JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Photosensitizing Effect of Riboflavin, Lumiflavin, and Lumichrome on the Generation of Volatiles in Soy Milk

RONGMIN HUANG,† HYUN JUNG KIM, AND DAVID B. MIN*

Department of Food Science and Technology, The Ohio State University, 2015 Fyffe Road, Columbus, Ohio 43210

Lumichrome and lumiflavin were formed from riboflavin under light. pH had a significant influence on the formation of lumichrome and lumiflavin from riboflavin. Lumichrome was the only major product from riboflavin under neutral or acidic pH values. Lumiflavin was also formed from riboflavin in basic pH. The maximum concentration of lumiflavin from 100 μM riboflavin at pH 8.5 was 30.9 μM, and it was reached after 2 h of exposure at 1500 lux. The maximum concentration of lumichrome formed from 100 μM riboflavin at pH 4.5, 6.5, or 8.5 was 79.9, 58.7, and 73.1 μM, respectively, after 8, 6, or 2 h of light exposure. The formation of lumichrome and lumiflavin from riboflavin was due to the type I mechanism of the riboflavin photosensitized reaction. Singlet oxygen was also involved in the photosensitized degradation of lumiflavin and lumichrome. The reaction rates of riboflavin, lumiflavin, and lumichrome with singlet oxygen were 9.66×10^8, 8.58×10^8, and 8.21×10^8 M^{-1} s^{-1}, respectively. The headspace oxygen depletion and headspace volatile formation were significant in soy milk containing lumichrome or lumiflavin under light ($p < 0.05$) and were insignificant ($p > 0.05$) in the dark. Ascorbic acid could inhibit the total volatile changes of soy milk under light. Soy milk should be protected from light to prevent the photodegradation of riboflavin and the oxidation of soy milk.

KEYWORDS: Riboflavin; lumiflavin; lumichrome; photosensitized oxidation; ascorbic acid

INTRODUCTION

Riboflavin (RF) exists in milk, eggs, meats, vegetables (*1*), and many other food products (*2*, *3*). Because of its complex photochemical properties (*4*−*9*), RF has been extensively studied as a photosensitizer in food (*10*) and biological systems (*11*−*13*). In addition to being an effective photosensitizer to produce singlet oxygen, RF itself is a good reactant for singlet oxygen (*14*). When exposed to light, RF is quickly degraded in aqueous solution (*15*). Similarly, 75% of the RF in cheese is degraded within 100 days under light (*16*). However, RF degradation in food items is insignificant when stored in the dark. Depending on the pH of the RF solution, RF is reported to be photodegraded into lumichrome (LC), lumiflavin (LF), and others (*17*, *18*).

Soy milk is one of the traditional nutritious beverages that has been used for thousands of years in oriental countries. In recent years, soy milk consumption among Western consumers has increased considerably due to the increased awareness of the healthy benefits from soybean products. Soy beverages were a 622 million market in 2003, and it is expected to grow to a 1.78 billion market in 2010 with a compounding annual growth rate of 17% in the United States (*19*). Soy milk contains 2.86% protein, 1.53% fat, 0.27% ash, 1.53% carbohydrate, and 93.81% moisture (*20*). The RF content in soy milk is 2−3 mg/kg, which is comparable to the RF content in cow's milk (*21*). Plain, unfortified soy milk is an excellent source of high-quality protein, B vitamins, and iron. Some brands of soy milk are fortified with vitamins and minerals and are good sources of calcium, vitamin D, and vitamin B-12.

While it is well-known that RF is an effective photosensitizer in food systems (*13*, *14*, *22*), there are no reports if LC and LF can be photosensitizers in foods. The objectives of this research were (i) to study the effect of pH on the photodegradation of RF by separation and identification; (ii) to determine the reaction rate between singlet oxygen and LC, LF, and RF; (iii) to study the effects of LC, LF, and RF as photosensitizers on the flavor stability of soy milk; and (iv) to study the effect of ascorbic acid (AA) on inhibiting the flavor changes of soy milk under light.

MATERIALS AND METHODS

Materials. Soy milk containing 3 mg/kg RF was purchased from a local organic food store in Columbus, Ohio. RF (98% purity) was purchased from Acros Organics (Jersey City, NJ). LC (∼95% purity), LF (≥97% purity), and sodium azide were purchased from Sigma Chemical Co. (St. Louis, MO). Water used in sample preparations was purified by a Milli-Q purification system (Millipore Co., Bedford, MA). Serum bottles (10 mL), aluminum caps, and Teflon-coated septa were purchased from Supelco, Inc. (Bellefonte, PA).

Sample Preparations. *Identification of Photodegradation Products from RF.* The 2 mL aliquot of the 100 μM RF solution in H_2O was

* To whom correspondence should be addressed. Tel: 614-292-7801. Fax: 614-292-0218. E-mail: min.2@osu.edu.
† Current address: R&D, Givaudan Flavors Corp., 1199 Edison Dr., Cincinnati, OH 45216.

