason for the color changes in milks exposed to light. Toba et al. (1980) reported that milk becomes slightly brown after 1−2 h exposure to the direct sunlight. The authors concluded that the color change was related to the degradation of tryptophan and tyrosine in milk. Under light, riboflavin reportedly produces superoxide anions and singlet oxygen (Aurand et al., 1975; Buettner and Need, 1985) and accelerates the oxidation of tryptophan and other amino acids (Kanner and Fennema, 1987; Toba et al., 1980). The photodecomposition of riboflavin might be related to the self-sensitized production of the active-oxygen species, superoxide anion and singlet oxygen. Ascorbic acid reportedly has strong quenching ability for both superoxide anion and singlet oxygen (Gotoh and Nike, 1992; Jung et al., 1995a,b) and effectively decreases riboflavin-sensitized photooxidation of tryptophan (Kanner and Fenneam, 1987). Thus, we expected that addition of ascorbic acid might protect the riboflavin degradation and color changes in milk during light illumination.

Even though the riboflavin reduction and color changes in milks under light have been extensively studied, no attempt has been previously made to reduce the photodegradation of riboflavin in milks by treating active-oxygen quenchers. The objective of this research is to study the qualitative and quantitative effects of addition of ascorbic acid, an effective active-oxygen quencher, on the riboflavin reduction and color changes in whole and skim milks during fluorescent light illumination.

MATERIALS AND METHODS

Materials. Riboflavin was purchased from Sigma Chemical Co. (St. Louis, MO). Whole milk was obtained from a Chungnam National University Dairy Farm, Daejeon, Korea. Skim milk was obtained by removing the oil layer after centrifugation 3 times at 6000 rpm.

Light Storage. To study the effects of ascorbic acid on the light-induced changes in riboflavin content and color in milks, calculated amounts of ascorbic acid (0.025, 0.05, and 0.1%, w/v) was added to whole milk and skim milk. The treated milk samples (30 mL) were, in duplicate, transferred into 35-mL transparent glass serum bottles. The bottles were sealed with rubber septa and aluminum caps and stored in a light storage box which was described in detail by Fakourelis et al. (1978) and Jung et al. (1991, 1995a). The light intensity at the sample level was 3300 lux. The light storage box was placed in a 4 °C working cooler, and the temperature within the light storage box was 7 ± 2 °C during light illumination. After 1, 2, 3, 5, and 10 h light illumination, the sample bottles were taken out, and riboflavin contents and color changes in the milks were determined.

Effects of sodium azide (5.68 mM), a well-known singlet oxygen quencher, on the changes in riboflavin and color in skim milk were also studied to monitor the singlet oxygen involvement in the light-induced riboflavin degradation and color change in milks.

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S0021-8561(97)00708-5 CCC: $15.00
Published on Web 01/06/1998

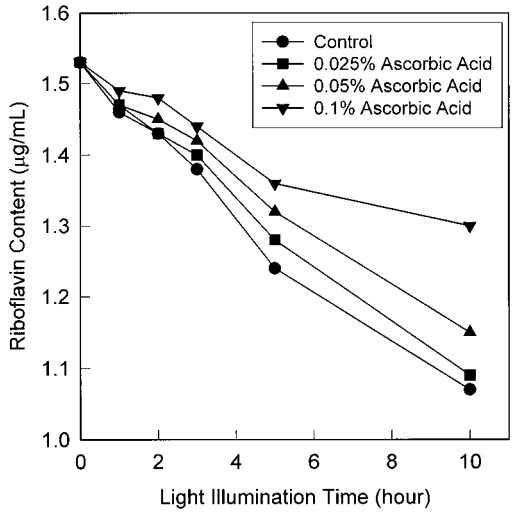

Figure 1. Effects of ascorbic acid on the riboflavin content in whole milk during fluorescent light illumination at 7 ± 2 °C. Mean values at the same light storage time with a different italicized letter are significantly different ($p < 0.05$).

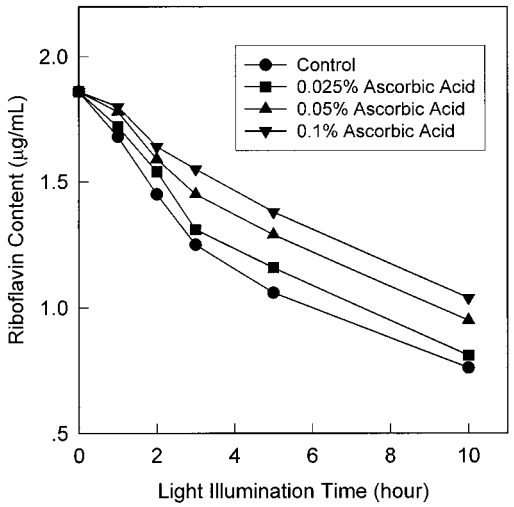

Figure 2. Effects of ascorbic acid on the riboflavin content in skim milk during fluorescent light illumination at 7 ± 2 °C. Mean values at the same light storage time with a different italicized letter are significantly different ($p < 0.05$).

