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MiniReview

Chlorophyll Degradation in Horticultural Crops

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Abstract

One of the symptoms of senescence in harvested horticultural crops is the loss of greenness that comes with the degradation of chlorophyll. With senescence, the chlorophyll-degrading enzyme activities such as chlorophyllase, Mg-dechelatase or Mg-dechelation activity, a new chlorophyll-degrading enzyme, pheophytinase, pheophorbidase and chlorophyll-degrading peroxidase, which are involved in chlorophyll degradation, affected greatly in stored horticultural crops. The chlorophyll derivatives, especially chlorophyllide, pheophytin, pheophorbide and C13^2-hydroxychlorophyll are accumulated as intermediates of chlorophyll degradation. In addition, chlorophyll degradation by the chlorophyll-degrading enzymes seems to occur in the thylakoid and envelope membrane of chloroplast and/ or the vacuole. The involvement of chlorophyll-degrading enzymes in senescing horticultural crops is also discussed.

Keywords: Chlorophyll, chlorophyll degradation, chlorophyll-degrading enzymes, horticultural crop

Introduction

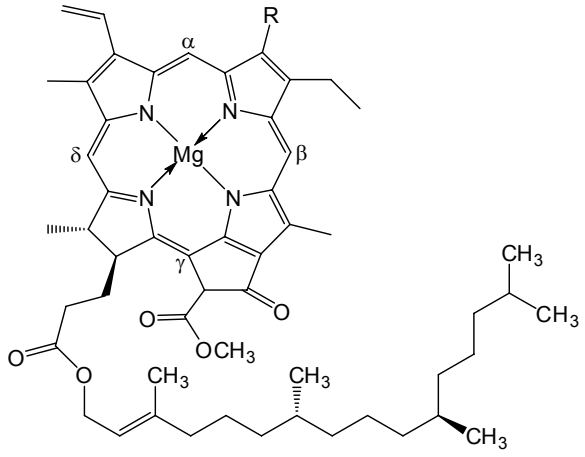
In general, the yellowing of leaves, florets and fruit pericarp is an important factor, indicating quality deterioration of stored horticultural products. Obviously, in spinach [1], parsley [2], broccoli [3] and lime [4,5], the most visible deterioration is the loss of sepal and peel greenness that usually occurs with chlorophyll (Chl) breakdown. On the other hand, in the fruit of an early-ripening cultivar, Wase Satsuma mandarin (*Citrus unshiu* Marc var. Tanaka), the peel is still green when the flesh matures and the fruit attains harvest maturity. To improve quality, ethylene is provided to the fruit to accelerate the degreening of the peel [6]. Thus, Chl degradation is a characteristic symptom of leaf senescence and fruit ripening, and elucidating the mechanism of Chl degradation is an important subject when considering the maintenance of the quality of harvested horticultural crops.

An early step of Chl *a* degradation seems to be the removal of the side chain attached to the tetrapyrrole macrocycle to form chlorophyllide (Chlide) *a* by chlorophyllase (Chlase). Chlide *a* formed still retains a green color [7,8]. The elimination of Mg^{2+} from Chlide *a* to produce pheophorbide (Pheide) *a* is induced by Mg-dechelatase (MD) [9-11] or a Mg-dechelating substance (MDS) [12-14], and the Pheide *a* formed then loses its green color. Finally, Pheide *a* is decomposed to fluorescent Chl catabolites, which are colorless, via a red Chl catabolite by both Pheide *a* oxygenase and red Chl catabolite reductase [15]. Chl-degrading peroxidase (POX) [11,16] is also suggested to be involved in Chl degradation as the 1st step enzyme with oxidizes Chl *a* to form 13^2-hydroxychlorophyll (C13^2-OHChl) *a*. In addition, a new Chl degrading enzyme, pheophytinase (pheophytin pheophorbide hydrolase, PPH) which would dephytylate the Mg-free Chl pigment, pheophytin (Phein) *a* to give Pheide has been recently reported [17].

In this review, we firstly deal with Chl structure and Chl derivatives, then the Chl degradation pathway by Chl-degrading enzymes, and, finally, with the characterization of Chl-degrading enzymes of postharvest horticultural crops.

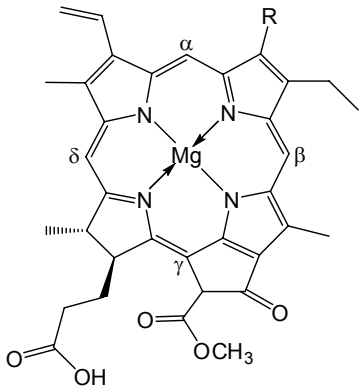
Chlorophyll structure

Chls are porphyrins containing basic tetrapyrrole rings, of which one is reduced. The 4 rings are coordinated to a Mg^{2+} ion. A 5th isocyclic ring E, is found near the 3rd pyrrole ring. At the 4th ring, the propionic acid substituent is esterified with diterpene alcohol phytol ($C_{20}H_{39}OH$), which is the hydrophobic side of the molecule, the rest of the molecule being hydrophilic. Chl *b* differs from Chl *a* only by having an aldehyde group (–CHO) in place of the methyl group at ring B position (**Figure 1**) [18].



