In Vitro Chlorophyll Degradation Involved in Flavonoid Radical Formed by Chlorophyll-degrading Peroxidase in Flavedo Extract of *Citrus nagato-yuzukichi* Fruit

Naoki Yamauchi* and Kuniyasu Eguchi**

Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515

Summary

Effects of flavonoid radical formed by a chlorophyll (Chl)-degrading peroxidase in the flavedo extract of the green sour citrus, Nagato-yuzukichi (*Citrus nagato-yuzukichi* hort. ex Y. Tanaka) fruit on *in vitro* Chl degradation were determined. Adding hydrogen peroxide accelerated the Chl breakdown by the flavedo extract which included flavonoids and a Chl-degrading peroxidase. During Chl degradation, naringin, hesperidin, and neohesperidin were rapidly oxidized, but only naringin was involved in Chl degradation by Chl-degrading peroxidase. This enzymatic degradation in the flavedo extract was suppressed by the addition of scavengers of radical and superoxide anions. The superoxide formed by a xanthine-xanthine oxidase system and a superoxide dismutase, however, had little effect on Chl degradation. MnCl_2 , which is a superoxide anion scavenger, also inhibited the flavonoid oxidation. These findings indicate that the naringin radical formed by a Chl-degrading peroxidase in the flavedo could be related to Chl degradation.

Key Words: chlorophyll-degrading peroxidase, citrus, flavonoid, Nagato-yuzukichi, radical.

Introduction

Nagato-yuzukichi, which is cultivated in Yamaguchi Prefecture, is a flavorful, acidic citrus fruit. It is harvested in late summer when the acidic fruit is small and eaten while the peel is still green, similar to kabosu (*Citrus sphaerocarpa* hort. ex Tanaka) and sudachi (*Citrus sudachi* hort. ex Shirai). For postharvest marketing, the green color of the peel should be retained as long as possible.

At an initial step of chlorophyll (Chl) degradation, chlorophyllase seems to be involved in chlorophyllide formation (Shimokawa et al., 1978; Amir-Shapira et al., 1987). The chlorophyllide is degraded further to colorless, low molecular weight compounds through pheophorbide (Matile et al., 1999) and pyropheophorbide (Shioi et al., 1991; Kurata et al., 1998). However, Chl-degrading peroxidase also seems to be involved in C13^2 -hydroxychlorophyll *a* (Chl *a-1*) formation as an initial step in Chl degradation as the fruit senesce (Kato and Shimizu, 1985; Yamauchi and Watada, 1994; Yamauchi et al., 1997).

We reported previously that in Satsuma mandarin, Chl was degraded concurrently with hesperidin oxidation by a Chl-degrading peroxidase. Chl content in ethylene-treated fruit decreased rapidly with the enhancement of

the peroxidase activity and the decrease of the hesperidin content in the fruit flavedo (Yamauchi et al., 1997). These results indicated that Chl-degrading peroxidase could be involved in the degreening of Satsuma mandarin fruit.

This paper deals with the involvement of flavonoid oxidation and the resulting flavonoid radical and superoxide anion in *in vitro* Chl degradation in Nagato-yuzukichi flavedo. The results elucidate the Chl degradation and the role of Chl-degrading peroxidase.

Materials and Methods

Preparation of Nagato-yuzukichi flavedo extract

The Nagato-yuzukichi (*Citrus nagato-yuzukichi* hort. ex Y. Tanaka) fruit were harvested at Hagi Citrus Research Institute Farm. The flavedo (2.5 g) was ground in liquid nitrogen by using a mortar and pestle and homogenized in 20 ml 20 mM phosphate buffer (pH 7.0). The homogenate was filtered through one layer of Miracloth (CALBIOCHEM) and the filtrate centrifuged at 16,000 $\times$ g for 15 min at 4 °C. The supernatant served as the flavedo extract.

Determination of chlorophyll degradation by flavedo extract

The reaction mixture containing 0.5 ml flavedo extract, 0.1 ml Chls acetone solution (400 μg Chl *a* $\cdot$ ml⁻¹), 0.1 ml 2% Triton X-100 and 1.7 ml 100 mM phosphate buffer (pH 6.0), was activated by the addition of 0.1 ml

Received; May 9, 2001. Accepted; August 13, 2001.