High Performance Liquid Chromatographic Analysis for Riboflavin. To extract the riboflavin, 0.3 mL of 33.3% aqueous acetic acid solution and 0.3 mL of 33.3% aqueous sodium acetate solution were added to the milks. Then, the sample was stirred slowly for 5 min. The samples then were centrifuged to obtain a top layer. Casein was precipitated at the bottom layer. The top layer was taken and filtered through 0.2 μm membrane filter. The filtered sample was injected into a HPLC (Spectra-Physics, Fremont, CA) (Ashoor et al., 1983). The column used was a Versapack C18 (10 μm, 4.1 × 250 mm, Alltech Associates, Inc., Deerfield, IL). The flow rate of mobile phase (water/methanol/acetic acid, 68:32: 0.1, v/v/v) was 1.0 mL/min. The detection was made at 270 nm by using a UV1000 Spectra System detector (Spectra-Physics). During the extraction and analysis, special care was taken to protect all the samples from light.

Color Parameters. To determine the color changes in skim milk during light illumination, the Hunter *L*, *a*, *b* values were determined by a Minolta CR 300 colorimeter (Tokyo, Japan).

Statistical Analysis. All the experiments were carried out in duplicate, and the statistical analysis was done by using the Statistical Analysis System (SAS Institute, Inc., 1982). Duncan's multiple range test was used to ascertain the treatment effects on the riboflavin contents and color parameters in skim milk.

RESULTS AND DISCUSSION

The effects of ascorbic acid addition on the riboflavin content in whole milk and skim milk during light storage are shown in Figures 1 and 2. As the light storage time increased, the riboflavin content in both whole milk and skim milk decreased considerably. The contents of riboflavin in whole milk after 0, 1, 2, 3, 5, and 10 h light storage were 1.53, 1.46, 1.43, 1.38, 1.24, and 1.07 μg/mL, respectively (Figure 1). The result showed that 1, 2, 3, 5, and 10 h fluorescent light illumination induced 4.6, 6.5, 19.0, and 30.1% reduction of riboflavin in whole milk, respectively. The contents of riboflavin in skim milk after 0, 1, 2, 3, 5, and 10 h light storage were 1.86, 1.68, 1.45, 1.25, 1.06, and 0.76 μg/mL, respectively (Figure 2). That is, the 1, 2, 3, 5, and 10 h light illumination induced 10.7, 22.0, 32.8, 43.0, and 59.1% reduction of riboflavin in skim milk, respectively. The results in both Figures 1 and 2 indicated that the light-induced destruction of riboflavin was slower in whole milk than in skim milk. Similar results were also previously reported by Allen and Park (1979). The riboflavin reduction in milks under light might be due to the self-sensitized production of active-oxygen species such as superoxide anion and singlet oxygen which are extremely reactive species (Aurand et al., 1975; Buettner and Need, 1985).

The addition of ascorbic acid, an active oxygen quencher, greatly inhibited the light-induced destruction of riboflavin in both whole and skim milks. The riboflavin contents in whole milk treated with 0, 0.025, 0.05, and 0.1% (w/v) ascorbic acid after 10 h storage were 1.07, 1.09, 1.15, and 1.30 μg/mL, respectively. That is, the 0.025, 0.05, and 0.1% ascorbic acid treatments resulted in 4.3, 17.4, and 50.0% inhibition of riboflavin reduction in the whole milk after 10 h fluorescent light illumination, respectively (Figure 1). Duncan's multiple range test showed that 0.05 and 0.1% ascorbic acid treatments were significantly different in the riboflavin content from the control after 10 h illumination ($p < 0.05$). Figure 2 showed that the 0.025, 0.05, and 0.1% ascorbic acid treatments resulted in 12.9, 28.7, and 40.0% inhibition of riboflavin reduction in the skim milk after 5 h light illumination, respectively. Duncan's multiple range test showed that each treatment was significantly different in the riboflavin content from each other at 5 h light storage ($p < 0.05$). After 10 h light illumination, the 0.025, 0.05, and 0.1% ascorbic acid treatment in skim milk inhibited 5.5, 17.3, and 25.5% riboflavin reduction in the skim milk, respectively. The riboflavin contents in the skim milks treated with 0.05% and 0.1% ascorbic acid were significantly higher than that of control after 10 h illumination ($p < 0.05$).