10.1021/jf052448v CCC: $33.50
Published on Web 02/18/2006

pipetted into a 10 mL serum bottle and sealed airtight with a Teflon-coated rubber septum and an aluminum crimp cap. The sample bottles were then stored under light for up to 72 h. The light in the storage box was provided by Sylvania fluorescent lamps (GE, Cleveland, OH), and the intensity was 1500 lux (*10*, *23*). Samples in duplicate were taken out from the light box at 2, 4, 8, 16, 24, 48, and 72 h for RF and its degradation products analyses.

Effect of pH on Photodegradation of RF. To study the effect of pH on the photodegradation of RF, 100 μM RF solutions were prepared in 100 mM phosphate buffer at pH 4.5, 6.5, or 8.5, respectively. Samples (2 mL) were then sealed airtight in 10 mL serum bottles for storage in the same light box as described above for up to 72 h. The changes of RF and its degradation products were analyzed by a high-performance liquid chromatography (HPLC) method.

Effect of Sodium Azide on the Photostability of RF, LF, and LC. To detect the involvement of singlet oxygen in photodegradation, 50 μM RF, LF, or LC solution containing 5 mM sodium azide was prepared. Samples (2 mL) were sealed airtight in 10 mL serum bottles and stored in the same light box as mentioned above for up to 24 h. Samples with no sodium azide were used as the control. The change of RF, LF, and LC was determined by the HPLC method.

Reaction Rate Constants of RF, LF, and LC with Singlet Oxygen. The reaction rate between singlet oxygen and RF, LC, or LF at 12.5, 25, 50, and 100 μM was determined. After the samples were stored under light for 24 h, the residual concentrations of RF, LF, and LC were determined by the HPLC method. The steady-state kinetic equation for singlet oxygen and the excited flavin quenching (*22*) were used to calculate the reaction rates of singlet oxygen with RF, LF, and LC.

Effect of RF, LF, and LC on Soy Milk Flavor Stability. To study the effect of RF, LF, and LC on the soy milk flavor stability, RF, LC, or LF was added to soy milk at 100 μM. Soy milk without the addition of the flavins was used as the control. A 2 mL aliquot of soy milk was pipetted into a 10 mL glass bottle and sealed airtight with a Teflon-coated rubber septum and an aluminum crimp cap. The sample bottles were stored in a light box of 1500 lux (*10*, *23*) at a temperature of 4 °C. The headspace oxygen and total volatiles were analyzed in duplicate by gas chromatography (GC) methods after 24, 48, or 72 h of storage.

Effect of AA on Soy Milk Flavor Stability. To study the effect of AA on the changes of volatile compounds, soy milk containing 100 μM RF, LC, or LF was prepared. AA was then added at 50 mM. The total volatile compounds in the headspace of soy milk bottles were analyzed with solid phase microextraction (SPME)/GC/flame ionization detection (FID) after the samples were stored under light at 4 °C for up to 72 h.

Analysis of RF and Its Photodegradation Products. An HPLC (HP 1100; Hewlett-Packard, Wilmington, DE) equipped with an Aqua C-18 column (5 μm, 150 mm × 4.60 mm) (Phenomenex, Torrance, CA) was used to monitor the change of RF and its degradation products. Samples removed from the light box were filtered with a 0.2 μm membrane filter before injection. The injection volume was 10 μL. The flow rate of the mobile phase [methanol:water = 3:7 (v/v)] was 1.0 mL/min. An HP 1100 diode array detector was used to measure the absorbance of RF and its degradation products. The concentration of RF, LC, and LF was calculated using a standard linear line of HPLC peaks of standard chemicals in aqueous solution. RF degradation products were identified by the combination of the retention time and UV−vis spectra comparison with the authentic standard chemicals and the HPLC/MS analysis.

Analysis of Headspace Oxygen. Headspace oxygen in the sample bottles was analyzed by injecting a 100 μL headspace air sample into a HP 5890 GC (Avondale, PA), equipped with a stainless steel molecular sieve column (13×, 80:100; Alltech, Deerfield, IL) and a thermal conductivity detector (*24*). High purity helium (99.99%) was used as the carrier gas. The flow rate was 40 mL/min. The GC oven temperature was maintained at 40 °C. The injector port and detector temperatures were maintained at 120 and 150 °C, respectively. Each sample was analyzed in duplicate. Oxygen contents were quantified by an HP 3396A integrator (*22*, *24*).

