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Effect of Heating Pattern on Pigment Degradation of Green Vegetable Leaf

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ABSTRACT: We investigate the effect of two heating patterns, quick and gradual, on the relative degradation rate of chlorophyll, carotenoid, and the three intermediates, protoporphyrin IX (PPIX), magnesium protoporphyrin IX (MGPP) and protochlorophyllide (Pchlide), of chlorophyll biosynthetic pathway in three vegetable leaves. The data showed that (1) all pigments declined under both patterns of heating, however, the relative degradation rate of quick heating is greater than that of gradual heating; (2) The mechanism of all investigated pigments is a two-step reaction; (3) the relative degradation rate of chlorophyll is slower than that of carotenoid; (4) the relative degradation rate of chlorophyll a is faster than of that of chlorophyll b; (5) the relative degradation rate of protochlorophyllide is slower than that of magnesium protoporphyrin IX, which is slower than that of protoporphyrin IX; (6) nutrient content after gradual heating within 30 min is better than quick heating within the same period. We concluded that the degradation of leaf in nature is different from that in the heating condition of water.

KEYWORDS: Chlorophyll, Carotenoid, Heating pattern, Magnesium protoporphyrin IX, Pigment, Protochlorophyllide, Protoporphyrin IX.

INTRODUCTION

Chlorophyll and carotenoid are the most important pigments involved in the photosynthetic light-harvesting in higher plants. All the two pigments are associated with specific peptides to form pigment-protein complexes located in the chloroplast thylakoid membrane (Markwell *et al.*, 1979). The pigment-protein complexes contain either only chlorophyll a or both chlorophyll a and b. Both types of pigment-protein complex also possess several carotenoids which are thought to protect their associated polypeptides from photodamage. The molar ratio of chlorophyll a to b in higher plants is approximately 3, commonly range from 2.5 to 3.5 (Lichtenthaler, 1968). Color is a major criteria for the quality of vegetables and processing of foods. The loss of fresh color implicates the decomposition of chlorophyll and carotenoid and degradation of chloroplast thylakoid (Schwartz and Lorenzo, 1990) and degradation of chloroplast thylakoid (Murphy, 1986).

Much research effort has been made for the pigment synthesis in the leaf development, degradation in the senescence process, physiological function in photosynthesis (Albertsson

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et al. 1990; Anderson, 1988), and the alterations of chlorophyll and carotenoid during food processing (Schwartz and Lorenzo, 1990; Chen, 1992; Chen and Chen, 1993), however, only very few work was done to analyze the alteration of chlorophyll biosynthetic precursors, such as protoporphyrin IX, magnesium protoporphyrin IX and protochlorophyllide. Therefore, the aim of this preliminary work is to study the effect of heating patterns on the relative degradation rate of pigments in green vegetables.

MATERIALS AND METHODS

Vegetable leaf

Three green vegetables were purchased from supermarket and punched as leaf disk with 1.5 cm in diameter. They are *Brassica pekinensis* Rupr, *B. chinensis* L. cv. Ching-geeng and *Ipomoea batatas* (L.) Lam. Fifty leaf disks of each species were quickly or gradually heated in 500 ml water held in a 2000 ml glass beaker heated with a Corning stirrer hotplate (25x26 cm2). Three leaf disks were randomly sampled each time for determining their pigment concentrations.

Heating pattern

Quick heating was that leaf disk was started boiled when water boiling point was reached, while gradual heating was defined as leaf disk was started heating at room temperature, approximately 25 °C (Fig. 1). It took about 30 min to boil 500 ml water from 25°C to 100 °C under heating condition as described above.

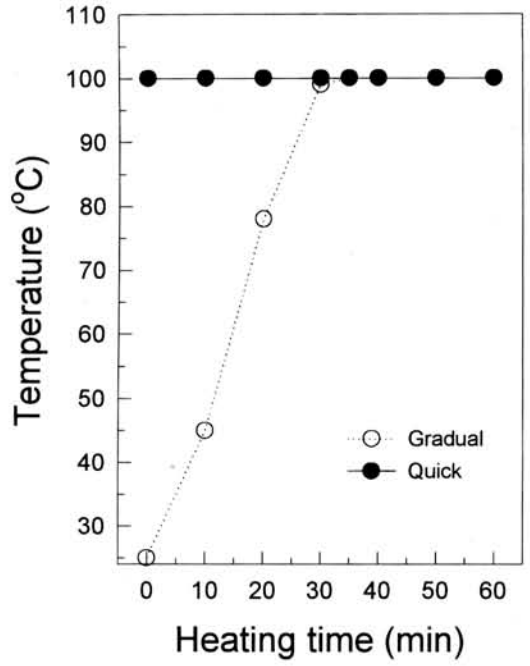

Fig. 1. The pattern of temperature change in the quick and gradual heating condition of green vegetable leaf.

