

PROTECTIVE ABILITY OF MELANOIDS ON RIBOFLAVIN PHOTO-DEGRADATION

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ABSTRACT

Effects of melanoidins, Maillard reaction products, on riboflavin photo-degradation were studied in riboflavin solution (1.5 mg L⁻¹) and Ultra high temperature treated milk (UHT-treated milk) during exposure to irradiation at 7000-lux light intensity for 2.5 h. In riboflavin solution, melanoidins showed the protective ability on the degradation of riboflavin under light exposure, and this ability depended on melanoidins concentration. In addition, the protective ability of melanoidins was higher than that of ascorbic acid which has the ability to reduce riboflavin photo-degradation. The study on milk sample clarified that heat-treatment of milk enhanced the protective ability on the riboflavin photo-degradation. Thus, it could be concluded that melanoidins were compounds which were responsible for the riboflavin photo-stability in UHT-treated milk.

Keywords: melanoidins, maillard reaction, riboflavin, photo-degradation and milk.

1. INTRODUCTION

Milk and dairy products are important sources of riboflavin in the diets [4]. Riboflavin is stable to heat and oxidation, but rapidly photo-degraded [19]. This strong photo-sensitizer is able to absorb visible and UV light and transfers this energy into highly reactive forms of oxygen such as superoxide anion and singlet oxygen (Fig. 1) (Min et al., 2002). The radicals and reactive oxygen species can accelerate oxidative damages of food components such as proteins, lipids, carbohydrates, and vitamins including riboflavin (Bradley et al., 2006) leading to significant nutrient losses (Choe et al., 2005). Additionally, the oxidation products also caused rancid off-flavor, sunlight off-flavor (King et al., 2002) and the color change in milk sample (Lee et al., 1998). The riboflavin photo-degradation and riboflavin photosensitized oxidation of milk can be reduced by treating active oxygen quenchers (Lee et al., 1998).

Maillard reaction, a reaction between amino groups and reducing sugars, can cause either deterioration or enhancement of food quality. Beside the reported formation of anti-nutritional and toxic products, the formation of beneficial compounds such as antioxidant compounds in the Maillard reaction has also been frequently found (Silvan et al., 2006). These compounds are

well known to have strong reducing properties that can prevent oxidative spoilage of processed foodstuffs and influence the oxidative stability and shelf life of foods such as cereals, milk and meat (Pischetsrieder et al., 1998). The antioxidant capacity of Maillard reaction products was observed for the first time by Franzke and Iwainsky (1954) and some products were reported to act as strong antioxidants comparable to commonly used food antioxidants (Lingert et al., 1986, Sun et al., 2006). In milk, the increasing of thermal treatment frequently occurs in concomitance with the increasing of Maillard reaction products as melanoidins. Saidi et al. (1995) recognized that a heat treatment of skim milk could increase riboflavin photostability and they proposed that this property might come from the increasing in casein micelle size and browning. Based on the Maillard reaction products property, we presumed that their antioxidant activity might be one of main important role in protecting riboflavin during illumination instead for the change in milk structure. Thus, it would be valuable to determine the effects of melanoidins on the light-induced riboflavin degradation. From this reason, the effect of melanoidins on riboflavin photo-degradation was investigated in the present study.

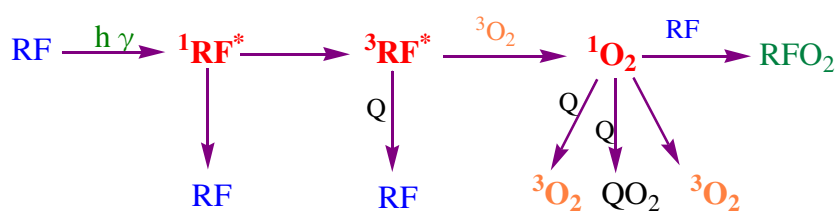


Figure 1. Formation of singlet oxygen from atmospheric triplet oxygen by riboflavin photosensitization. RF, riboflavin; $^1\text{RF}^*$, excited singlet stage riboflavin; $^3\text{RF}^*$, excited triplet state riboflavin; Q, quencher; $^3\text{O}_2$, triplet oxygen; $^1\text{O}_2$, singlet oxygen.