Light illumination also induced the changes in color parameters of the whole milk (Table 1). The Hunter *L* (lightness) value slightly decreased from 71.84 to 70.81 after 10 h light illumination. The greenness ($-a$) and the yellowness ($+b$) of whole milk, however, considerably decreased after 10 h light illumination. The greenness ($-a$) decreased from −1.91 to −0.55, and yellowness ($+b$) decreased from 4.73 to 3.16 after 10 h light illumination. The ascorbic acid treatment greatly inhibited the light-induced color changes in greenness ($-a$) and yellowness ($+b$) of the whole milk during fluorescent light illumination. The color parameters of

Table 1. Effects of Ascorbic Acid on the Color Parameters in Whole Milk during 10 h of Fluorescent Light Illumination at 7 ± 2 °C[a]

color property	ascorbic acid treatment (%)	light storage time (h) 0	2	5	10
lightness (L)	0	71.84[a]	71.27[a]	71.08[a]	70.81[a]
	0.025	71.81[a]	71.44[a]	71.79[a]	70.75[a]
	0.05	71.64[a]	71.36[a]	72.04[a]	71.47[a]
	0.1	71.87[a]	71.77[a]	71.41[a]	70.90[a]
greenness ($-a$)	0	−1.91[a]	−1.48[c]	−1.23[b]	−0.55[b]
	0.025	−1.85[a]	−1.70[b]	−1.47[a]	−1.21[a]
	0.05	−1.84[a]	−1.74[ab]	−1.49[a]	−1.22[a]
	0.1	−1.86[a]	−1.77[a]	−1.50[a]	−1.24[a]
yellowness ($+b$)	0	4.73[a]	4.49[a]	3.85[b]	3.16[b]
	0.025	4.63[a]	4.50[a]	3.97[ab]	3.29[b]
	0.05	4.64[a]	4.53[a]	4.03[a]	3.46[a]
	0.1	4.61[a]	4.51[a]	4.00[a]	3.49[a]

[a] Means within each column with the different superscript letters are significantly different at $p < 0.05$.

Table 2. Effects of Ascorbic Acid on the Color Parameters in Skim Milk during 10 h of Fluorescent Light Illumination at 7 ± 2 °C[a]

color property	ascorbic acid treatment (%)	light storage time (h) 0	2	5	10
lightness (L)	0	65.52[b]	65.44[a]	64.30[c]	63.80[d]
	0.025	65.47[b]	65.07[b]	64.89[b]	65.17[b]
	0.05	65.05[c]	64.58[c]	65.46[c]	64.37[c]
	0.1	66.01[a]	64.96[c]	65.35[a]	65.39[a]
greenness ($-a$)	0	−2.44[a]	−2.10[a]	−1.53[d]	−1.06[d]
	0.025	−2.44[a]	−2.11[a]	−1.80[c]	−1.46[c]
	0.05	−2.30[c]	−1.96[b]	−1.84[b]	−1.61[b]
	0.1	−2.36[b]	−2.12[a]	−1.88[a]	−1.68[a]
yellowness ($+b$)	0	3.03[b]	2.44[c]	1.76[c]	1.30[d]
	0.025	3.10[a]	2.47[b]	1.72[d]	1.31[c]
	0.05	2.95[c]	2.36[d]	1.99[b]	1.43[b]
	0.1	3.01[b]	2.52[a]	2.08[a]	1.71[a]

[a] Means within each column with the different superscript letters are significantly different at $p < 0.05$.

the whole milk treated with 0.1% ascorbic acid were 70.90 (L), −1.24 (a), 3.49 (b) after 10 h fluorescent light illumination. Duncan's multiple range test showed that the ascorbic acid treatments were significantly effective in the prevention of color (greenness and yellowness) change in whole milk after 5 and 10 h fluorescent light illumination (Table 1).

The Hunter L (lightness) value decreased from 65.52 to 63.80 in skim milk after 10 h light illumination (Table 2). This result indicated that skim milk became darker after light illumination. The similar result was reported by Toba et al. (1980). The authors (1980) reported that the color changes in skim milk after light exposure were directly related to the oxidation of tryptophan and tyrosine in the milk. The absolute values of greenness ($-a$) and yellowness ($+b$) of skim milk also decreased considerably during light storage. The greenness ($-a$) decreased from −2.44 to −1.06, and yellowness ($+b$) decreased from 3.03 to 1.30 after 10 h light illumination. Addition of ascorbic acid also effectively protected the light-induced color changes in skim milks, and its effectiveness was concentration dependent. The color parameters of the skim milk treated with 0.1% ascorbic acid were 65.39 (L), −1.68 (a), 1.71 (b) after 10 h fluorescent light illumination. Duncan's multiple range test showed that the ascorbic acid treatments were significantly effective in the prevention of color change in skim milk after 5 and 10 h fluorescent light illumination (Table 1). The protective activity of ascorbic acid for the color changes in skim milk is obviously, to some extent, due to the protection of riboflavin in skim milk during light illumination. Since ascorbic acid effectively reduced the riboflavin-sensitized photooxidation of tryptophan (Kanner and Fennema, 1987), the protection of tryptophan by ascorbic acid might be an another possible explanation for the ascorbic acid activity for decreasing the color changes in skim milk during light illumination.