Pigment determination

Chlorophyll concentration and a/b ratios were determined using the spectra-photometric method of Porra *et al.* (1989) following extraction of liquid nitrogen-dried leaf with 80% acetone. The same acetone extract was measured at 480 nm to determine the content of total caro-tenoid. Room temperature absorbance was determined with a Hitachi U2000 UV-visible spectrophotometer.

Porphyrin assay

The concentration of protoporphyrin IX (PPIX), magnesium-protoporphyrin IX (MGPP) and protochlorophyllide (Pchlide) was determined using the method of Kahn *et al.* (1976). The experimental materials were extracted with 80% ammoniacal acetone (ammonia : acetone, 20:80, v/v) and then partition with equal volume of hexane to remove the majority of chlorophyll molecules before measuring the porphyrins contents.

Mole percent of total porphyrins

Individual mole percent of each porphyrin was defined as PPIX (or MGPP or Pchlide)/(PPIX+MGPP+Pchlide) x100%.

RESULTS AND DISCUSSION

In this study two patterns of heating treatment were used to investigate the decomposition of chlorophyll and its three biosynthetic precursor (Fig. 1). The quick pattern started heating leaf disk when temperature reached water boiling point. The gradual pattern cooked the samples at 25 ℃ and reached water boiling point within 30 min. The two heating patterns led to much different decomposition process of the same pigment.

The content of either chlorophyll or carotenoid dramatically decreased under the two heating conditions (Fig. 2). Within the first 30~40 min, the relative degradation rate of the two pigments is much slower in gradual than in quick heating, but, reverse thereafter. In spite of the two pigments, the degradation process of gradual heating is in contrast to that of quick heating. The degradation rate of either chlorophyll or carotenoid proceed slowly under gradual heating condition within the first 20 min but much linearly faster thereafter, however, that under quick heating condition proceed linearly fast for almost 40 min but much slower thereafter. These result suggest that the mechanism of chlorophyll and carotenoid decomposition, in spite of the two heating conditions, is a two-step process. This is in contrast to the finding of other research on the food processing or canning (Schwartz and Lorenz, 1990), which demonstrated that the kinetic of either chlorophyll a or b is

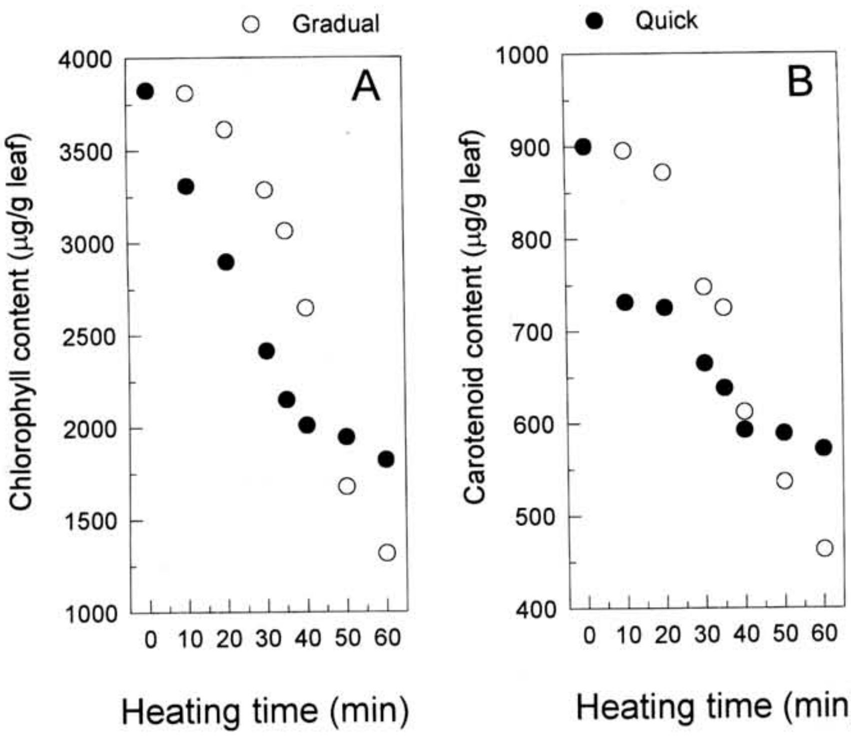

Fig. 2. Effect of heating on the chlorophyll and carotenoid content in the leaf of *Brassica chinensis* L. cv. Ching-Geeng.

completely linear. Since all chlorophyll and carotenoid molecules are located in the pigment-protein complexes, it is reasonable that the two pigments demonstrated similar degradation kinetic under the same condition. Since carotenoid such as lutein form an internal cross-brace in the center of light-harvesting complex, providing a direct, strong link between the peptide loops at both sides (Kuhlbrandt *et al.* 1994), it is also reasonable that chlorophyll is more easily released from light-harvesting complex than carotenoid.