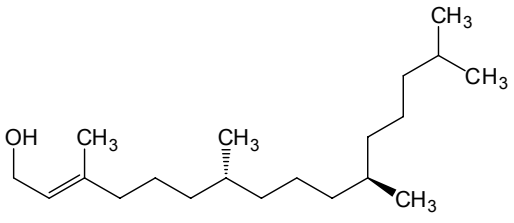
Chl

(a)



Chlide

+



Phytol

(b)

Figure 1 Structure formulae (a) Chl *a* (R=CH_3), Chl *b* (R= CH=O); (b) Chlide *a* (R=CH_3), Chlide *b* (R= CH=O) and phytol.
Source: Hörtensteiner and Kräutler [18]

Chlorophyll derivatives

Chls can be readily transformed, both *in vivo* and *in vitro*, into a series of derivatives.

1. Chlorophyllides *a* and *b*

The phytyl ester can be easily hydrolyzed to give Chlide and phytol. The hydrolysis takes place under mild conditions in the presence of either an acid or alkali. Chlides *a* and *b* are prepared enzymatically, the hydrolysis being catalyzed by Chlase, an enzyme commonly found in green plant tissues. Leaves that are especially rich in Chlase such as sugar beet [19], common cocklebur (*Xanthium pennsylvanicum*) [20], goosefoot (*Chenopodium album)* [12] and *Citrus unshiu* fruit [21] are used as enzyme sources.

2. Pheophytins *a* and *b*

Pheins are the magnesium-free derivatives of Chls. Pheins *a* and *b* are easily obtained from Chlase by the action of dilute acids, which remove the magnesium. The reaction lasts 1 - 2 min, and the concentration of HCl used is 13 % [20,22].

3. Pheophorbides *a* and *b*

Pheides *a* and *b* are hydrolyzed Chl without phytol (Chlides) that have also lost the magnesium. The reaction may be prepared from Chls treated with concentrated acid (30 % HCl) or from acidified Chlides [20,22].

4. C13^2-hydroxychlorophyll *a* and *b*

Chl *a* is oxidized with the oxygen atom being located at the position C-13^2 and hydroxychlorophyll being formed. C13^2-OHChl *a* was indentified in senescing excised leaves [23] and broccoli, which was prepared by adding peroxidase with the addition of H_2O_2 and *p*-coumaric acid to a Chl *a* solution [24].

5. Pyrochlorophylls

Pyroderivatives of Chls or their derivatives are compounds that have lost the carbomethoxy group –$COOCH_3$ at C-10 of the isocyclic ring, the group being replaced by hydrogen. Chl *a*, methyl Chlide *a*, Phein *a*, or methyl Pheide *a* when heated in pyridine at 100 °C give rise to 'pyro' derivatives by decarbomethoxylation [20].

The chlorophyll degradation pathway

The generally accepted pathway of Chl degradation comprises 2 stages, before (early stage) and after (late stage) cleavage of the tetrapyrrole macrocyclic rings. The products of the early stage are greenish, whereas those of the late stage are essentially colorless. The early stage includes modification of the side chain of the tetrapyrrole macrocycle; hydrolysis of a phytol residue in ring IV (dephytylation), release of Mg^{2+} from the tetrapyrrole macrocycle by displacement with $2H^+$ (dechelation) and some modifications of the macrocycle that are probably specific for the plant species. The late stage includes the cleavage of the tetrapyrrole macrocycle by an oxygenase and subsequent reactions, such as reduction to yield colorless fluorescent and further nonfluorescent catabolites. The late stage is thus essential in the degreening of the Chl molecule and therefore it determines Chl degradation in leaf senescence and fruit ripening. In most cases of leaf senescence and fruit ripening, degradation intermediates do not accumulate to an appreciable extent, suggesting that there is a series of degradation reactions (**Figure 2**) [25].