2. MATERIALS AND METHODS

2.1. Reagents and milk sample

Lactose monohydrate was from Nacalai tesque, Inc. (Kyoto, Japan). Riboflavin, *n*-butylamine, methanol (HPLC grade), acetic acid (HPLC grade), and ascorbic acid (Vitamin C) were obtained from Wako Pure Chemical Industries (Osaka, Japan). All other reagents were of the highest grade commercially available. Milli-Q water was used in all procedures. Milk sample (Ultra high temperature -treated milk (UHT-treated milk); 130 °C , 2 s) was supplied from Japan Milk Community, co. Ltd.

2.2. Preparation of melanoidins

The preparation of melanoidins were carried out according to the method of Trang et al. (2008). Lactose monohydrate (262 mM) and butylamine (1.16 M) were dissolved in 1.28 M phosphate buffer (pH 7.0). The sample solution (1.2 ml) was heated at 100 °C. Immediately after heating for an indicated time, the sample was cooled by ice. After that it was extracted three times with double volume of ethyl acetate, and the solvent was evaporated. The residue was diluted with 10 ml of 20 % methanol (MeOH) and filtered through Sep-pak Plus Cartridged C18 column (activated by 5 ml of ethanol (EtOH) and equilibrated by Milli-Q water) to collect the brown components. The melanoidin remained in Sep-pak Cartridged C-18 column was washed by Mili-Q water and collected by 100 % ethylacetate. After that, the eluent was evaporated.

Residual melanoidin was also diluted by Mili-Q water until suitable concentration for following experiment. Melanoidins were estimated by spectrophotometer at 420 nm.

2.3. Light exposure

To study the effects of melanoidins on riboflavin photo-degradation, melanoidins were added into the solution containing riboflavin at concentration of 1.5 mg L⁻¹. The solution was transferred into 5 mL transparent glass bottle, and sealed with caps. Then, the bottles were left in a light storage box at 25 °C and 7000 lux light intensity (Temperature Gradient chamber, model 11HP-A2P, type LH-100-RDS, NK system, Japan). During 2.5 h illumination, the sample solution was collected every 30 min and the remaining riboflavin content was determined. UHT-treated milk containing riboflavin (1.75 mg L⁻¹) that was supplied from Japan Milk Community was also applied for this study. The sample was heated at 120°C for an indicated of time. The heated solution was used for riboflavin photochemical degradation.

2.4. Riboflavin determination

To identify the remaining riboflavin after illumination, sample solution was injected into the HPLC column after filtration with 0.45 µm filter membrane. In the case of milk sample, as a pretreatment for HPLC analysis, 0.5 mL of 2.5 % trichloroacetic acid was added into 0.5 mL of milk sample. After centrifugation at 5000 rpm for 5 min (TOMY High speed micro centrifuge MC – 150, Tomy Seiko CO., LTD, Tokyo Japan), the supernatant was filtered with 0.45-µm filter membrane.

The prepared sample solution containing riboflavin (20 µL) was injected into HPLC system equipped with a LC–10 ADVP liquid chromatograph pump (Shimadzu, Kyoto, Japan) and SCL-10AVP Shimadzu system controller. The column used was a Capcell pak C18 (Type: MG II S-5 µm, 4.6 mm ID x 150 mm, Shiseido fine chemicals, Japan). The flow rate of mobile phase (acetic acid/methanol/water, 1/43/56, v/v/v) was 1.5 mL min⁻¹. Fluorescent intensity was detected at 450 nm (λ excitation) and 510 nm (λ emission) with Shimadzu RF – 10 AXL Fluorescence detector (Woodcock et al., 1982).