As heating continue to proceed, the ratio of chlorophyll a/b under both heating conditions decreased from about 3.1 to 1.5 for quick heating or to 2.5 for gradual heating within 60 min (Fig. 3A). While the chlorophyll content under both heating conditions began to decrease just after heating, the chlorophyll a/b ratio still remained at the original level for about 20 and 30 min, respectively. The decline rate of chlorophyll ratio under quick heating condition is much faster than that under gradual heating condition. The result suggest that the relative degradation rate of chlorophyll a is faster than that of chlorophyll b and the difference between the two pigments is widen as heating proceed, causing the decrease in the ratio of chlorophyll a/b. Since most of chlorophyll b is located in the light-harvesting protein II of photosystem II (PSII) inside the thylakoid grana and most photosystem I (PSI) is located in the margin of grana and intergranar lamellar, the decrease of chlorophyll a/b ratio imply that chlorophyll a and b in PSI is destroyed earlier that those in PSII. However, the change in chlorophyll a/b ratio in this two heating system is in contrast to that in the natural degradation process in which chlorophyll a/b ratio increases from 2.5 to 4.5 for the exocarp of orange fruit (Hsu *et al.* 1995) or from 3 to 7 for the exocarp of papaya (Chen *et al.* 1996). It seem that granar center degrades earlier than its margin in the natural ripening process but reverse in this study. It requires further investigation to explore the cause of difference.

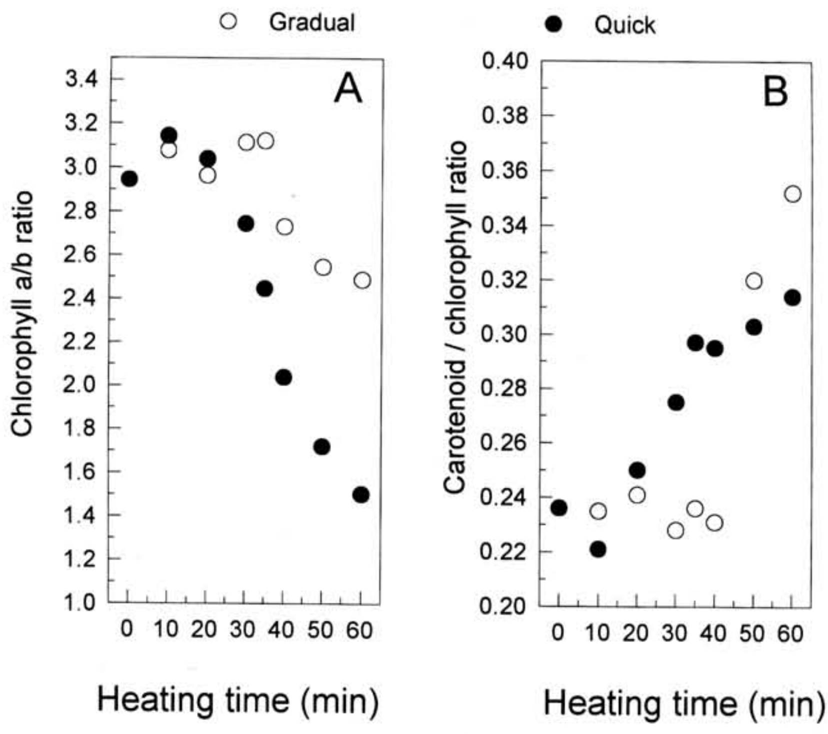

Fig. 3. Effect of heating on the ratios of chlorophyll a/b and chlorophyll/carotenoid in the leaf of *Brassica chinensis* L. cv. Ching-Geeng.

The ratio of carotenoid/chlorophyll increased from about 0.23 to 0.33 within 60 min (Fig. 3B). No significant change was found within the first 40 min under gradual heating condition but dramatically increased from 0.23 to 0.36 thereafter. As heating further proceed, more chlorophyll are released from the pigment-protein complexes than carotenoid, leading to the increase of ratio.