* Corresponding author. E-mail: yamauchi@agr.yamaguchi-u.ac.jp

**Present address: Hiroshima Branch, Agro-Kanesho Co., Ltd., 2-8-29 Tokaichi-machi, Naka-ku, Hiroshima 730-0802

0.3% hydrogen peroxide to give a total volume of 2.5 ml. The reaction was carried out for 10 min at 25 °C and stopped by the addition of 5.0 ml acetone. The remaining Chls were extracted with 5.0 ml hexane and determined spectrophotometrically at 663 nm. The amount of degraded Chls was calculated by subtracting this value from the blank (distilled water instead of hydrogen peroxide). One unit of Chl degradation was defined as the change of 0.01 absorbance per min.

Partially purified Chls used as substrate were prepared according to Yoshiura and Iriyama (1979).

Crude enzyme extract from flavedo

Flavedo (5.0 g) was ground in liquid nitrogen using a mortar and pestle and homogenized in 20 ml of 100 mM phosphate buffer (pH 7.5) containing 10 mM cysteine and 0.5 g polyclar AT. The homogenate was filtered through one layer of Miracloth and the filtrate centrifuged at 16,000 x g for 15 min at 4 °C. Granular ammonium sulfate was added to the supernatant at 0 °C to give 90% saturation. One hour later, the sample was centrifuged at 16,000 x g for 15 min at 4 °C. The pellet was suspended in 90% saturated ammonium sulfate (pH 7.0) and stored at -20 °C.

Determination of chlorophyll degradation by crude enzyme extract

An aliquot of the saturated ammonium sulfate suspension was dissolved in a 20 mM phosphate buffer (pH 7.0) and the solution centrifuged at 16,000 x g for 15 min at 4 °C. The supernatant was passed through a Sephadex PD-10 column (Amersham Pharmacia Biotech) equilibrated with a 5 mM phosphate buffer (pH 7.0) and the enzyme eluted. The reaction mixture containing 0.5 ml crude enzyme solution (0.48 mg · ml⁻¹), 0.1 ml Chls acetone solution (400 µg Chl a · ml⁻¹), 0.1 ml 2% Triton X-100, 0.1 ml 5 mM naringin and/or hesperidin, and 1.7 ml 100 mM phosphate buffer (pH 6.0), was activated by adding 0.1 ml 0.3% hydrogen peroxide to yield a total volume of 2.5 ml. After 10 min at 25 °C, the reaction was stopped by adding 5.0 ml acetone. The residual Chls were determined as above.

The enzyme protein content was assayed by the method of Bradford (1976).

Determination of chlorophyll degradation by a superoxide anion

Chl degradation was assayed by using the xanthine-xanthine oxidase system, which forms a superoxide anion. The reaction mixture containing 0.1 ml of 2.5 or 12.5 mM xanthine, Chls acetone solution (400 µg Chl a · ml⁻¹), 0.1 ml 1% Triton X-100, 0.2 ml distilled water, and 2.0 ml potassium phosphate buffer (pH 7.0), was activated by adding 20 µl xanthine oxidase (SIGMA, Grade I, 1 Unit · ml⁻¹). After 10 min at 25 °C, the reaction was stopped by the addition 5.0 ml acetone. The remaining Chls were determined as above. Superoxide

anion production by the xanthine-xanthine oxidase system was estimated by measuring the cytochrome c (WAKO Chemical) reduction according to Ogita and Oura (1987).

Flavonoid analysis by HPLC

The Chl degradation reaction with the flavedo extract was stopped by the addition of acetone, after which hexane was added to remove the remaining Chls. The acetone fraction was filtered through a 0.5 µm filter (Katayama Chemical), and the filtrate analyzed for flavonoids by HPLC using a slightly modified procedure of Yamauchi et al. (1997). An aliquot of filtrate (50 µl) was injected into the HPLC fitted with a LiChrospher C18 column (Merck), 4 x 250 mm. The column was eluted with a linear gradient of 40 to 70% methanol over 50 min and then kept isocratic for an additional 10 min; the flow rate was 0.6 ml per min. The absorbance which was monitored at 283 nm distinguished narirutin, naringin, hesperidin, and neohesperidin by their retention times and the absorption spectra using standards.

All analyses were performed in duplicate or triplicate.

Results and Discussion

Chlorophyll degradation by chlorophyll-degrading peroxidase in a flavedo extract

Chls were degraded rapidly in the presence of hydrogen peroxide (Table 1). Some Chls might have been degraded by chlorophyllase in the flavedo extract. The reaction showed very little Chl degradation without an enzyme. Additionally, the Chl degradation was suppressed by removing the low molecular weight compounds.