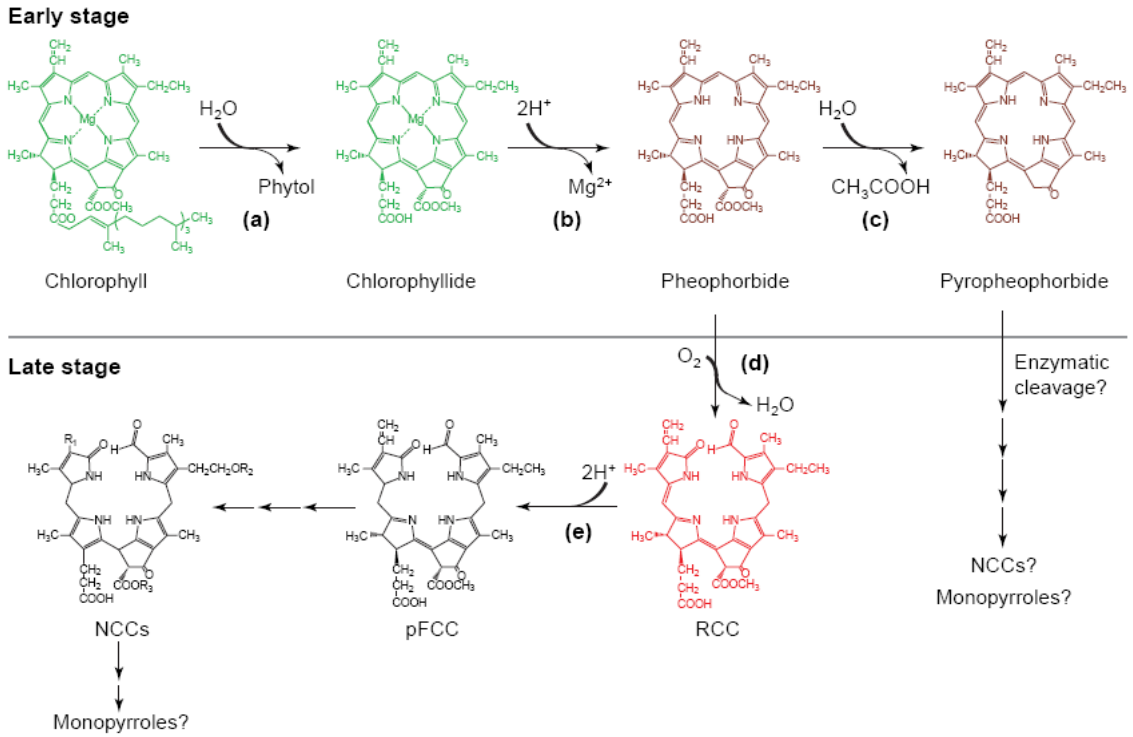


Figure 2 Chlorophyll degradation in higher plants (a) chlorophyllase (b) Mg-dechelatase (c) Pheophorbidase (d) Pheophorbide *a* oxygenase. (e) Red chlorophyll catabolite reductase. Abbreviations: NCCs, nonfluorescent chlorophyll catabolites; pFCC, primary fluorescent chlorophyll catabolite; RCC, red chlorophyll catabolite.
Source: Takamiya *et al* [25]

Characterization of Chlorophyll-degrading enzymes in relation to chlorophyll degradation

1. Chlorophyllase

The enzyme catalyzing the dephytylation, Chlase was one of the 1st plant enzymes to be studied [26]. There are many reports on Chlase activity, including the properties of crude enzymes and the effect of internal and external factors such as phytohormones and temperature stresses on the activity [27]. In spite of repeated isolations of Chlase from various plants and algae [28-30], molecular properties such as the entire amino acid sequence, functional domain, homology among Chlase and regulation of the expression of Chlase were unclear. The Chlase reaction is the 1st step of Chl degradation and therefore the location of Chlase is a factor in determining the site of Chl degradation. There is more than one compartment for Chlase localization. In most cases, the Chlase activity was latent in chloroplasts and *in vitro* which was considered to be thylakoid bound. Activity detection of Chlase using chloroplast subfractions localized Chlase activity to the envelope, probably in the inner membrane [31]. The latency appeared to be merely the result of the spatial inaccessibility of Chlase to Chl in the Chl–protein complex in thylakoid. Thus, based on the envelope location of Chlase, it was recently proposed that, *in vivo*, an as-yet unknown carrier protein for chlorophyll is synthesized in the senescent cell and transported to senescing chloroplasts and then it shuttles between the thylakoid and envelope membrane [15,31]. The Chl molecule or Chl–protein complex released from such plastoglobuli could be attacked by Chlase in the vacuole more than the chloroplast [32,33].

2. Mg-dechelation activity

Mg-dechelation takes place after dephytylation to yield Pheide *a* and pheophorbin *a* using Chlide *a* and Chlorophyllin (Chlin) *a* as substrates (**Figure 3**) [34]. Initially, the *in vivo* and *in vitro* accumulation of pheopigments during Chl degradation of algae and higher plants suggested the presence of MD enzymes [9,12,35,36]. Furthermore, *in vitro* assays of the dechelatase activity revealed that it was associated with thylakoid membranes in a latent form of rape cotyledon [9]. By contrast, in *Chenopodium album*, the activity could still be detected in a soluble low molecular mass (900 Da.) fraction after gel filtration of the enzyme, and it was heat stable. This activity was thus designated MDS [12]. Costa *et al* [37] reported that the Mg-dechelation activity was associated with a compound with a low molecular weight substance of 2,180 ± 20 Da. Suzuki *et al* [38] and Kunieda *et al* [34] demonstrated that the low molecular weight substances in radish cotyledons and mature leaves of *Chenopodium album* play a role in catalysis of the Mg-dechelation reaction using Chlide *a* as a substrate. Specifically, the low molecular weight substances in the mature leaves of *Chenopodium album* were found to have molecular masses of 3.3 and 1.1 kDa [34]. Lastly, the characterization of Mg-dechelation activity of stored broccoli florets was investigated to clarify the mechanism of Chl degradation [14]. Mg-dechelation activity in floret extracts was found in 2 different molecular weight fractions - a low molecular weight (< 5,000) fraction (LMWF) and a high molecular weight (> 5,000) fraction (HMWF), which seemed to be MD, using Chlin *a* or Chlide *a* as a substrate. The activity of the HMWF, which was partially purified by molecular exclusion chromatography (Sephacryl S-200) and using Chlin *a* as a substrate (**Figure 4a**), increased in yellowing broccoli florets after 6 days of storage (**Figure 4b**), whereas heat treatment reduced the enhancement of the activity concurrently with the inhibition of yellowing (**Figure 4c**). Only one peak of the activity was detected in fresh broccoli extracts and no other isozyme with Mg-dechelating action was found in the yellow broccoli extract which had a molecular mass of about 70 KDa. This high-molecular weight substance shows strong activity with the artificial substrate, Chlin *a*, but hardly an activity with native substrates, Chlide *a*. This means that the high-molecular weight substance (HMWS) does not have an activity with Chlide *a*. It is interpreted by the results obtained in this study. HMWS is not involved in the Chl degradation pathway of broccoli florets. It is necessary to purify the low molecular substance and clarify the characterization in the future.

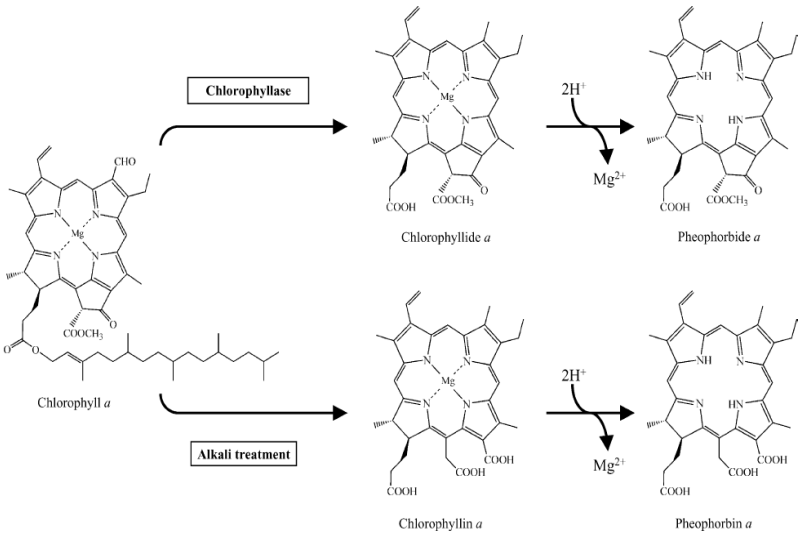


Figure 3 Structure of chlorophyll *a* and its derivative in relation to Mg-dechelation reaction. Chlorophyllide *a* is a native substrate, whereas chlorophyllin *a* is an artificial substrate in Mg-dechelation reaction.
Source: Kunieda *et al* [34]

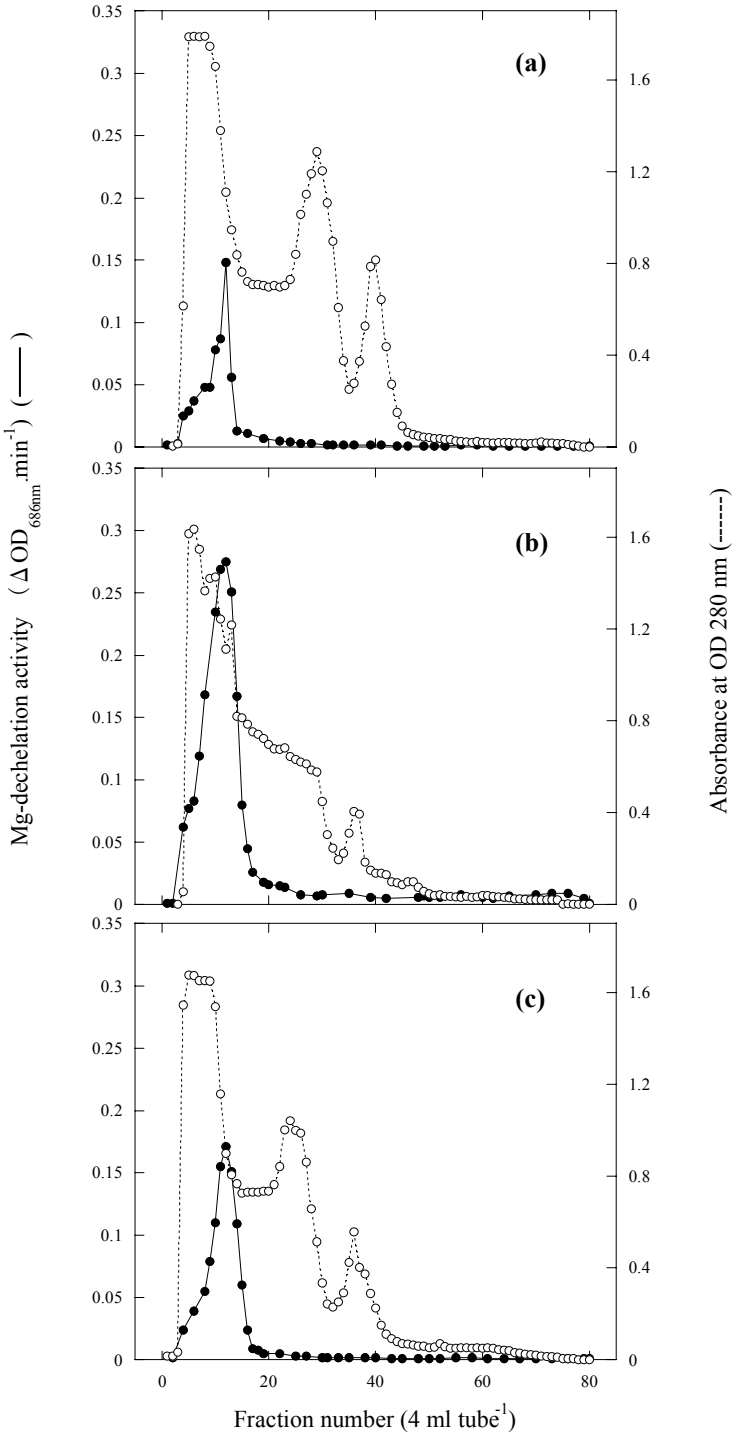


Figure 4 Elution profile on a Sephacryl S-200 column of Mg-dechelation activity in broccoli florets. (a) fresh broccoli florets, (b) broccoli florets stored for 6 days at 15 °C and (c) heat-treated broccoli florets stored for 6 days at 15 °C. a: Blue Dextran 2000.
Source: Kaewsuksaeng *et al* [14]