Analysis of Headspace Volatile Compounds. Headspace volatiles were analyzed by SPME and GC (*23*, *25*−*27*). Headspace volatile compounds were isolated by SPME with a 75 μm Carboxen/poly-(dimethylsiloxane) fiber. The fiber was exposed to the bottle headspace for 30 min while the bottle was incubated in a 40 °C water bath (*25*, *26*). The extracted volatiles were desorbed in the GC injection port for 3 min at 250 °C. The injection port was fitted with a 0.75 mm internal diameter splitless glass liner. Volatile compounds were separated using an Rtx-5 (Crossbond 5% diphenyl−95% dimethyl polysiloxane) column (30 m × 0.32 mm × 0.1 μm film thickness) and detected with a FID at Hewlett-Packard 6890 GC using hydrogen as the carrier gas. The GC oven temperature was programmed to hold at 60 °C for 3 min, increase to 120 °C at 4 °C/min, increase to 240 °C at 10 °C/min, and hold at 240 °C for 2 min (*25*, *26*).

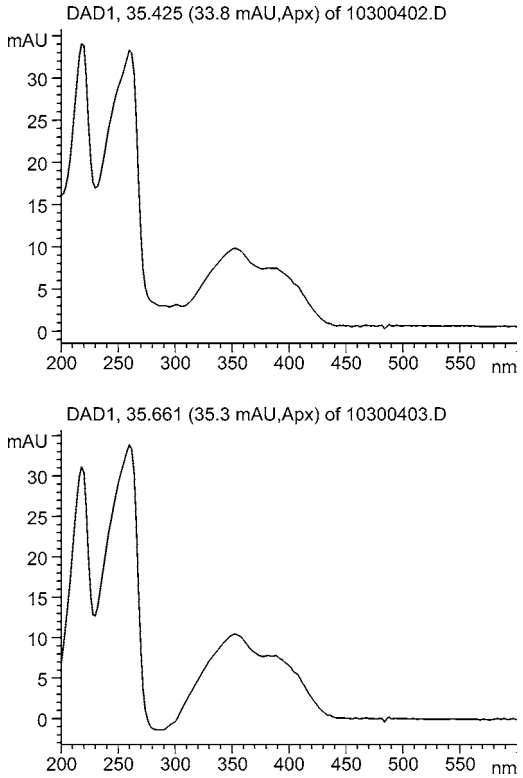

Figure 1. Spectra comparison of RF degradation product (top) and authentic LC (bottom).

Data Analysis. All of the treatments were duplicated for this study. Data were analyzed using Microsoft Office Excel Program. Comparisons for mean value differences were done by *T*-test. A p value of ≤ 0.05 was considered to be significant.

RESULTS AND DISCUSSION

Identification of the Photodegradation Products of RF in Aqueous Solution. An aqueous solution of RF (100 μM) was prepared and stored under light for up to 72 h. The sample vials wrapped with aluminum foils were designated as the samples stored in dark. RF was quickly degraded under light and stayed unchanged in dark throughout the study as was found by Huang and others (*15*). As the RF peak decreased under light, a peak with a retention time of 35.6 min increased progressively. The 35.6 min retention time of the peak was the same as that of the authentic LC standard. The UV−vis spectrum of the peak was the same as that of the LC standard (**Figure 1**). HPLC/MS analysis indicated the degradation product had m/z 243 $[M + H]^+$, which represented the molecular cation of LC. The combined results of retention time, UV−vis spectrum comparison (*28*), and the HPLC/MS analysis indicated that the RF degradation product with the retention time of 35.6 min was LC.

Effect of pH on the Photodegradation Products of RF. RF was quickly degraded under light. LC was the only major

Table 1. Effect of pH on the Change of RF, LF, and LC under Light[a]

under light (h)	pH 4.5			pH 6.5			pH 8.5		
	RF	LF	LC	RF	LF	LC	RF	LF	LC
0	97.3			101.4			99.3		
2	48.1		47.3	35.8		48.8	3.7	30.9	73.1
4	11.2		73.9	3.7		56.4		15.1	63.8
6			79.1			58.7		9.9	52.7
8			79.9			58.1		5.6	53.0
16			78.2			56.9			41.7
24			72.3			50.7			31.5
48			57.3			40.8			23.5
72			31.7			30.1			9.9

[a] Blank cells, not detectable. Numbers (μM) were the average of duplicated determinations and were calculated from the respective calibration curves.

Table 2. Effects of Sodium Azide (NaN_3) on the Retention of 50 μM RF, LF, or LC under Light[a]

storage time (h)	RF (μM)		LF (μM)		LC (μM)	
	0 mM NaN_3	5 mM NaN_3	0 mM NaN_3	5 mM NaN_3	0 mM NaN_3	5 mM NaN_3
0	49.9	50.1	50.2	49.8	49.7	50.2
2	28.8	43.5	42.3	46.8	44.3	48.0
4	7.1	38.1	28.3	43.4	39.7	45.4
8	0.2	28.1	13.5	36.1	29.9	41.2
24	0.3	10.7	0.4	25.8	14.5	35.3

[a] Numbers (μM) were the average of duplicated determinations and were calculated from the respective calibration curves.