Flavonoids were oxidized by the addition of hydrogen peroxide to a reaction mixture containing the flavedo

Table 1. Characteristics of chlorophyll degradation by the flavedo extract in the presence of hydrogen peroxide.

	Chl degradation	Relative activity(%)
Control ^z	23.1 ^y	100
-H ₂ O ₂	4.86	21
-Enzyme ^x	0.72	3
-LMWC ^w	2.02	9

Chl: Chlorophyll

^zControl: Reaction mixture=flavedo extract+Chls acetone solution+hydrogen peroxide+Triton X-100 +phosphate buffer (pH 6.0)

^yUnits · g⁻¹ flavedo, Unit= Δ O.D. 663 nm 0.01 · min⁻¹.

^xThe flavedo extract was filtered through Ultrafree-MC (Millipore) for removing the high molecular weight compounds (>MW 5,000).

^wLMWC: Low molecular weight compounds (<MW 5,000)
The flavedo extract was passed through PD-10 column (Amersham Pharmacia Biotech).

Fig. 1. Flavonoid changes by the addition of hydrogen peroxide to a reaction mixture containing Nagato-yuzukichi flavedo extract. Closed symbol: + hydrogen peroxide, open symbol: - hydrogen peroxide. The vertical bars represent SE (n=3).

extract and Chls (Fig. 1). Four flavonoid pigments, naringin, and narirutin, which have a hydroxyl group at the *p*-position in the B ring of naringenin as an aglycone, and hesperidin and neohesperidin, which have a methyl group at the *p*-position in the B ring of hesperetin as an aglycone, were detected in the flavedo extract. Levels of naringin, hesperidin, and neohesperidin decreased markedly with the addition of hydrogen peroxide, whereas the level of narirutin remained almost unchanged. Chl degradation by the flavedo enzyme-hydrogen peroxide system occurs in the presence of naringin and/or hesperidin (Table 2). Apparently, Chls were degraded rapidly by the addition of naringin but only slightly by the addition of hesperidin. Moreover, the Chl degradation by naringin was suppressed in the presence of hesperidin. Neohesperidin, which has the hesperetin as an aglycone as well as hesperidin, seems to react similarly to hesperidin during Chl degradation by peroxidase. These findings indicate that only naringin is primarily involved in peroxidase catalyzed Chl degradation, and that the process is also suppressed in the presence of hesperidin.

Yamauchi and Minamide (1985) reported that flavonoid, like apigenin, was an important substrate in Chl degradation catalyzed by peroxidase, whereas Yamauchi

Table 2. Chlorophyll degradation by the flavedo enzyme-hydrogen peroxide system in the presence of naringin and/or hesperidin.

Flavonoid	Chl degradation (%)
Naringin (0.2 mM)	72.3 ^z (100)
Naringin (0.2 mM) + Hesperidin (0.2 mM)	47.3 (65)
Hesperidin (0.2 mM)	5.97 (8)

Chl: Chlorophyll

Reaction mixture=flavedo enzyme+Chls acetone solution
+flavonoid+hydrogen peroxide+Triton X-100
+phosphate buffer (pH 6.0)

^zUnits $\cdot \text{mg}^{-1}$ protein, Unit= Δ O.D. 663 nm $0.01 \cdot \text{min}^{-1}$.

Table 3. Effects of various chemicals on chlorophyll degradation by the flavedo extract-hydrogen peroxide system.

Chemicals	Concentration (mM)	Chl degradation ^z (%)
Control ^y		26.5 $\pm$ 0.03 ^x (100)
Histidine	1.0	26.8 $\pm$ 0.22 (101)
	5.0	25.2 $\pm$ 0.46 (95)
2, 2'-Bipyridyl	1.0	18.1 $\pm$ 0.19 (68)
Ascorbate	1.0	16.3 $\pm$ 0.59 (62)
	5.0	0.70 $\pm$ 0.31 (3)
Tiron	1.0	2.99 $\pm$ 0.24 (11)
Hydroquinone	1.0	28.0 $\pm$ 3.59 (106)
2, 5-Dimethylfulan	1.0	18.9 $\pm$ 0.09 (71)
	5.0	19.3 $\pm$ 0.38 (73)
NBT	1.0	13.9 $\pm$ 0.27 (52)
SOD	338 ^w	26.2 $\pm$ 0.25 (99)
MnCl ₂ ^v	1.0	2.22 $\pm$ 0.28 (8)
n-Propyl gallate	1.0	7.72 $\pm$ 0.51 (29)
	5.0	0 (0)