3. Pheophorbidase

Chl derivatives are found with modified side chains of a tetrapyrrole macrocycle. Recently, an enzyme named pheophorbidase (28 - 29 KDa) has been purified from *Chenopodium album*, which catalyzes the hydrolysis of the methyl ester bond of the isocyclic ring of Pheide to yield $C13^2$-carboxypyropheophorbide [39]. This is not stable and therefore is nonenzymatically converted to pyropheophorbide. Interestingly, pheophorbidase is located outside the chloroplast [34]. If Pheide *a* is a true substrate of the enzyme, it suggests that there is another degradation pathway whose early steps occur outside the chloroplast. Because the pheophorbidase activity is found in several, but not in all species of higher plants tested, this reaction might be specific for certain plants. $C13^2$-OHChl *a* is reported to be accumulated in ethylene-treated *Citrus* species and other plants [40,41] and might be an intermediate in the oxidative Chl bleaching pathway as well [41].

4. Chlorophyll-degrading peroxidase

The mechanism of *in vitro* Chl degradation by peroxidase can be summarized as shown in **Figure 5**. Peroxidase oxidizes the phenolic compounds, which have the hydroxyl group at the *p*-position, to form the phenoxy radical and superoxide anion; then, the radical and/or superoxide anion attacks Chl *a* to form $C13^2$-OHChl *a*. Chl *a* may be ultimately degraded in sequence to colorless low molecular weight compounds through Chl catabolites such as $C13^2$-OHChl *a* and bilirubin-like compounds [16]. Martinoia *et al* [42] demonstrated that peroxidative Chl bleaching activity was present in the thylakoid membrane of barley seedlings. Abeles *et al* [43] reported that in cucumber (*Cucumis sativus* L.) cotyledons treated with ethylene, cationic peroxidase (33KD, pI = 8.9), which degrades Chl *in vitro*, increased. Yamauchi and Watada [44] reported the involvement of peroxidase in Chl degradation of stored broccoli florets. The activity of peroxidase, which is involved in Chl degradation, showed a sharp increase concurrently with floret yellowing [24,45]. By the method of native-PAGE, 6 anionic and 2 cationic isoperoxidases were detected in fresh broccoli florets. In these isoperoxidases, only one cationic isoperoxidase (Rf 0.3) was related to Chl degradation. The cationic isoperoxidase was further purified by means of molecular exclusion chromatography and cationic exchange chromatography. Two Chl-degrading peroxidase isozymes (Type I and Type II) were contained in the cationic isoperoxidase [46]. This finding indicates that peroxidase might play a role in Chl oxidation. Further study is necessary to clarify the speculations about the action of peroxidase on Chl degradation and the mechanism of peroxidase-mediated Chl degradation in postharvest horticultural crops.

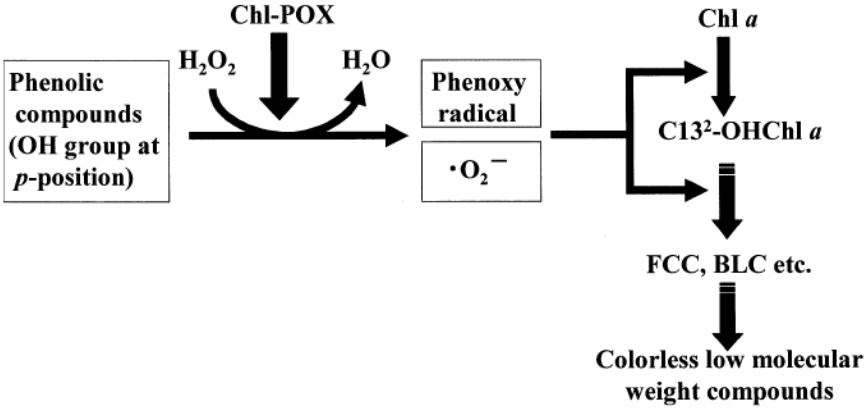


Figure 5 Pathway of peroxidase-mediated chlorophyll degradation. Chl-POX: Chlorophyll-degrading peroxidase, Chl: Chlorophyll, $C13^2$-OHChl: $C13^2$-Hydroxychlorophyll, FCC: Fluorescent chlorophyll catabolite, BLC: Bilirubin-like compounds.
Source: Yamauchi *et al* [16]