photodegradation product of RF under neutral and acidic pH values, which is consistent with information in the literature (*29*, *30*). Ahmad et al. (*30*) reported that LC was the major RF degradation product in neutral to acidic pH values. When the pH of the solution was 7−9, LF was also one of the major degradation products. Formylmethylflavin and carboxymethylflavin were reported as the other minor products. At pH 4.5, the maximum LC concentration (79.9 μM) was reached after 8 h under 1500 lux light. At pH 6.5, the maximum LC concentration (58.7 μM) was reached after 6 h of storage under light. At pH 8.5, the maximum LC concentration (73.1 μM) was reached after only 2 h under light. In addition to LC, LF was also detected as the RF photodegradation product at a pH of 8.5 (**Table 1**). The maximum concentration was 30.9 μM after 2 h under light. Both LC and LF started to degrade after they reached their respective maximum concentrations at various pH values (**Table 1**). This suggested the unstable nature of LF and LC under light. LC seemed to be relatively more stable in lower pH than in a higher pH solution.

Effect of Sodium Azide on the Photostability of RF, LF, and LC. To study the involvement of singlet oxygen in the photodegradation of RF, LF, or LC, sodium azide, a well-known singlet oxygen quencher (*31*), was added to the RF, LF, and LC solution. If the addition of sodium azide could increase the stability of RF, LF, and LC, singlet oxygen was involved in the photodegradation of RF, LF, and LC. Sodium azide increased the stability of RF, LF, and LC under light (**Table 2**). After 8 h, RF was nearly undetectable without sodium azide, while 56.2% of the RF still remained with 5 mM sodium azide in solution. Similarly, a higher percentage of LF and LC remained in the solution when sodium azide was included in the sample preparations (**Table 2**). These results suggested that singlet oxygen was involved in the photodegradation of RF, LF, and LC.

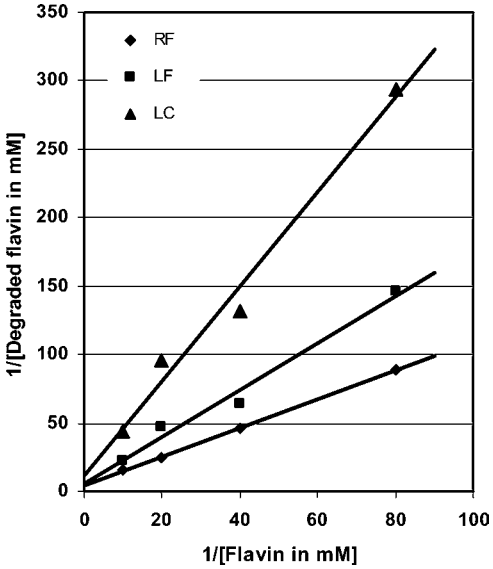

Figure 2. Reciprocal plot of the degradation of RF, LF, and LC under light at different initial concentrations.

Reaction Rate between Singlet Oxygen and RF, LC, and LF. LC and LF were identified as the major photodegradation products from RF under light. On the basis of their molecular structure, it is expected that these molecules are also good reactants for singlet oxygen. As described previously, singlet oxygen was involved in the photodegradation of RF, LF, and LC. To study the reaction rate between the singlet oxygen and the RF, LF, or LC, the steady-state kinetic equation for singlet oxygen and the excited flavin quenching (*22*) can be used (*32*):

$$\left(\frac{\mathrm{d}[FO_2]}{\mathrm{d}t}\right)^{-1} = K^{-1}\left(1+\frac{k_{\mathrm{Q}}[\mathrm{Q}]}{k_{\mathrm{o}}[^3\mathrm{O}_2]}\right)\left\{1+\frac{(k_{\mathrm{q}}+k_{\mathrm{ox-Q}})[\mathrm{Q}]+k_{\mathrm{d}}}{k_{\mathrm{r}}}\times[\mathrm{F}]^{-1}\right\}$$

where $[FO_2]$ is the concentration of oxidized flavins; K is the quantum yield of intersystem crossing; k_{Q} is the rate constant for triplet flavin quenching; [Q] is the quencher concentration; k_{o} is the rate constant for singlet oxygen formation by energy transfer from excited triplet flavin to triplet oxygen; $[^3O_2]$ is the triplet oxygen concentration; k_{q} is the rate constant of singlet oxygen physical quenching; $k_{\mathrm{ox-Q}}$ is the rate constant of singlet oxygen chemical quenching; k_{d} is the reaction rate for singlet oxygen decay in a solvent; k_{r} is the rate constant between flavins and singlet oxygen; and [F] is the initial concentration of RF, LF, or LC.