Chl: Chlorophyll, Tiron: 1, 2-Dihydroxybenzene-3, 5-disulphonic acid, NBT: Nitroblue tetrazolium, Ascorbate: Sodium ascorbate, SOD: Superoxide dismutase (Cu, Zn-type)

^zChl degradation: Units $\cdot \text{g}^{-1}$ flavedo, Unit= Δ O.D. 663 nm $0.01 \cdot \text{min}^{-1}$.

^yControl: Reaction mixture=flavedo extract+Chls acetone solution+hydrogen peroxide+Triton X-100+phosphate buffer (pH 6.0)

^xMean $\pm$ SE (n=3).

^wUnits.

^vMES buffer (pH 6.0) was used in this reaction since the precipitate of MnCl₂ was formed in the phosphate buffer.

et al. (1997) found that hesperidin and narirutin in the flavedo of Satsuma mandarin fruit were major flavonoid, but that only hesperidin decreased, accompanied by peroxidase activity, during the degreening of Satsuma mandarin fruit. We infer that the substrate affinity of the Satsuma mandarin Chl-degrading peroxidase is different from that of the Nagato-yuzukichi fruit. We also suggest that in Satsuma mandarin fruit, there is another phenolic compound that has a *p*-position of a

hydroxyl group that might be involved in degreening. Further study is needed to support this speculation.

Involvement of flavonoid oxidation and superoxide generation in chlorophyll degradation

The inhibition of Chl degradation by several chemicals was determined to clarify the involvement of flavonoid oxidation (Table 3). Histidine, a hydroxy radical scavenger, and hydroquinone, a reducing agent, had little effect on Chl degradation by peroxidase. The radical scavengers, ascorbate and *n*-propyl gallate, and the Fe^{2+} chelating agent, 2, 2'-bipyridyl, effectively inhibited the reaction, which indicates that ascorbate and *n*-propyl gallate might reduce the naringin radical formed by a Chl-degrading peroxidase and that 2, 2'-bipyridyl inhibits the action of the enzyme. The scavengers of the superoxide anion, Tiron, NBT, MnCl_2 , and 2, 5-dimethylfulan, strongly suppressed Chl breakdown, but the superoxide dismutase (Cu, Zn-SOD), which also scavenges the superoxide anion, had little effect.

The superoxide anion formed by the xanthine-xanthine oxidase system did not degrade Chls during the reaction time, even when naringin or flavedo extract was added in the reaction mixture (Table 4). This suggests that the superoxide anion is probably not involved in Chl degradation. Allomerization of Chl in acetone-methanol or acetonitrile is induced in the presence of active oxygen species (Hynninen, 1981; Merzlyak et al., 1985). However, only a small amount of Chl was degraded by the superoxide anion in our reaction condition, and SOD also did not inhibit the Chl degradation. More study is necessary to clarify the effect of superoxide anion on Chl degradation.

Naringin and hesperidin contents decreased when SOD was added in the reaction mixture, whereas their

levels were unchanged when MnCl_2 was added (Table 5). This suggests that SOD is only a scavenger of the superoxide anion, whereas MnCl_2 inhibits flavonoid oxidation as well as scavenges for the superoxide anion. Thus, the scavengers of the superoxide anion, except for SOD, may function both as a scavenger of the superoxide anion and an inhibitor of flavonoid oxidation.

Adachi and Shimokawa (1995) demonstrated that some scavengers of the superoxide anion inhibited peroxidase catalyzed Chl degradation in radish cotyledon, indicating that the superoxide anion plays an important role in enzymatic Chl α bleaching reactions. Ma and Shimokawa (1998) also reported that the superoxide anion and radical could be involved in Chl degradation by peroxidase in banana peel. In Nagato-yuzukichi flavedo, however, the naringin radical seems to be mainly involved in Chl degradation by peroxidase. Takahama and Oniki (1998) demonstrated that, in broad bean leaves, 3, 4-dihydroxyphenylalanine (DOPA) was oxidized by phenoxyl radicals of the hydroxycinnamic acid ester, which has a hydroxyl group at the *p*-position, formed by peroxidase in the presence of both DOPA and hydroxycinnamic acid ester; peroxidase mainly oxidizes hydroxycinnamic acid ester; and the radical formed reacts with DOPA. Kato and Shimizu (1985) also reported that the free radicals produced from the phenolic compounds could mediate Chl degradation when the hydrogen peroxide was added in the Chl-peroxidase-phenolic compound system. Chl degradation by peroxidase in the Nagato-yuzukichi flavedo extract seems to indicate that naringin is mainly oxidized by the enzyme; hence, the naringin radical is related to the Chl degradation.