5. Pheophytinase

A new Chl degrading enzyme, PPH which would dephytylate the Mg-free Chl pigment, Phein *a* to give Pheide *a* has recently been reported [17]. They identified PPH, a chloroplast-located and senescence-induced hydrolase widely distributed in algae and land plants. *In vitro*, *Arabidopsis* PPH specifically dephytylates the Mg-free chlorophyll pigment, phein, yielding Pheide *a*. An *Arabidopsis* mutant deficient in PPH (*pph-1*) is unable to degrade chlorophyll during senescence and therefore exhibits a stay-green phenotype. Furthermore, *pph-1* accumulates Phein during senescence. Moreover, the *in vitro* activity of PPH in lime fruit was recently determined. The absorption spectrum of the product as Pheide *a* was recorded at 665 nm. The activity of PPH increased with Pheide *a* formation based on the total peak area (**Figure 6**). Therefore, PPH is an important component of the Chl breakdown machinery of senescent leaves, and we propose that the sequence of early Chl catabolic reactions be revised. Removal of Mg most likely precedes dephytylation, resulting in the following order of early breakdown intermediates: Chl → Phein → Pheide. Chlide, the last precursor of Chl biosynthesis, is most likely not an intermediate of breakdown. Thus, Chl anabolic and catabolic reactions are metabolically separated. Kaewsuksaeng *et al* [47] recently showed PPH activity gradually increased during storage in lime fruit, while the UV-B treatment effectively suppressed PPH activity. Further study needs to be conducted to establish a correct measurement of PPH and clarify the role of PPH in Chl degradation of horticultural crops.

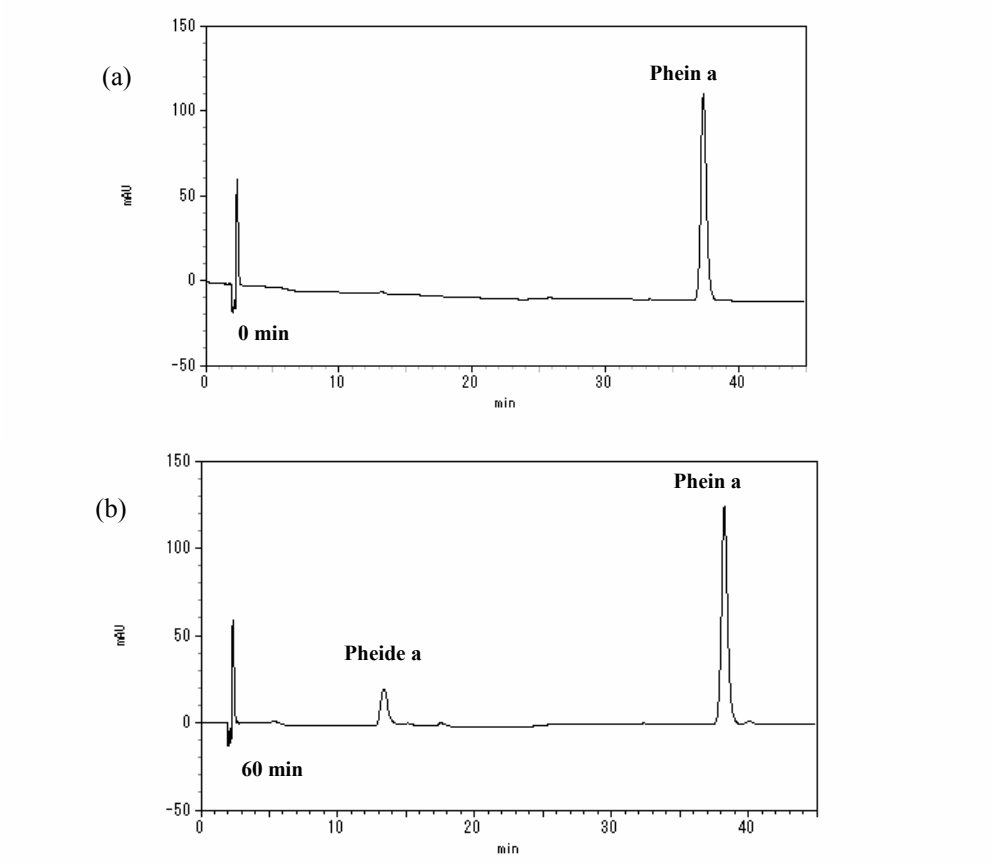


Figure 6 Analysis of Pheide formation of lime fruit. The *in vitro* pheophytinase reaction was held at 25 °C for 0 min (a) and 60 min (b), Pheide - Pheophorbide and Phein - Pheophytin.

Conclusions

A series of the reactions involved in Chl-degrading enzymes such as Chlase, MD or MDS, pheophytinase, pheophorbide *a* oxygenase and pheophorbidase, is thought to be the main pathway of Chl *a* degradation in horticultural crops and this occurs in the chloroplasts. On the other hand, Chl-degrading peroxidase is also reported to be involved in Chl oxidation to form $C13^2$-OHChl *a*. The initial step of Chl *a* degradation seems to be the formation of Chlide *a* by Chlase or Phein *a* by MD or that of $C13^2$-OHChl *a* by oxidation related to Chl-degrading peroxidase. Both Chlide *a,* Phein *a* and $C13^2$-OHChl *a* can be finally degraded to colorless, low molecular weight compounds.

References

[1] N Yamauchi and AE Watada. Regulated chlorophyll degradation in spinach leaves during storage. *J. of Amer. of Chem. Soc*. 1991; **116**, 58-62.

[2] N Yamauchi and AE Watada. Pigment changes in parsley leaves during storage in controlled or ethylene containing atmosphere. *Food Sci.* 1993; **58**, 104-8.

[3] ML Costa, PM Civello, AR Chaves and GA Martínez. Effect of hot air treatments on senescence and quality parameters of harvested broccoli (*Brassica oleracea* L var *Italica*) heads. *J. of the Sci. of Food and Agri*. 2005; **85**, 1154-60.