The relative degradation rate of total porphyrins (PPIX, MGPP and Pchlide) is very similar to that of chlorophyll and carotenoid (Fig. 2 and 4). The individual porphyrin (data not shown) demonstrated the same pattern as the total porphyrins. As heating proceed, while the mole percent of PPIX in total porphyrins under both heating conditions demonstrated no significant change within 20 min and then dramatically decreased from about 41% to 18% thereafter, that of Pchlide also demonstrated no significant change within 20 min but then dramatically increased. The mole percent of MGPP is much different from those of PPIX and Pchlide. While the mole percent of MGPP decreased under quick heating condition, it increased under gradual heating condition. It is apparent that the relative degradation rate of Pchlide is much slower than that of MGPP, which is much slower than that of PPIX. This data is in contrast to that found in the exocarp of orange fruit and papaya (Hsu *et al.* 1995; Chen *et al.* 1996), suggesting that the order of relative degradation rate of pigments in green vegetable heated in this study is chlorophyll a, chlorophyll b, protoporphyrin IX, magnesium protoporphyrin IX, protochlorophyllide. It is therefore concluded that the mechanism of pigment degradation in nature is different from that in boiling water.

At present time it is known that chlorophyllase and Mg-dechelatase are two major integral part of the chlorophyll degradation system (Drazkiewicz, 1994). Based on the combination of the above data, we propose that the two-step reaction for the mechanism of chlorophyll and carotenoid degradation may be resulted from the sensitivity of the two enzymes to the change of water temperature. This requires further investigation.

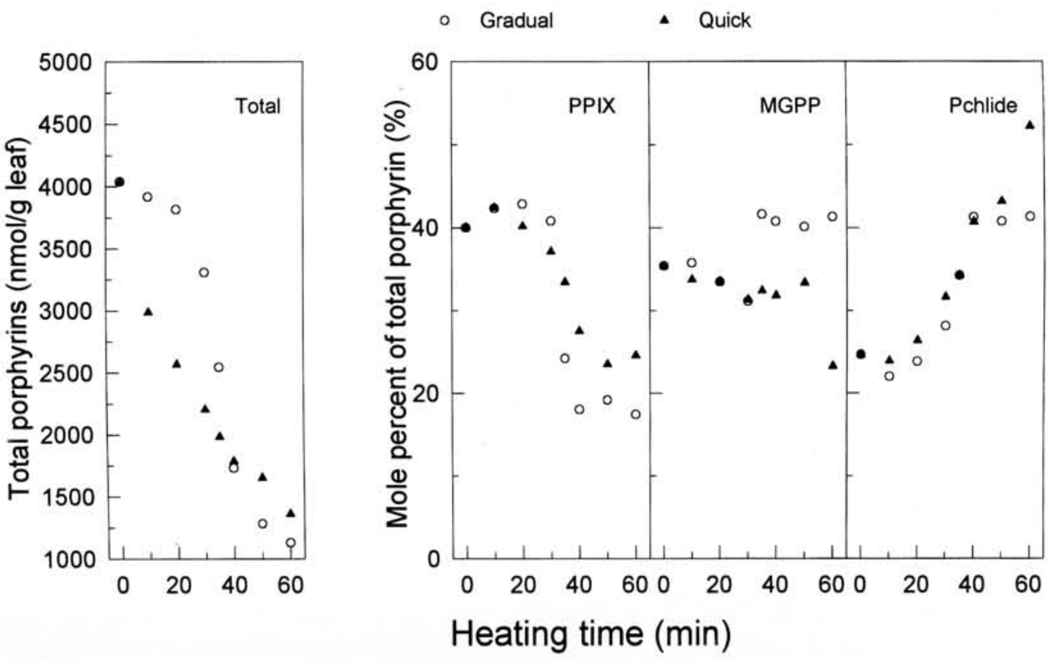

Fig. 4. Effect of heating on the content and mole percent of total porphyrins in the leaf of *Brassica chinensis* L. cv. Ching-Geeng.

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加熱方式對綠色蔬菜色素崩解的影響

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摘　　要

本文探討急速加熱和漸進加熱對三種綠色蔬菜的葉綠素、類胡蘿蔔素及葉綠素的三種前驅物質原 卟啉 IX (PPIX)、原 卟啉鎂 IX (MGPP) 及原脫植醇葉綠素 (Pchlide) 之相對崩解速度的影響。資料顯示：(1)不論何種加熱方式，急速加熱的相對崩解速度都比漸進加熱快；(2)所有被檢測的色素之崩解速度是兩階段反應；(3)葉綠素的相對崩解速度比類胡蘿蔔素慢；(4)葉綠素 a 的相對崩解速度比葉綠素 b 快；(5) 卟啉的相對崩解速度分別是原 卟啉 IX>原 卟啉鎂 IX>原脫植醇葉綠素。顯然的，自然界色素的崩解速度過程和水煮法造成的崩解不同。

關鍵詞：葉綠素，類胡蘿蔔素，加熱方式，原 卟啉鎂 IX，色素，原脫植醇葉綠素，原 卟啉 IX。

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