3. RESULTS AND DISCUSSION

3.1. Effect of melanoidins on riboflavin photo-degradation

In the final stage of Maillard reaction, the browning of the color is generated due to low molecular weight colored compounds and to high molecular weight conjugated chromophores, commonly referred as melanoidins (Lee, 1998). In general terms, the formation of melanoidins have been frequently associated to the formation of compounds with high antioxidant capacity (Manzocco et al., 2001). Because of this reason, the effect of melanoidins on riboflavin photodegradation was studied. However, the missing information on melanoidin formation and melanoidin structure makes it very difficult to quantify melanoidin. (Carline, M.J.B. et al., 2002). Previously, browning has been measured spectrophotometrically as the absorbance at 420nm. Besides that, Carline has reported the relationship between the absorbance of melanoidin fraction and the concentration of melanoidin as determined using ¹⁴C-labeled sugar. In this experiment, we also used the absorbance at 420 nm for indicating the melanoidins contents in samples (Fig. 2).

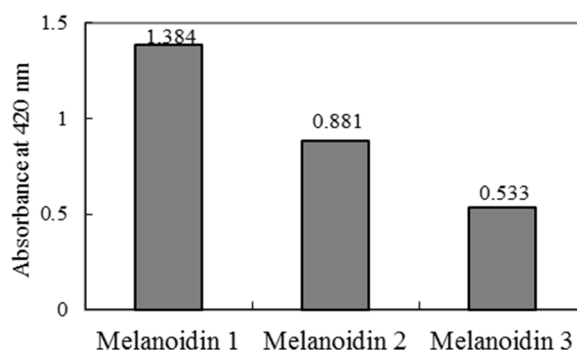


Figure 2. The absorbance of melanoidin solution used in the experiment at 420 nm.

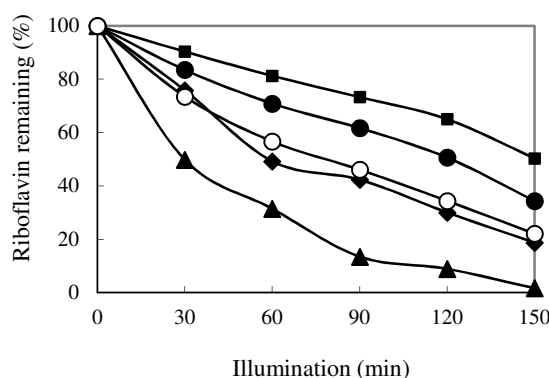


Figure 3. The photo-degradation of riboflavin (1.5 mgL^{-1}) during exposure to irradiation at 7000 lux.

Effects of Melanoidin and 5 mM ascorbic acid on photodegradation of 1.5 mg/l riboflavin during fluorescent light illumination at 7000 lux: Control (▲); melanoidin 1 (■); melanoidin 2 (●); melanoidin 3 (○) and 5 mM ascorbic acid (◆).

In order to investigate the protective ability of melanoidins on riboflavin photo-degradation, melanoidins isolated from the Maillard reaction solution of lactose and butylamine were added into the solution containing riboflavin (1.5 mg L^{-1}). In the literature, ascorbic acid had strong quenching ability against active oxygen species and effectively prevents the light-activated off-flavor formation and the riboflavin reduction in milk and aqueous solution (Lee et al., 1998, Jung et al., 2000). Based on these facts, ascorbic acid was used as a positive control in this study. Under light irradiation, riboflavin content considerably decreased depending on the illumination time in the negative control sample containing only riboflavin, and it was almost completely disappeared after 2.5 h illumination (Fig. 3 and table 1). However, the presence of melanoidins or ascorbic acid in riboflavin solutions resulted in the reduction of riboflavin photo-degradation (Fig. 3 and table 1). The degradation rate of riboflavin in the solution containing melanoidins or ascorbic acid was slower than that in the negative control. To be able to estimate the ability of melanoidins and ascorbic acid on riboflavin photo-degradation, “Half-life period”, the illumination period to reduce 50 % of riboflavin in the solution during the exposure at 7000 lux, was chosen as an index. In this case, the longer “half-life period” showed the higher protective

ability on riboflavin photo-degradation. Using this index, the effect of melanoidins on photo-degradation was examined (Fig. 4).

Table 1. The photo-degradation of riboflavin (1.5 mgL^{-1}) during exposure to irradiation at 7000 lux in the presence of melanoidins and ascorbic acid.