When there is no quencher added in an experimental system, the equation can be simplified to the following (*32*−*34*):

$$\left(\frac{\mathrm{d}[\mathrm{FO}_2]}{\mathrm{d}t}\right)^{-1} = K^{-1}\left(1+\frac{k_{\mathrm{d}}}{k_{\mathrm{r}}}\times[\mathrm{F}]^{-1}\right)$$

From a plot of $[FO_2]^{-1}$ against $[F]^{-1}$, the ratio of the slope to intercept would equal $k_{\mathrm{d}}/k_{\mathrm{r}}$. Because the singlet oxygen decay rate k_{d} in water solution is known, k_{r}, the reaction rate constant between the flavins and singlet oxygen, can be calculated (*32*).

The reciprocal plots of the degraded flavin concentration against the initial flavin concentrations are shown in **Figure 2**. The slope and intercept for the regression lines in **Figure 2** are summarized in **Table 3**. The singlet decay rate k_{d} in water was reported at 2.56×10^5 s^{-1} (*35*, *36*). Thus, the reaction rate (k_{r})

Table 3. Slopes and Intercepts from the Regression Lines in **Figure 2**

	slope (*S*) (1/mM)	intercept (*I*)	*S*/*I* (mM)	k_r ($\times 10^8$ $M^{-1}s^{-1}$)
RF	1.0619	4.005	0.2651	9.66
LF	1.7109	5.732	0.2985	8.58
LC	3.4665	11.115	0.3119	8.21

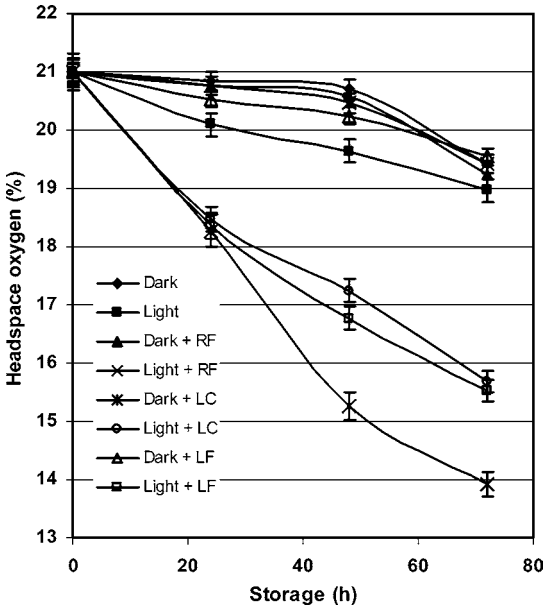

Figure 3. Effect of RF, LF, and LC on the headspace oxygen (%) of soy milk under light or in dark. The concentration of RF, LF, or LC in soy milk was 100 μm.

between the flavins and the singlet oxygen can be calculated as 2.56×10^5 s^{-1}/S/I. The results are shown in **Table 3**.

The results showed that RF had a slightly higher reaction rate constant than LC and LF. The k_r for RF is in general agreement with the data from the literature (*15*, *37*). To the best of our knowledge, this is the first report of the rate constant between singlet oxygen and LC or LF.

Effect of RF, LF, and LC on the Headspace Oxygen of Soy Milk under Light. Considering the quick degradation of RF in aqueous solution under light, one would hypothesize that the degradation products from RF may also act as the photosensitizers, which in turn cause the oxidative deterioration in food products. To test such a hypothesis, soy milk was used as the model system and the effect of RF, LF, and LC on the headspace oxygen depletion of soy milk under light was compared.

RF, LF, or LC was added to soy milk at the final concentration of 100 μM. Soy milk without the addition of the flavins was used as the control. Results in **Figure 3** showed the changes of headspace oxygen in soy milk. Light played a role on the headspace oxygen depletion. The addition of RF, LC, or LF did not influence the change of headspace oxygen of soy milk in the dark. After 48 h in the dark, the headspace oxygen remained between 20.2 and 20.7%. The supplementation of RF, LC, or LF caused significant headspace oxygen depletion in soy milk under light (**Figure 3**). The headspace oxygen in sample bottles under light for 72 h was 13.9, 15.5, and 15.7%, respectively, for soy milk supplemented with 100 μM RF, LC, or LF. Without the supplementation of the flavins, soy milk under light showed much less depletion on the headspace oxygen. The headspace oxygen in soy milk was 19.1%. Among