Table 4. Effects of superoxide anion formed by xanthine-xanthine oxidase system on chlorophyll degradation.

Superoxide generation ^z	Chl degradation ^y
-Xanthine (Control)	0.04
+Xanthine (0.1 mM)	0.12
+Xanthine (0.5 mM)	0.14
+Xanthine (0.1 mM) + Naringin (0.2 mM)	0
+Xanthine (0.1 mM) + Flavedo extract	0

Chl: Chlorophyll

^zReaction mixture=xanthine+xanthine oxidase (0.02 Unit)+Chls acetone solution+Triton X-100+phosphate buffer (pH 6.0)

The level of superoxide anion generation by xanthine (0.1 or 0.5 mM)-xanthine oxidase system was approximately $7.0 \text{ nmole} \cdot \text{min}^{-1}$.

^yUnit, Unit= $\Delta \text{O.D. } 668 \text{ nm } 0.01 \cdot \text{min}^{-1}$.

The activity of Chl degradation was determined spectrophotometrically by reading the decrease of absorbance at 668 nm as Chl was degraded.

Table 5. Changes in flavonoid content with chlorophyll degradation by the flavedo extract-hydrogen peroxide system in the presence of scavengers of superoxide anion, SOD and MnCl_2 .

Treatments	+ H_2O_2	- H_2O_2
	Naringin content ($\mu\text{g} \cdot \text{ml}^{-1}$)	
Control ^z	0.452	1.98
+ MnCl_2 (1 mM)	1.92	2.20
+ SOD (338 Units)	0.454	2.16
	Hesperidin content ($\mu\text{g} \cdot \text{ml}^{-1}$)	
Control	0.037	1.27
+ MnCl_2 (1 mM)	1.12	1.20
+ SOD (338 Units)	0.014	1.38

Naringin and hesperidin contents at the beginning of the reaction were $1.94 \mu\text{g} \cdot \text{ml}^{-1}$ and $1.24 \mu\text{g} \cdot \text{ml}^{-1}$, respectively. The reaction was carried out for 5 min.

^zControl: Reaction mixture=flavedo extract+Chls acetone solution+hydrogen peroxide+Triton X-100+phosphate buffer (pH 6.0)

Acknowledgements

We are grateful to Hagi Citrus Research Institute of Yamaguchi Prefecture for supplying the Nagato-yuzukichi fruit. We thank Dr. Umeo Takahama, Professor of Kyushu Dental College, for his useful comments. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 11834011) from Japanese Society for the Promotion of Science.

