

CHAPTER 5

CONCLUSION

The yellowing at the tip of Japanese bunching onion during storage is a major postharvest problem. This has been attributed to chlorophyll degradation that is commonly thought to occur in the chloroplast. However, it may also occur inside the vacuole. Earlier, chlorophyll degradation in Japanese bunching onion has been reported to occur in the chloroplast. This study illustrates that chlorophyll degradation also occurs in the vacuole.

Base on results of experiments, the main findings can be concluded as follows:

1. Suitable procedures for extraction protoplasts from Japanese bunching onion by method B and got a yield of protoplasts about $1.8 \pm 0.7 \times 10^5$ cells.
2. The protoplasts were further purified to avoid possible contamination by enzyme contained in digestion solution. The purification of protoplasts was centrifuged on 2, 5, 10, 15% Ficoll discontinuous gradient.
3. The isolation and purification of vacuoles from purified protoplasts by using the method B (method for purified vacuole was modified from Asami et al., 1985) was got a yield of vacuole about 10-20%.
4. The purification of vacuole was found a low activity of the marker enzyme of other organelles and high activity of the marker enzyme of vacuole. This indicated that the purified vacuoles were good enough for further step of analysis.

5. *In vitro* test, the non-boiled vacuoles and protoplasts had higher of chlorophyllide *a*, pheophytin *a*, pheophorbide *a*, pyropheophorbide *a* and C13²-hydroxychlorophyll *a* contents compared boiled sample.

6. The chlorophyll *a* and chlorophyll *b* content decreasing in Japanese bunching onion storage at 25 °C faster than that Japanese bunching onion storage at 4 °C

7. Vacuole of Japanese bunching onion of storage at 25 °C shown chlorophyll *a* level decreasing on day 1 and stored at 4 °C decreased on day 2. Chlorophyllide *a*, pheophytin *a*, pheophorbide *a* and pyropheophorbide *a* content in leaves stored at 25 °C were increased on day 2 but C13²-hydroxychlorophyll *a* content showed the lower than that Japanese bunching onion stored at 4 °C.

From the results in this study obtained technique of isolation vacuole from Japanese bunching onion for examine on chlorophyll degradation in vacuole. *In vitro* test presence of chlorophyll catabolite was observed in vacuole of Japanese bunching onion. Moreover, *in vivo* test on vacuole of Japanese bunching onion during storage in high and low temperatures were different mechanisms of chlorophyll loss. Therefore, the chlorophyll degradation occurs in the vacuole of Japanese bunching onion especially at storage temperature of 25 °C induced chlorophyll breakdown faster than at low temperature.