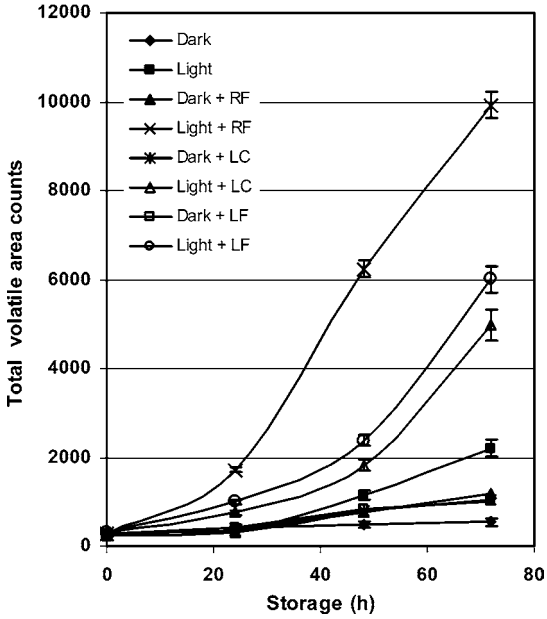

Figure 4. Effect of RF, LF, and LC on the total volatiles of soy milk under light or in dark. The concentration of RF, LF, or LC in soy milk was 100 μm.

the three compounds studied during this research, RF was the most efficient in depleting the headspace oxygen in soy milk.

When compared to those with flavin supplementation in the dark, the headspace oxygen depletion was higher in soy milk with no flavin supplementation under light. It was believed that this oxygen depletion was caused by the endogenous RF (~3 mg/kg) in the soy milk used for this study.

It has recently been reported that chemicals should exhibit several specific properties in order to qualify as effective photosensitizers (*38*). Some of these specific properties include (i) a high absorption coefficient in the spectral region of the excitation light and (ii) a high quantum yield of the triplet state and long triplet state lifetimes. RF as well as its degradation products LC and LF had very high absorption coefficients in the near UV wavelength region. Therefore, they are likely the candidates to act as photosensitizers.

Effect of RF, LF, and LC on the Headspace Volatile Compounds of Soy Milk. When soy milk was supplemented with RF and stored under light, the total volatile and beany flavor compounds such as hexanal increased significantly. Singlet oxygen was believed to be involved in the flavor changes of soy milk stored under light (*10*). We have further compared the effect of RF, LF, and LC on the total volatiles of soy milk stored under light or in dark (**Figure 4**). When soy milk with or without the addition of RF, LF, or LC was stored in the dark for up to 72 h, the change of total headspace volatile in those samples was less than 2-fold. When stored under light, however, soy milk showed significant changes in total headspace volatile compounds. The total volatile was about 7-fold of the initial volatile concentration after being stored under light for 72 h. When soy milk was supplemented with 100 μM RF, LF, or LC and stored under light, the change of the total headspace volatile was even more significant. After 72 h, the final total headspace volatile was 30-, 20-, and 17-fold of the initial levels, respectively. These results support the hypothesis that RF, LF, and LC act as the photosensitizer to cause more significant flavor changes of soy milk under light. As can be seen in **Figure 4**, RF is more effective than LF or LC in causing the headspace total volatile accumulations. This is consistent with the finding from the headspace oxygen depletion analysis.

Table 4. Effects of AA on Total Volatile Compounds of Soy Milk Supplemented with RF, LF, or LC and Stored under Light[a]

	soy milk + RF		soy milk + LF		soy milk + LC	
storage time (h)	0 mM AA	50 mM AA	0 mM AA	50 mM AA	0 mM AA	50 mM AA
0	288	268	322	250	302	285
24	1715	675*	1003	353*	750	325*
48	6235	922*	2378	536*	1818	480*
72	9925	1538*	6008	920*	4988	773*

[a] The concentration of RF, LF, or LC in soy milk was 100 μM. Volatile compounds are expressed in electronic counts of GC peaks. Numbers are the averages from SPME/GC analysis for duplicated samples. Numbers with the * indicated that it was significantly ($p < 0.05$) different from that of treatment without AA at the same storage condition.

Effect of AA on the Formation of Headspace Volatiles in Soy Milk Supplemented with RF, LF, or LC. It has been reported that singlet oxygen was involved in the flavor deterioration of soy milk (*10*) and whole fat cow milk (*23*) when they were supplemented with RF under light. AA is an excellent singlet oxygen and excited triplet sensitizer quencher (*14*) and has been used widely as an antioxidant in food formulations. Inclusion of AA in soy milk under light inhibited the formation of hexanal and total volatile compounds in a dose-dependent manner (*10*). In our current study, we tested the effect of AA on volatile compound changes of soy milk supplemented with RF, LC, or LF. Shown in **Table 4** is the effect of AA in soy milk containing RF, LC, or LF on the total volatile changes under light. The addition of AA significantly inhibited the formation of total headspace volatile compounds in soy milk. After 72 h under light at 4 °C, the total headspace volatiles in soy milk with 50 mM AA were only 15% of those containing no AA (**Table 4**).

The results suggested that the RF degradation products, LC and LF, have similar photosensitization properties as RF. Under light, RF in soy milk was photodegraded to LC and/or LF. In the meantime, all three acted as photosensitizers to form singlet oxygen. The singlet oxygen thus formed can react with the lipid and protein components in soy milk to cause the flavor deteriorations.