Literature Cited

- Adachi, M. and K. Shimokawa. 1995. Evidence for the involvement of superoxide anion in the ethylene-induced chlorophyll *a* catabolism of *Raphanus sativus* cotyledons. *Phytochemistry* 39: 527–530.
- Amir-Shapira, D., E. E. Goldschmidt and A. Altman. 1987. Chlorophyll catabolism in senescing plant tissue: *In vivo* breakdown intermediates suggest different degradative pathways for *citrus* fruit and parsley leaves. *Proc. Natl. Acad. Sci. USA*. 84: 1901–1905.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254.
- Hynninen, P. H. 1981. Mechanism of the allomerization of chlorophyll: Inhibition of the allomerization by carotenoid pigments. *Z. Naturforsch.* 36b: 1010–1016.
- Kato, M. and S. Shimizu. 1985. Chlorophyll metabolism in higher plants. VI. Involvement of peroxidase in chlorophyll degradation. *Plant Cell Physiol.* 15: 187–190.
- Kurata, H., Y. Maeda, M. Adachi and K. Shimokawa. 1998. A possible participation of pyropheophorbide *a* in chlorophyll catabolism during ripening of *Citrus unshiu* Fruits. *J. Japan. Soc. Hort. Sci.* 67: 651–654.
- Ma, X. W. and K. Shimokawa. 1998. *In vitro* studies on ethylene-enhanced chlorophyll degrading peroxidase and its reaction products in banana (*Musa sapientum* L.) fruits. *J. Japan. Soc. Hort. Sci.* 67: 261–269. (In Japanese with English summary)
- Matile, P., S. Hörtensteiner and H. Thomas. 1999. Chlorophyll degradation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50: 67–95.
- Merzlyak, M. N., V. A. Kovrighnikh, N. S. Kuprianova and I. B. Afanas'ev. 1985. The allomerization of chlorophylls *a* and *b* with superoxide anion. *J. Inorg. Biochem.* 24: 239–242.
- Ogita, Z. and H. Oura. 1987. Kasseisan-sono rinshou-igaku heno ouyou. p. 42–43. Kyouritushuppan. Tokyo (In Japanese).
- Shimokawa, K., S. Shimada and K. Yaeo. 1978. Ethylene-enhanced chlorophyllase activity during degreening of *Citrus unshiu* Marc. *Sci. Hortic.* 8: 129–135.
- Shioi, Y., Y. Tatsumi and K. Shimokawa. 1991. Enzymatic degradation of chlorophyll in *Chenopodium album*. *Plant Cell Physiol.* 32: 87–93.
- Takahama, U. and T. Oniki. 1998. 3, 4-Dihydroxyphenylalanine is oxidized by phenoxyl radicals of hydroxycinnamic acid esters in leaves of *Vicia faba* L. *J. Plant Res.* 111: 487–494.
- Yamauchi N. and T. Minamide. 1985. Chlorophyll degradation by peroxidase in parsley leaves. *J. Japan. Soc. Hort. Sci.* 54: 265–271.
- Yamauchi, N. and A. E. Watada. 1994. Effectiveness of various phenolic compounds in degradation of chlorophyll by *in vitro* peroxidase-hydrogen peroxide system. *J. Japan. Soc. Hort. Sci.* 63: 439–444.
- Yamauchi, N., X. Xia and F. Hashinaga. 1997. Involvement of flavonoid oxidation with chlorophyll degradation by peroxidase in wase Satsuma mandarin fruits. *J. Japan. Soc. Hort. Sci.* 66: 283–288.
- Yoshiura, M. and K. Iriyama. 1979. Methods for the preparation and qualitative and quantitative analysis of chlorophylls. *Protein, Nucleic acid & Enzyme* 24: 612–620 (In Japanese).

長門ユズキチ果実フラベド抽出物のクロロフィル分解ペルオキシダーゼにより生じたフラボノイド・ラジカルによるクロロフィル分解

山内直樹・江口國安*

山口大学農学部 753-8515 山口市吉田 1677-1

摘 要

緑色香酸柑橘の長門ユズキチ (*Citrus nagato-yuzukichi hort. ex Y. Tanaka*) 果実フラベド抽出物を用い、フラボノイド酸化が関与するクロロフィル分解過程について調べた。フラベド抽出物に過酸化水素を添加すると、抽出物に存在するフラボノイドとクロロフィル分解ペルオキシダーゼによりクロロフィルの分解が促進された。クロロフィル分解中、ナリンギン、ヘスペリジンおよびネオヘスペリジンが酸化されたが、ナリンギンのみがクロロフィル分解に直接関与することを認めた。

数種阻害剤を用いクロロフィル分解ペルオキシダーゼによるクロロフィル分解過程について検討したところ、ラジカル並びにスーパーオキシドアニオン消去剤により抑制が認めら

れた。しかしながら、キサンチン-キサンチンオキシダーゼ系によるスーパーオキシドアニオン生成下でクロロフィル分解が生じないことを認め、また、スーパーオキシドジスムターゼがクロロフィル分解反応を阻害しないことがわかった。さらに、スーパーオキシドアニオン消去剤の一つである MnCl_2 はフラボノイド酸化抑制作用も併せ持つことがわかった。以上の結果から、長門ユズキチ果実フラベド抽出物のクロロフィル分解ペルオキシダーゼによるクロロフィル分解は、まずナリンギンが酸化され、生じたナリンギンラジカルがクロロフィル分解に関与しているものと推察された。

---

*現在: アグロカネショウ株式会社広島営業所 730-0802 広島市中区十日市町2-8-29