

Title Involvement of Mg-dechelation Substances in Relation to Chlorophyll Degradation in Broccoli (*Brassica oleracea* L. Italica Group) Florets

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Abstract

Involvement of Mg-dechelataase in relation to chlorophyll (Chl) degradation of stored broccoli (*Brassica oleracea* L. Italica Group) florets was investigated to clarify the mechanism of Chl degradation. Mg-dechelation activity in floret extracts was found in two 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 Mg-dechelataase, using chlorophyllin (Chlin) *a* or chlorophyllide (Chlide) *a* as a substrate. Mg-dechelation activity of the extract from broccoli florets, which were stored for 4 days at 15 °C, was higher than that of extracts from fresh broccoli florets using Chlin *a* or Chlide *a* as substrate. Chelating compounds, radical scavengers and reducing agents had different inhibitory effects. Ascorbate, and especially quercetin, had more effective inhibitory activity on both yellow and fresh broccoli extracts. High molecular weight substance (HMWS), which has a Mg-dechelation activity, was purified by (NH₄)₂SO₄ precipitation (20-60% saturation), molecular exclusion chromatography (Sephacryl S-200), anionic exchange chromatography (DEAE-650M), hydrophobic interaction chromatography (Butyl-650M) and molecular exclusion chromatography (HW-55F). Only one peak of the activity was detected in fresh broccoli extract and no other isozyme with Mg-dechelating action was found in the yellow broccoli extract and molecular mass was about 70 KDa. This high-molecular weight substance shows strong activity with artificial substrate, Chlin *a*, but hardly have an activity with native substrates, Chlide *a*. Moreover, low molecular weight substance from 5,000 to 10,000 also had a Mg-dechelation activity. This means that HMWS does not have an activity with Chlide *a*. It is interpreted by the results obtained in this study that HMWS is not involved in Chl degradation of broccoli florets.