Illumination time (min)	Riboflavin remaining (%)				
	Control (Riboflavin 1.5 mg/l)	Riboflavin and 5 mM ascorbic acid	Riboflavin and melanoidin 1	Riboflavin and melanoidin 2	Riboflavin and melanoidin 3
0	100	100	100	100	100
30	49.84	75.81	90.35	83.50	73.45
60	31.47	49.23	81.25	70.86	56.63
90	13.50	42.35	73.28	61.67	46.10
120	8.91	30.07	65.02	50.64	34.38
150	1.67	18.74	50.35	34.38	22.10

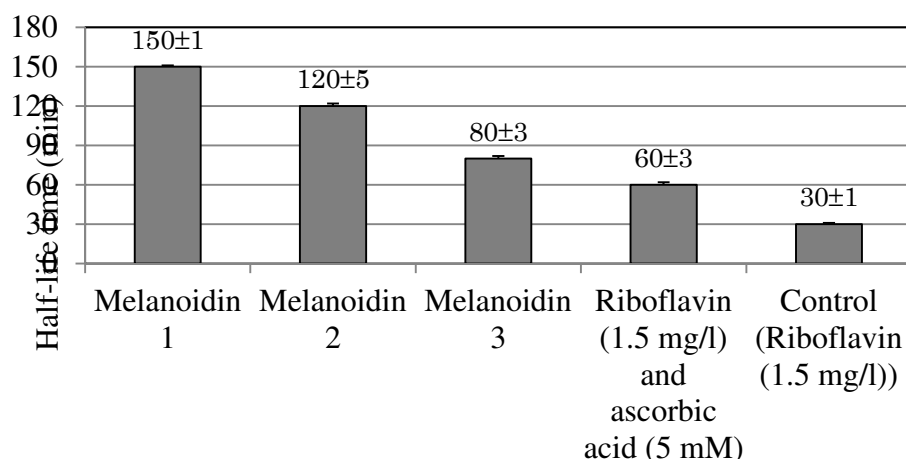


Figure 4. Effect of melanoidin and ascorbic acid on photo-degradation of riboflavin (1.5 mgL^{-1}) during exposure to irradiation at 7000 lux.

Melanoidins showed the protective effect on riboflavin photo-degradation, since the solutions containing melanoidins exhibited longer half-life period comparing with negative control sample. Additionally, the increase in protective effect of melanoidins on riboflavin photo-degradation directly related to the increase of melanoidins concentration which was manifested by the absorbance at 420 nm (Fig. 4). It is interesting to observe that a positive correlation between protective ability to photooxidation and melanoidin amount ($r = 0.98$) (Fig. 5). This result strongly suggested that melanoidins had protective ability on riboflavin photo-degradation and those effects depended on melanoidins concentration. The riboflavin degradation under light

was explained due to the self-sensitized production of active-oxygen species which were extremely reactive (Aurand et al., 1977) (Fig. 1). The generation mechanism of the singlet oxygen by the interaction of riboflavin under light and atmospheric triplet oxygen was also explained as follows: the ground singlet state riboflavin receives the energy from the light and becomes excited singlet state riboflavin. Next, the excited singlet state riboflavin becomes excited triplet state riboflavin by an intersystem crossing mechanism. The excited triplet state riboflavin reacts with atmosphere triplet oxygen to produce a superoxide anion or singlet oxygen. Finally, these active-oxygen species accelerate the oxidation of riboflavin (Bradley et al., 2006). The addition of melanoidins which has the antioxidant activity into the test solution might scavenge these active species. For that reason, it was thought that the photo-decomposition of riboflavin was reduced due to the effect of melanoidins.

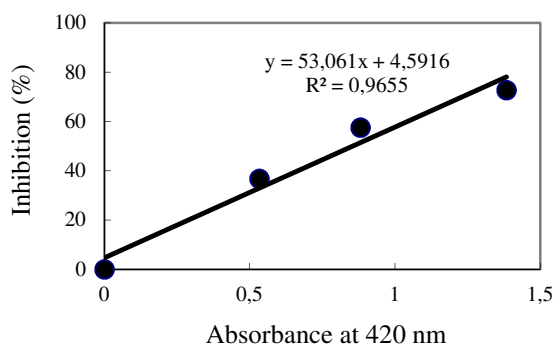


Figure 5. The relationship between the absorbance at 420 nm and the inhibition on riboflavin photodegradation of melanoidin at 60 min light oxidation.

