

Title Characterization of senescence-associated proteases in postharvest broccoli florets
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Abstract

We characterized the senescence-associated proteases of postharvest broccoli (*Brassica oleracea* L. var Green King) florets, using class-specific protease inhibitors and gelatin-polyacrylamide gel electrophoresis. Different classes of senescence-associated proteases in broccoli florets were partially characterized for the first time. Protease activity of broccoli florets was depressed by all the inhibitors and showed different inhibition curves during postharvest. The hydrolytic activity of metalloprotease (EC 3.4.24. –) and serine protease (EC 3.4.21. –) reached a maximum, 1 day after harvest (DAH), then decreased, while the hydrolytic activity of cysteine protease (EC 3.4.22. –) and aspartic protease (EC 3.4.23. –) increased throughout the postharvest senescence based on the calculated inhibition percentage of protease activity. The senescence-associated proteases were separated into seven endoprotease (EP) groups by gelatin-polyacrylamide gel electrophoresis and classified into EP1 (metalloprotease), EP2 (metalloprotease and cysteine protease), EP3 (serine protease and aspartic protease), EP4, EP5, EP7 (cysteine protease), and EP6 (serine protease) based on the sensitivity of class-specific protease inhibitors. The proteases EP2, EP3, and EP4 were present throughout the postharvest stages. EP3 was the major EP at all times during senescence; EP4 intensity of activity increased after 2 DAH; EP6 and EP7 clearly increased after 4 DAH. Our results suggest that serine protease activity contributes to early stage (0–1 DAH) and late stage (4–5 DAH) of senescence; metalloprotease activity was involved in the early and intermediate stages (0–3 DAH) of senescence; and cysteine protease and aspartic protease activities participated in the whole process of broccoli senescence.

Abbreviations: DAH, day after harvest; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EP, endoprotease; IA, iodoacetate; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid