CHAPTER 1

INTRODUCTION

1.1 Background

The yellowing is the loss of green color in leaves, florets and fruit pericarp. It is considered as a major factor in the quality deterioration of horticultural products during storage, especially on leafy vegetables. The progress of yellowing obviously shorten their shelf life and highly affect the acceptance of consumers. The yellowing in leaves is a result of the chlorophyll (Chl) breakdown which occurs in the chloroplast during Chl degradation process.

In general of Chl degradation, the Chl *a* is degraded into chlorophyllide (Chlide) *a* by the activity of chlorophyllase (Chlase). The Chlase removes the side chain that attached to tetrapyrrole macrocycle to form Chlide *a*, which still retains a green color (Shimokawa et al., 1978; Amir-Shapira et al., 1987). The elimination of Mg^{2+} from Chlide *a* to produce pheophorbide (Pheide) *a* is catalysed by Mg-dechelatase (MD) (Langmeier et al., 1993). After removal of Mg^{2+} from the center of Chlide *a*, resulting of the change on its green into brown color. Recently, Tang et al., 2000 report that Mg-dechelatase activity can be remove directly Mg atom from Chl *a* to form pheophytin (phy) *a*. After that, the side chain of Phy *a* is removed by the activity pheophytinase and formed Pheide *a*. Finally, Pheide *a* decomposes to reduce fluorescent chlorophyll catabolites, which are primary colorless catabolites, through a red chlorophyll catabolits by both Phide a oxygenase and red chlorophyll catabolite reductase (Matile et al., 1999) These reactions are thought to form the main pathway of chlorophyll *a* degradation which occurs in the chloroplasts.

Furthermore, during leaf senescence, chloroplasts are transformed into gerontoplast. This process is characterized by changes in ultrastructure and in the biochemical and functional properties of the plastids. Changes in ultrastructure have been found in chloroplasts senescence size shape during progressive senescence plastids (Śesták 1985, Synková et al., 1997, Kutik et al., 1998). Many stromal and membrane components in chloroplasts gradually degrade during senescence (Grumbach and Lichtenthalar 1973, Grover and Mohanty 1993). A typical feature of chloroplast in senescing leave is the increase in the number and size of plastoglobuli (Butler and Simon 1971, Noodén 1988, Guiamet et al., 1999, Kura-Hotta et al., 1990, Matile 1992, Inada et al., 1998).

However, the good of postharvest handing techniques can help retarding the shelf life of horticultural products. Temperature is the characteristic of the postharvest environment that has the greatest impact on storage life of vegetable. Good temperature management is the most important and simplest procedure for delaying product deterioration. In addition, optimum temperature storage retards the aging of vegetable, softening textural and color changes as well as slowing undesirable metabolic change, moisture loss and losses due to pathogen invasion (Cecília et al., 2003). Chlorophyll content is indicating of color change in many vegetable during storage. Yellowing of mostly vegetable is very often due to storage above the recommended temperature such as broccoli, barley leaves, spinach and green beans. The yellowing of leaves is major cause of product denial from consumer (Makhlouf et al., 1991; Tovivonen, 1997; Zhuang et al., 1997).

In Japanese bunching onion have relatively short shelf life due to their external appearance. The storage life of at 5°C is approximately one week and at higher temperature rapidly increases the yellowing of the leaves. Dissanyake et al. (2009)

reported that Japanese bunching onion leaves stored at 25 °C turned progressively yellow starting from the leaf tip towards the base during 3 day of storage, whereas storage at 4 °C could maintain the green color of the leaf tip. The yellowing of leaves is the attribute symptom of senescence with caused by chlorophyll degradation as mentioned above. The changes of other organelles, however, may take in part on chlorophyll catabolism. The chloroplast is the organelle that exhibits senescence through chloroplast transformation as a result of dissociation of grana, increased size and number of plastoglobuli, and disruption of the chloroplast envelope (Mancera et al., 1999). After chloroplast envelop was released plastoglobuli to the cytoplasm and vacuole (Butler and Simon, 1971; Hurkman, 1979). Plastoglobuli were possibly containing chlorophyll movement from chloroplast thylakoid to the vacuole (Dissanyake et al., 2009). In general, chlorophyll degradation in mostly vegetable has been reported to occur in the chloroplast. Recently, Dissanyake et al. (2009) have investigated chlorophyll and observe the intracellular structural changed during storage Japanese bunching onion. During storage of Japanese bunching onion occur yellowing of leave and intracellular structural change in chloroplasts by plastoglobuli remove from chloroplast to vacuole. However, the chlorophyll degradation of Japanese bunching onion may also occur in the vacuole during storage. Therefore, we examined the effect of temperature on chlorophyll degradation and determined chlorophyll degradation in vacuole of during storage.

1.2 Objectives of study

1.2.1 To study the techniques of protoplast extraction, purification and isolation of vacuoles.

1.2.2 To study the chlorophyll and its derivative contents in vacuole which relative to chlorophyll degradation of Japanese bunching onion.

1.2.3 To study the effect of storage temperature on chlorophyll degradation of Japanese bunching onion.

1.3 Scopes of study

1.3.1 To study on extraction, purification of protoplasts and isolation of vacuole from Japanese bunching onion. The protoplasts extraction from Japanese bunching onion leaves will be done by osmotic balanced method using enzyme digestion at different concentration and vary digestion time. After the protoplasts are purified the various gradient and speed of centrifugation are applied to get the vacuole. Purified vacuole should have no contamination with other organelles. Therefore, confirmation of the vacuole purity will be checked with the maker enzymes.

1.3.2 To study *in vitro* test on changes of chlorophyll derivatives in protoplasts and vacuole. The experiment is subjected to changes in chlorophyll derivative such as chlorophyllide *a*, pheophytin *a*, C13^2-hydroxychlorophyll *a*, pheophorbide *a* and pyropheophorbide *a* by HPLC analysis.

1.3.3 To study the effect of storage temperature on chlorophyll degradation of Japanese bunching onion. The bunching onion was storage at 4 and 25 °C in the dark condition. The change of the color will be determined using colorimeter. The chlorophyll and chlorophyll derivatives contents in vacuole during storage will be analyzed by HPLC.

1.4 Benefits

1.4.1 This research will help to better understanding on mechanism of chlorophyll degradation in Japanese bunching onion.

1.4.2 The knowledge from this research would be a useful technique for extraction of protoplasts and vacuoles by using osmotic balance method.

1.4.3 Understand the effect of temperature on chlorophyll degradation in vacuole of Japanese bunching onion during postharvest period.