In conclusion, RF is not stable under light and can be quickly degraded. LC and LF were identified as the major photodegradation products from RF depending on the pH of the solution. Similar to RF, LC and LF act as the photosensitizers. They generate singlet oxygen when exposed to light in aqueous food systems. All three had significant effects on the depletion of headspace oxygen and the formation of total headspace volatile in soy milk under light. AA minimized the formation of volatile compounds of soy milk containing RF, LC, or LF under light. Most importantly, soy milk should be kept under conditions with no light to minimize the possible flavor changes.

ACKNOWLEDGMENT

We thank Dr. Q. Tian and Professor S. Schwartz for their support on the HPLC/MS analysis during this research.

LITERATURE CITED

(1) Szczesniak, T.; Karabin, L.; Szczepankowska, M.; Wituch, K. Biosynthesis of riboflavin by *Ashbya* gossypii. I. Influence of fats of animal origin on riboflavin production. *Acta Microbiol. Pol., Ser. B: Microbiol. Appl.* **1971**, *3*, 29−34.

(2) Gliszczynska, A.; Koziolowa, A. Flavins and flavoproteins. Proceedings of the 13th International Symposium, Konstanz, Germany, Aug. 29−Sept. 4, 1999; pp 875−878.

(3) U.S. Department of Agriculture. *USDA National Nutrient Database for Standard Reference*, Release 16 Nutrient Lists; U.S. Department of Agriculture: Washington, DC, 2004.

(4) Kim, H.; Kirschenbaum, L. J.; Rosenthal, I.; Riesz, P. Photosensitized formation of ascorbate radicals by riboflavin: An ESR study. *Photochem. Photobiol.* **1993**, *57*, 777−784.

(5) Crank, G.; Pardijanto, M. S. Photooxidations and photosensitized oxidations of vitamin A and its palmitate ester. *J. Photochem. Photobiol., A: Chem.* **1995**, *85*, 93−100.

(6) Li, T.; Min, D. B. Stability and photochemistry of vitamin D_2 in model system. *J. Food Sci.* **1998**, *63*, 413−417.

(7) Edwards, A. M.; Bueno, C.; Saldano, A.; Silva, E.; Kassab, K.; Polo, L.; Jori, G. Photochemical and pharmacokinetic properties of selected flavins. *J. Photochem. Photobiol., B: Biol.* **1999**, *48*, 36−41.

(8) Criado, S.; Castillo, C.; Yppolito, R.; Bertolotti, S.; Garcia, N. A. The role of 4- and 5-aminosalicylic acids in a riboflavin-photosensitized process. *J. Photochem. Photobiol., A: Chem.* **2003**, *155*, 115−118.

(9) Viteri, G.; Edwards, A. M.; De la Fuente, J.; Silva, E. Study of the interaction between triplet riboflavin and the α-, β_H- and β_L-crystallins of the eye lens. *Photochem. Photobiol.* **2003**, *77*, 535−540.

(10) Huang, R.; Choe, E.; Min, D. B. Effects of riboflavin photosensitized oxidation on the volatile compounds of soymilk. *J. Food Sci.* **2004**, *69*, C733−C738.

(11) Grzelak, A.; Rychlik, B.; Bartosz, G. Light-dependent generation of reactive oxygen species in cell culture media. *Free Radical Biol. Med.* **2001**, *30*, 1418−1425.

(12) Lucius, R.; Mentlein, R.; Sievers, J. Riboflavin-mediated axonal degeneration of postnatal retinal ganglion cells in vitro is related to the formation of free radicals. *Free Radical Biol. Med.* **1998**, *24*, 798−808.

(13) Hlavinka, D. J.; Goodrich, R. P.; Goodrich, L.; McGraw, D. Reduction of contaminants in blood and blood products using photosensitizers and peak wavelengths of light. WO A1 2003063915, 2003.

(14) Min, D. B.; Boff, J. M. Chemistry and reaction of singlet oxygen in foods. *Comput. Rev. Food Sci. Food Saf.* **2002**, *1*, 58−72.

(15) Huang, R.; Choe, E.; Min, D. B. Kinetics for singlet oxygen formation by riboflavin photosensitization and the reaction between riboflavin and singlet oxygen. *J. Food Sci.* **2004**, *69*, C726−C732.

(16) Kristensen, D.; Hansen, E.; Arndal, A.; Trinderup, R. A.; Skibsted, L. H. Influence of light and temperature on the color and oxidative stability of processed cheese. *Int. Dairy J.* **2001**, *11*, 837−843.

(17) Ahmad, I.; Fasihullah, Q.; Vaid, F. H. M. A study of simultaneous photolysis and photoaddition reactions of riboflavin in aqueous solution. *J. Photochem. Photobiol., B: Biol.* **2004**, *75*, 13−20.

(18) Ansari, I. A.; Vaid, F. H. M.; Ahmad, I. Chromatographic study of photolysis of aqueous cyanocobalamin solution in the presence of vitamins B and C. *Pak. J. Pharm. Sci.* **2004**, *17*, 19−24.

(19) Savitry, A.; Prakash, M. Sing a song of soy. *Prepared Foods* **2004**, 25−28.

(20) Rosenthal, A.; Deliza, R.; Cabral, L. M. C.; Cabral, L. C.; Farias, C. A. A.; Domingues, A. M. Effect of enzymatic treatment and filtration on sensory characteristics and physical stability of soymilk. *Food Control* **2003**, *14*, 187−192.

(21) Kwok, K.-C.; Shiu, Y.-W.; Yeung, C.-H.; Niranjan, K. Effect of thermal processing on available lysine, thiamine and riboflavin content in soymilk. *J. Sci. Food Agric.* **1998**, *77*, 473−478.

(22) Yang, W. T.; Lee, J. H.; Min, D. B. Quenching mechanisms and kinetics of α-tocopherol and β-carotene on the photosensitizing effect of synthetic food colorant FD&C Red No. 3. *J. Food Sci.* **2002**, *67*, 507−510.

(23) Lee, J. H. Photooxidation and photosensitized oxidation of linoleic acid, milk and lard. Ph.D. Dissertation, The Ohio State University, Columbus, OH, 2002.

(24) King, J. M.; Min, D. B. Riboflavin-photosensitized singlet oxygen oxidation product of vitamin D_2. *J. Am. Oil Chem. Soc.* **2002**, *79*, 983−987.

(25) Steenson, D. F.; Lee, J. H.; Min, D. B. Solid-phase microextraction of volatile soybean oil and corn oil compounds. *J. Food Sci*. **2002**, *67*, 71−76.

(26) Lee, J.-H.; Diono, R.; Kim, G.-Y.; Min, D. B. Optimization of solid phase microextraction analysis for the headspace volatile compounds of parmesan cheese. *J. Agric. Food Chem*. **2003**, *51*, 1136−1140.

(27) Suratman, L.; Jeon, I.; Schmidt, K. Ability of cyclodextrins to entrap volatile beany flavor compounds in soymilk. *J. Food Sci.* **2004**, *69*, FCT109−113.

(28) Zeng, K.; Hwang, H.-M.; Zhang, Y.; Yu, H. Identification of 6-aminochrysene photoproducts and study of the effect of a humic acid and riboflavin on its photolysis. *J. Photochem. Photobiol, B: Biol.* **2003**, *72*, 95−100.

(29) Woodcock, E. A.; Warthesen, J. J.; Labuza, T. P. Riboflavin photochemical degradation in pasta measured by high performance liquid chromatography. *J. Food Sci.* **1982**, *47*, 545−549, 555.

(30) Ahmad, I.; Fasihullah, Q.; Noor, A.; Ansari, I. A.; Ali, Q. N. M. Photolysis of riboflavin in aqueous solution: a kinetic study. *Int. J. Pharm*. **2004**, *280*, 199−208.

(31) Fujita, H.; Matsuo, I. In vitro phototoxic activities of new quinolone antibacterial agents: Lipid peroxidative potentials. *Photodermatol., Photoimmunol. Photomed.* **1994**, *10*, 202−205.

(32) Foote, C. S. Quenching of singlet oxygen. In *Singlet Oxygen*; Wasserman, H., Murray, R. W., Eds.; Academy Press: New York, 1979; pp 139−171.

(33) Jung, M. Y.; Kim, S. K.; Kim, S. Y. Riboflavin-sensitized photooxidation of ascorbic acid: Kinetics and amino acid effects. *Food Chem.* **1995**, *53*, 397−403.

(34) Lee, K. H.; Jung, M. Y.; Kim, S. Y. Quenching mechanism and kinetics of ascorbyl palmitate for the reduction of the photosensitized oxidation of oils. *J. Am. Oil Chem. Soc*. **1997**, *74*, 1053−1057.

(35) Hurst, J. R.; Schuster, G. B. Nonradiative relaxation of singlet oxygen in solution. *J. Am. Chem. Soc*. **1983**, *105*, 5756−5760.

(36) Rodgers, M. A.; Snowden, P. T. Lifetime of singlet delta dioxygen in liquid water as determined by time-resolved infrared luminescence measurements. *J. Am. Chem. Soc.* **1982**, *104*, 5541−5543.

(37) Haag, W. R.; Mill, T. Rate constants for interaction of singlet oxygen (^1D_g) with azide ion in water. *Photochem. Photobiol.* **1987**, *45*, 317−321.

(38) DeRosa, M. C.; Crutchley, R. J. Photosensitized singlet oxygen and its applications. *Coord. Chem. Rev*. **2002**, *233*−*234*, 351−371.

Received for review October 3, 2005. Revised manuscript received January 6, 2006. Accepted January 22, 2006.

JF052448V