On the positive control sample (the riboflavin solution containing ascorbic acid), the prolongation of half-life period was also recognized (Fig. 4). It was coincident with the results reported by Lee et al. (1998). They demonstrated that the 0.1 % (approximately 5 mM) ascorbic acid treatments resulted in 50 and 25.5 % inhibition of riboflavin reduction in whole milk and skim milk, respectively after 10 h light illumination at 3300 lux. In another study, Jung et al. (2000) also observed that 0.1 % ascorbic acid treatment exhibited the positive effect on the riboflavin-sensitized photochemical changes in milk protein degradation. Notably, melanoidin 3 that absorbance is 0.533 at 420 nm showed the protective ability as much as ascorbic acid (5 mM). From this result, it was demonstrated that melanoidin had showed the higher protective activity than ascorbic acids on riboflavin photo-degradation.

3.2. Effect of melanoidins formed during the Maillard reaction in milk sample on riboflavin photo-degradation

In order to evaluate the effect of melanoidins formed in milk on the riboflavin photo-degradation, UHT-treated milk was heated at 120 °C for 0 - 30 min. As shown in Fig. 6, the prolongation of a half-life period from 53.5 to 90.5 min depended on heating time and the increasing of absorbance at 420 nm (table 2). In other words, the heat-treatment of milk increased the riboflavin photo-stability. This result was of the same tendency with the finding of Saidi et al. (1995) who reported that heat-treatment of skim milk increased the riboflavin photo-stability. However, the authors did not mention about the effect of Maillard reaction products

which were produced during heat-treatment on riboflavin photo-degradation. Calligaris et al. (2004) also suggested that the antioxidant activity of milk increased after the heat-treatment because of the formation of Maillard reaction products. In particular, the Maillard reaction products also performed free radical scavenging ability in milk (Morales et al., 2001; Calligaris et al., 2004). From these reasons, it could be suggested that melanoidins - Maillard reaction products could be potentially responsible for the increase of riboflavin stability. Furthermore, to make the protective ability of melanoidins on riboflavin photo-degradation in milk more clearly, the extracted melanoidins were added into milk and it was subjected to light exposure test. Under the same illuminating condition, riboflavin contained in milk was more easily degraded than that in the presence of melanoidins with the concentration equal to 0.73 value at 420 nm (table 2). These results clearly demonstrated that melanoidins themselves showed the protective effect against riboflavin photo-degradation in milk.

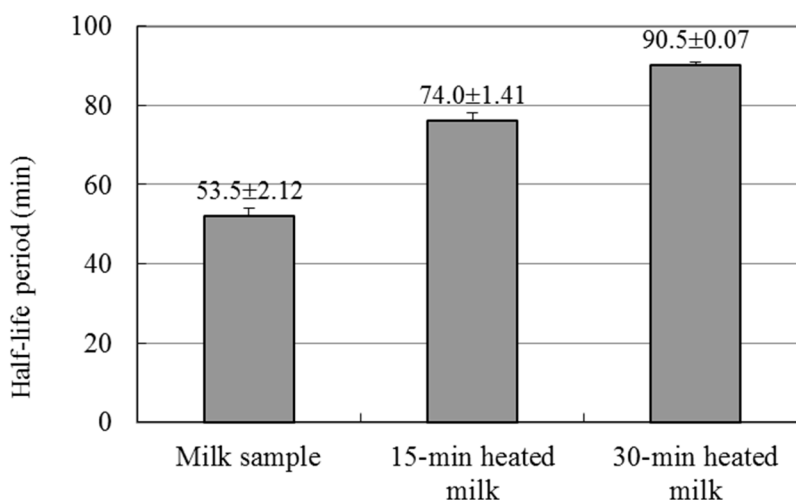


Figure 6. Effect of melanoidin formation during heating at 120 °C on photo-degradation of riboflavin in milk.

Table 2. Effect of melanoidins formed by Maillard reaction in heated-milk sample on photo-degradation of riboflavin.

