

Mechanism of Riboflavin Destruction under Light

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ABSTRACT

Riboflavin, an essential vitamin, is stable during processing and storage in dark, but it is very sensitive to light. The mechanism for the rapid destruction of riboflavin in foods under light and the protection of riboflavin under light have been extensively studied since riboflavin was discovered in 1930s. The mechanisms of the destruction of riboflavin under light were studied by the combination of HPLC, SPME-GC and mass spectrometry. The mechanisms for the formation of compounds from riboflavin under light were studied to minimize the destruction of riboflavin in foods under light. Lumichrome, lumiflavin and 2,3-butanedione formed from riboflavin under light were positively identified. Lumichrome was the major product under neutral or acidic pH. Lumiflavin was also formed in basic pH. 2,3-Butanedione was the major volatile compound. Lumichrome and lumiflavin were formed from unstable diradical excited triplet riboflavin by dealkylation. 2,3-Butanedione was produced from the reaction between electrophilic singlet oxygen and electron-rich riboflavin. Our study showed that sodium azide and ascorbic acid minimized the destruction of riboflavin under light.

INTRODUCTION

FAD - Flavin Adenine Dinucleotide

- Riboflavin functions in body (Choe and others 2005)
 - Vitamin B₂
- · An important part of coenzyme FAD
- · Essential in red blood cell formation, metabolism of proteins, fats and
- carbohydrates, and resistance to stress
- Riboflavin is stable in the absence of light even at high temperature.
- Riboflavin is very unstable to light even at room temperature.
- The rapid destruction of riboflavin in foods under light has significantly adverse nutritional impact and lowers food quality.
- Chemical mechanisms of rapid destruction of riboflavin under light and how to protect it have been extensively studied without success since riboflavin was discovered in 1930s (Warburg and Christian 1932).

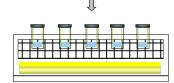
OBJECTIVES

- To separate and identify the compounds formed from riboflavin under light
- To study the mechanisms for the formation of compounds formed from riboflavin under light
- To minimize the destruction of riboflavin under light



Riboflavin in phosphate buffer

MATERIALS AND METHODS



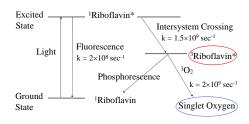
Storage under Fluorescence light (3,000 lux, 25°C)



Separation of compounds formed from Riboflavin by HPLC or SPME-GC



Identification by HPLC or GC retention time and Mass Spectrometry



Scheme 1. Chemical mechanism of riboflavin under light (Choe and Others 2005)

Storage	Destroyed Riboflavin (%)				
	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs
Light	0	50.8	78.4	90.6	98.0
Dark	0	0.2	0.6	1.8	3.6

Table 1. Effect of light on riboflavin stability

RESULTS

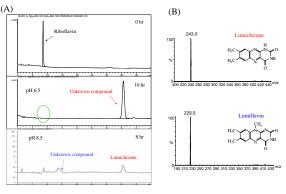


Figure 1. (A) HPLC chromatograms of unknown compounds formed from riboflavin under light at pH 6.5 and 8.5, (B) Mass spectra of unknown compounds

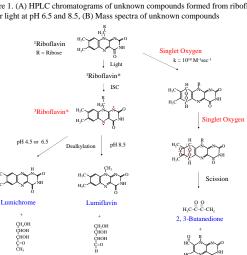


Figure 3. Mechanisms for the formation of lumichrome, lumiflavin, and 2,3butanedione

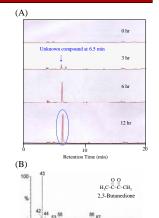


Figure 2. (A) GC chromatograms of volatile compounds formed from riboflavin under light, (B) Mass spectrum of unknown compound

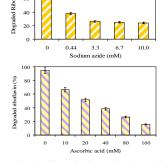


Figure 4. Effects of sodium azide and ascorbic acid on the degradation of riboflavin under light

CONCLUSIONS and DISCUSSION

- The 98% of riboflavin was destroyed under light while only 4% of riboflavin destroyed in the dark.
- Lumichrome, lumiflavin and 2,3-butanedione formed from the riboflavin under light were positively identified by the combination of HPLC or GC retention time and mass spectrometry.
- Riboflavin produced very unstable and reactive excited diradical triplet riboflavin under light.
- The reactive and unstable diradical triplet riboflavin produced lumichrome under neutral or acidic pH and lumiflavin under only basic pH by dealkylation.
- Riboflavin produced electrophilic singlet oxygen under light.
- Singlet oxygen reacted with electron-rich riboflavin and produced 2,3-butanedione.
- Sodium azide and ascorbic acid minimized the destruction of riboflavin under light.

REFERENCES

- Choe E, Huang R, Min DB. 2005. Chemical reactions and stability of riboflavin in foods. J Food Sci. 70:R28-R36.
- Warburg O, Christian W. 1932. A second oxygen-transfer enzyme and its absorption spectrum. Naturwissenschaften 20:688.