Table 3. Effects of Ascorbic Acid (5.68 mM) and Sodium Azide (5.68 mM) on the Riboflavin Content in Skim Milk during 10 h of Fluorescent Light Illumination at 7 ± 2 °C[a]

treatment	light storage time (hour) 0	1	2	3	5	10
control	1.56[a]	1.32[b]	1.31[b]	1.08[b]	0.95[b]	0.68[b]
ascorbic acid	1.56[a]	1.45[a]	1.45[a]	1.32[a]	1.20[a]	1.00[a]
sodium azide	1.56[a]	1.45[a]	1.45[a]	1.34[a]	1.27[a]	1.02[a]

[a] Means within each column with the different superscript letters are significantly different at $p < 0.05$.

Table 4. Effects of Ascorbic Acid (5.68 mM) and Sodium Azide (5.68 mM) on the Color Parameters in Skim Milk during 10 h Fluorescent Light Illumination at 7 ± 2 °C[a]

color property	treatment (5.68 mM)	light storage time (h) 0	2	5	10
lightness (L)	control	67.38[a]	66.94[b]	66.75[b]	66.26[b]
	ascorbic acid	67.67[a]	67.04[b]	66.91[b]	67.74[a]
	sodium azide	67.24[a]	67.41[a]	67.58[a]	67.56[ab]
greenness ($-a$)	control	−2.47[a]	−2.14[a]	−1.61[c]	−0.83[b]
	ascorbic acid	−2.52[a]	−2.24[a]	−1.98[b]	−1.72[a]
	sodium azide	−2.55[a]	−2.24[a]	−2.13[a]	−1.84[a]
yellowness ($+b$)	control	3.34[b]	2.73[c]	2.12[c]	1.40[c]
	ascorbic acid	3.32[b]	2.85[b]	2.27[b]	1.71[b]
	sodium azide	3.45[a]	2.95[a]	2.53[a]	1.83[a]

[a] Means within each column with the different superscript letters are significantly different at $p < 0.05$.

To study the singlet oxygen involvement in the riboflavin reduction and color changes in milk during light illumination, skim milk treated with 5.68 mM NaN_3, a well known singlet oxygen quencher, was stored for 10 h under fluorescent light, and the changes in riboflavin content and color in skim milk were determined. Table 3 showed the effects of 5.68 mM ascorbic acid and 5.68 mM sodium azide on the riboflavin content in skim milk during 10 h fluorescent light illumination. The 5.68 mM sodium azide and 5.68 mM ascorbic acid (equivalent to 0.1% ascorbic acid) treatments resulted in 38.6 and 36.4% inhibition of riboflavin degradation in skim milk after 10 h illumination. It is well known that sodium azide does not possess oxygen scavenging activity, radical scavenging activity, and superoxide anion quenching ability, but that sodium azide has a strong singlet oxyen quenching ability. Thus, this present result suggested that riboflavin degradation during light illumination was, to a considerable degree, due to the formation of singlet oxygen in the milk system. Since the singlet oxygen quenching ability of ascorbic acid and sodium azide is reportedly close at the same molar basis (Bodannes and Chan, 1979), it is interesting to note that the effectiveness of 5.68 mM ascorbic acid, which is equivalent to 0.1% (w/v) ascorbic acid, was very close to that of 5.68 mM sodium azide. Both 5.68 mM ascorbic acid and 5.68 mM sodium azide also greatly inhibited the changes in greenness and

yellowness in skim milk during fluorescent light illumination as shown in Table 4. The results in Tables 3 and 4 suggested that singlet oxygen are involved in the changes in riboflavin content and color in milk, and that singlet oxygen quenching ability of ascorbic acid might play an important role in its protective activity on riboflavin content and color in milk. However, it can not be excluded that some other mechanisms are involved in the protective activity of ascorbic acid on the light-induced changes in riboflavin content and color changes in milks. Nevertheless, the present paper clearly showed, for the first time, that ascorbic acid effectively inhibited riboflavin reduction and color change in both whole milk and skim milk during illumination with fluorescent light.

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Received for review August 18, 1997. Revised manuscript received October 21, 1997. Accepted October 24, 1997.[⊗]

JF9707086

[⊗] Abstract published in *Advance ACS Abstracts*, December 15, 1997.