Sample	Half-life period (min)	Absorbance at 420 nm
UHT-milk	53.50 ± 2.12	0.08
UHT-milk heated at 120 °C for 15 min	74.00 ± 1.41	0.26
UHT-milk heated at 120 °C for 30 min	90.50 ± 0.07	0.52
UHT-milk with added melanoidin	120 ± 0	0.73

The effect of heat-treatment on the riboflavin photo-degradation was also observed in previous study, in which the increase in riboflavin photo-stability after heat-treatment was explained due to the change of casein micelle size and the effect of the absorption light (Saidi et al., 1995). However, from the results obtained in our study, it could be proposed that the protection ability of milk on riboflavin photo-degradation was derived from melanoidins generated during pasteurization rather than the effect of modification of milk structure. In

addition, because the degradation of riboflavin was prevented by melanoidins, the riboflavin-sensitized photochemical changes would be reduced in milk sample. For that reason, melanoidins can play an important role in protecting milk nutrients during storage.

4. CONCLUSION

In conclusion, melanoidins had the protective activity on the riboflavin photolysis. The preventing ability of melanoidins on riboflavin photolysis increased with the increasing of its concentration. Moreover, this study also clarified that the protective ability on photo-degradation of riboflavin in commercial milk is derived from the formation of melanoidins rather than the denaturizing of milk protein. In consequences, it was revealed that melanoidins could contribute greatly to protect the important nutrient in UHT-treated milk. This study could suggest for milk processing engineers to select the suitable technology conditions which not only keep good quality products, but also improve the generation of benefit chemicals for the products.

REFERENCES

1. Aurand W., Boone H., and Giddings G. - Superoxide and singlet oxygen in milk lipid peroxidation, *Journal of Dairy Science* **60** (1977) 363-3699.
2. Bradley G., Kim J., and Min B. - Effects, quenching mechanisms, and kinetics of water soluble compounds in riboflavin photosensitized oxidation of milk, *Journal of Agricultural and Food Chemistry* **54** (2006) 6016-6020.
3. Calligaris S., manzocco, L., Anese, M., & Nicoli, M. C. - Effect of heat-treatment on the antioxidant and pro-oxidant activity of milk, *International Dairy Journal* **14** (2004) 421-427.
4. Cardoso D. R., Homem-de-mello P., Olsen K., Silva A. B. F., Franco D. W., and Skibsted L. H. - Deactivation of triplet-excited riboflavin by purine derivatives: important role of uric acid in light-induced oxidation of milk sensitized by riboflavin, *Journal of Agricultural and Food Chemistry* **53** (2005) 3679-3684.
5. Choe E., Huang R., and Min B. - Chemical reactions and stability of riboflavin in foods, *Journal of Food Science* **70** (2005) 28-36.
6. Jung Y., Lee H., and Kim Y. - Riboflavin-sensitized photochemical changes in β -lactoglobulin and aqueous buffer solution as affected by ascorbic acid, *Journal of Agricultural and Food Chemistry* **48** (2000) 3847-3850.
7. King J., Kim H., and Min B. - Riboflavin photosensitized singlet oxygen oxidation products of vitamin D, *Journal of the American Oil Chemist's Society* (2002) 983-987. (Thiếu số Tập)
8. Lee H., Jung Y., and Kim Y. - Effects of ascorbic acid on the light-induced riboflavin degradation and color changes in milks, *Journal of Agricultural and Food Chemistry* **46** (1998) 407-410.
9. Lingnert H., and Hall G. - Formation of antioxidative Maillard reaction products during food processing, *The 3rd International Symposium on the Maillard reaction*, Susono, Shizuoka, Japan, July 1-5, 1985. Tokyo, Japan: Elsevier.