[4] TO Win, V Srilaong, KL Kyu, K Poomputsa and S Kanlayanarat. Biochemical and physiological changes during chlorophyll degradation in lime (*Citrus aurantifolia* Swingle cv. 'Paan'). *J. Hort. Sci. Biotech*. 2006; **81**, 471-7.

[5] V Srilaong, S Aiamla-or, A Soontornwat, M Shigyo and N Yamauchi. UV-B irradiation retards chlorophyll degradation in lime (*Citrus latifolia* Tan.) fruit. *Postharvest Biol. Technol*. 2011; **59**, 110-2.

[6] K Shimokawa. *Coloring*. *In*: Japanese Society for Horticultural Science (ed.). Shin-engeigaku zenpen, Youkendo, Tokyo, 1998, p. 585-8.

[7] K Shimokawa, S Shimada and K Yaeo. Ethylene-enhanced chlorophyllase activity during degreening of *Citrus unshiu* Marc. *Scientia Horti*. 1978; **8**, 129-35.

[8] D Amir-Shapira, EE Goldschmudt and A Altman. Chlorophyll catabolism in senescing plant tissues: *in vivo* breakdown intermediates suggest different degradative pathways for citrus fruit and parsley leaves. *In*: Proceedings of the National Academy of Sciences, USA, 1987, p. 1901-5.

[9] M Langmeier, S Ginsburg and P Matile. Chlorophyll breakdown in senescent leaves: demonstration of Mg-dechelatase activity. *Physiol. Plant.* 1993; **89**, 347-53.

[10] S Kaewsuksaeng, N Yamauchi, Y Funamoto, M Shigyo and S Kanlayanarat. Effect of Mg-dechelation activity on Chlorophyll Degradation in Stored Broccoli Florets. *Acta Hort.* 2006; **712**, 705-9.

[11] S Kaewsuksaeng, N Yamauchi, Y Funamoto, T Mori, M Shigyo and S Kanlayanarat. Effect of heat treatment on catabolites formation in relation to chlorophyll degradation during storage of broccoli (*Brassica oleracea* L. italica group) florets. *J. Jap. Soc. Hort. Sci.* 2007; **76**, 338-44.

[12] Y Shioi, N Tomita, T Tsuchiya and K Takamiya. Conversion of chlorophyllide to pheophorbide by Mg-dechelating substance in extracts of *Chenopodium album*. *Plant Physiol. Biochem.* 1996; **34**, 41-7.

[13] S Aiamla-or, S Kaewsuksaeng, M Shigyo and N Yamauchi. Impact of UV-B irradiation on chlorophyll degradation and chlorophyll-degrading enzyme activities in stored broccoli (*Brassica oleracea* L. Italica Group) florets. *Food Chem.* 2010; **120**, 645-51.

[14] S Kaewsuksaeng, N Yamauchi, Y Funamoto, S Aiamla-or, T Mori, M Shigyo and S Kanlayanarat. Partially purification of Mg-dechelatase in relation to chlorophyll degradation in broccoli (*Brassica oleracea* L. Italica Group) florets. *Acta Hort.* 2010; **875**, 509-14.

[15] P Matile, S Hörtensteiner and H Tomas. Chlorophyll degradation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 1999; **50**, 69-75.

[16] N Yamauchi, Y Funamoto and M Shigyo. Peroxidase-mediated chlorophyll degradation in horticultural crops. *Phytochem. Review*s. 2004; **3**, 221-8.

[17] S Schelbert, S Aubry, B Burla, B Agne, F Kessler, K Krupinska and S Hörtensteiner. Pheophytin pheophorbide hydrolase (Pheophytinase) is involved in chlorophyll