10. Manzocco L., Calligaris S., Mastrocola D., Nicoli M. and Lericci C. - Review of non-enzymatic browning and antioxidant capacity in processed food, *Food Science and Technology* **11** (2001) 340-346.
11. Min B., and Boff M. - Chemistry and reaction of singlet oxygen in foods, *Journal of Food Science* **70** (9) (2002) 142-159.
12. Morales F., and Jimenez-Perez S. - Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescence, *Food Chemistry* **72** (2001) 119-125.
13. Pischetsrieder M., Rinaldi F., Gross U., and Severin T. - Assessment of the antioxidative and prooxidative activities of two aminoreductones formed during Maillard reaction: Effects on the oxidation of β -Carotene, N^α-Acetylhistidine, and cis-Alkenes, *Journal of Agricultural and Food Chemistry* **46** (1998) 2945-2950.
14. Saidi B., and Warthesen J. - Effect of heat and homogenization on riboflavin photolysis in milk, *International Dairy Journal* **5** (1995) 635-645.
15. Shimamura T., Ukeda H., and Sawamura M. - Reduction of tetrazolium XTT by aminoreductone formed during the Maillard reaction of lactose, *Journal of Agricultural and Food Chemistry* **48** (2000) 6227-6229.
16. Silvan J. M., Lagemaat J. V. D., Olano A., and Castillo M. D. D. - Analysis and biological properties of amino acid derivatives formed by Maillard reaction in foods, *Journal of Pharmaceutical and Biomedical Analysis* **41** (2006) 1543-1551.
17. Sun Y., Hayakawa S., Puangmanee S., and Izumori K. - Chemical properties and antioxidant activity of glycated α -lactalbumin with a rare sugar, D-allose, by Maillard reaction, *Food Chemistry* **95** (2006) 509-517.
18. Ukeda H., Shimamura T., Hosakawa T., Goto Y., and Sawamura M. - Monitoring of the Maillard reaction based on the reduction of tetrazolium salt XTT, *Food Science and Technology International* **4** (1998) 258-263.
19. Woodcock A., Warthesen J., and Labuza P. - Riboflavin photochemical degradation in Pasta measured by high performance liquid chromatography, *Journal of Food Science* **47** (1982) 545-549.
20. Trang V., Kurogi Y., Katsuno S., Shimamura T. and Ukeda H. - Protection riboflavin against photo-degradation by aminoreductone, *International Dairy Journal* **18** (2008) 344-348.

TÓM TẮT

KHẢ NĂNG BẢO VỆ CỦA MELNOIDINS ĐỐI VỚI SỰ PHÂN HỦY RIBOFLAVIN BỞI ÁNH SÁNG

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Nghiên cứu về tác động của melanoidins, sản phẩm của phản ứng Maillard, lên quá trình phân hủy bởi ánh sáng của riboflavin được tiến hành trên dung dịch mẫu riboflavin ($1,5 \text{ mg L}^{-1}$) và sữa tiệt trùng trong điều kiện chiếu sáng với cường độ sáng 7000 lux trong 2,5 giờ. Trong dung dịch mẫu chứa riboflavin, melanoidins thể hiện khả năng bảo vệ riboflavin dưới sự phân hủy bởi ánh sáng và khả năng bảo vệ này phụ thuộc vào nồng độ melanoidins. Hơn thế nữa, melanoidins thể hiện khả năng bảo vệ cao hơn ascorbic axit, vốn là chất có tác dụng bảo vệ riboflavin tốt dưới sự phân hủy bởi ánh sáng. Trong mẫu sữa, sự hình thành melanoidin trong quá trình gia nhiệt sữa cũng làm nâng cao khả năng bảo vệ sự phân hủy riboflavin dưới tác dụng của ánh sáng. Vì vậy, có thể kết luận rằng, melanoidins chính là hợp chất làm tăng tính ổn định của riboflavin dưới tác động của ánh sáng trong sữa tiệt trùng UHT.

Từ khóa: melanoidins, maillard reaction, riboflavin, photo-degradation and milk.

Ý kiến TBT:

- Tài liệu tham khảo:
 - + Dùng cách viết trích dẫn bằng số [1]..
 - + Sắp lại thứ tự trong danh sách sao cho tài liệu trích dẫn lần đầu là [1], [2], [3].. không phải [4]...[9] như trong bài.
- Chỉnh lại (thu nhỏ) cỡ chữ hình 4, 5, 6 cho đúng như qui định của định dạng. Tên đại lượng và số thang đo của trục x bị đè nhau.
- Đề nghị dịch ra tiếng Việt các Từ khoa (sau Tóm tắt).