breakdown during leaf senescence in *Arabidopsis*. *Plant Cell.* 2009; **21**, 767-85.
[18] S Hörtensteiner and B Kräutler. Chlorophyll breakdown in oilseed rape. *Photosyn. Res.* 2000; **64**, 37-146.
[19] M Holden. The breakdown of chlorophyll by chlorophyllase. *J. of Biochem*. 1961; **78**, 359-64.
[20] FC Pennington, HH Strain, WA Svec, JJJ and Katz. Preparation and properties of pyrochlorophyll *a*, methyl pyrochlorophyllide *a*, pyropheophytin *a*, and methyl pyropheophorbide *a* derived from chlorophyll by decarbomethoxylation. *J. of Amer. of Chem. Soc.* 1964; **86**, 1418-26.
[21] N Yamauchi, K Eguchi, Y Tokuhara, Y Yamashita, A Oshima, M Shigyo and K Sugomota. Control of degreening by sucrose laurate ester and heat treatment of *Citrus nagato-yuzukichi* fruit. *J. of Hort. Sci. Biotech.* 2003; **78**, 563-7.
[22] PH Hynninen. Chlorophylls IV. Preparation and purification of some derivatives of chlorophylls *a* and *b*. *Acta Chem. Scand*. 1973; **27**, 1771-80.
[23] MJ Maunders, SB Brown and HW Woolhouse. The appearance of chlorophyll derivatives in senescing tissue. *Phytochem.*1983; **22**, 2443-6.
[24] Y Funamoto, N Yamauchi, T Shigenara and M Shigyo. Effects of heat treatment on chlorophyll degrading enzymes in stored broccoli (*Brassica olearacea* L.). *Postharvest Biol. Technol*. 2002; **24**, 163-70.
[25] K Takamiya, T Tsuchiya and H Ohta. Degradation pathway(s) of chlorophyll: what has gene cloning revealed? *Trends in Plant Sci*. 2000; **5**, 1380-5.
[26] R Willstätter and A Stoll. *Die Wirkungen der Chlorophyllase*. *In*: Untersuchungen über Chlorophyll. Springer, Berlin, 1913, p. 172-87.
[27] M Drazkiewice. Chlorophyllase: occurrence, function, mechanism of action, effects of external factors. *Photosynt.* 1994; **30**, 301-9.
[28] T Trebitsh, EE Goldschmidt and J Riov. Ethylene induces de novo synthesis of chlorophyllase, a chlorophyll degrading enzyme, in *Citrus* fruit peel. *In:* Proceeding of National Academic Science, USA, 1993, p. 9441-5.
[29] A Khalyfa, S Kermasha, P Marsot, and M Goetghebeur. Purification and characterization of chlorophyllase from algae *Phaeodactylum tricornutum* by preparative native electrophoresis. *Apply Biochem. and Biotech*. 1995; **53**, 11-27.
[30] T Tsuchiya, H Ohta, K Okawa, A Iwamatsu, H Shimada and T Masuda, and K Takamiya. Cloning of chlorophyllase, the key enzyme in chlorophyll degradation: finding of a lipase motif and the induction by methyl jasmonate. *In*: Proceedings of the National Academy of Sciences, USA, 1999, p. 15362-7.
[31] P Matile, M Schellenberg and F Vicentini. Localization of chlorophyllase in the chloroplast envelope. *Planta.*1997; **201**, 96-9.
[32] B Parthier. Gerontoplasts - the yellow end in the ontogenesis of chloroplasts. *Endo. Cell Resource.* 1988; **5**, 163-90.
[33] H Satoh, K Nakayama and M Okada. Molecular cloning and functional expression of a water-soluble chlorophyll protein, a putative carrier of chlorophyll molecules in cauliflower. *J. of Bio. and Chem.* 1998; **273**, 30568-75.
[34] T Kunieda, T Amano and Y Shioi. Search for chlorophyll degradation enzyme, Mg-dechelatase, from extracts of *Chenopodium album* with native and artificial substrates. *Plant Sci*. 2005; **169**, 177-83.
[35] TG Owensand and PG Falkowski. Enzymatic degradation of chlorophyll *a* by marine phytoplankton *in vitro*. *Phytochem.* 1982; **21**, 979-84.
[36] Y Shioi, Y Tatsumi and K Shimokawa. Enzymatic degradation of chlorophyll in *Chenopodium album*. *Plant Cell Physio.* 1991; **32**, 87-93.
[37] ML Costa, PM Civello, AR Chaves and GA Martínez. Characterization of Mg-dechelatase activity obtained from *Fragaria x ananassa* fruit. *Plant Physio. and Biochem.* 2002; **40**, 111-8.
[38] T Suzuki, T Kunieda, F Murai, S Morioka and Y Shioi. Mg-dechelation activity in radish cotyledons with artificial and native substrates, Mg-chlorophyllin *a* and chlorophyllide *a*. *Plant Physio. and Biochem.* 2005; **43**, p. 459-64.
[39] K Watanabe, H Ohta, T Tsuchiya, B Mikami, T Masuda, Y Shioi, and K Takamiya.

Purification and some properties of pheophorbidase in *Chenopodium album*. *Plant Cell and Physio.* 1999; **40**, 104-8.

[40] Y Maeda, H Kurata, M Adachi and K Shimokawa. Chlorophyll catabolism in ethylene-treated *Citrus unshiu* fruits". *J. Jap. Soc. Hort. Sci.* 1998; **67**, 497-502.

[41] MT Janave. Enzymic degradation of chlorophyll in Cavendish bananas: *in vitro* evidence for two independent degradative pathways. *Plant Physio. and Biochem.* 1997; **35**, 837-46.

[42] E Marutinoia, MJ Dalling and P Matile. Catabolism of chlorophyll: Demonstration of chloroplast-localized peroxidative and oxidative activities. *Z. Pflanzenphysiol.* 1982; **107**, 269-79.

[43] FB Abeles, LJ Dunn, P Morgens, A Callahan, RE Dinterman and J Schmidt. Induction of 33-KD and 60-KD peroxidases during ethylene-induced senescence of cucumber cotyledons. *Plant Physio*. 1988; **87**, 609-15.

[44] N Yamauchi and AE Watada. Chlorophyll and xanthophylls changes in broccoli florets stored under elevated CO_2 or ethylene-containing atmosphere. *Hort. Sci.* 1998; **33**, 114-7.

[45] N Yamauchi, Y Funamoto and Y Kanetsune. Involvement of chlorophyll degrading enzymes with chlorophyll degradation in stored broccoli (*Brassica oleracea* L.) florets. *Food Sci. Technol*. *Res.* 1999; **5**, 300-3.

[46] Y Funamoto, N Yamauchi and M Shigyo. Purification and characterization of chlorophyll-degrading peroxidase in broccoli florets. *J. Jap. Soc. Hort. Sci.* 2003; **72**, 137.

[47] S Kaewsuksaeng, Y Urano, S Aiamla-or, M Shigyo and N Yamauchi. Effect of UV-B irradiation on chlorophyll-degrading enzyme activities and postharvest quality in stored lime (*Citrus latifolia* Tan.) fruit. *Postharvest Biol. Technol*. 2011; **61**, 124-30.