CHAPTER IV

CONCLUSION

For the identification of black rice anthocyanins, the methodology was established based on the combination of LC-DAD, LC-ESI-MS and LC-ESI-MS/MS techniques. The LC-ESI-MS offered the advantage of screening for anthocyanin components in the separation profile of the crude rice extracts through the use of data processing in mass chromatogram mode. The UV-Vis spectra could partially confirm the identities of these anthocyanins, whereas the informative mass spectra obtained from scanning of product ion using LC-ESI-MS/MS technique facilitated the identification and characterization of the anthocyanins of interest. These product ion mass spectra provided sufficient fragmentation patterns based on the characteristic structures of mono-, di-, tri-glycosides or acylate groups linked with the anthocyanidins.

Ten anthocyanins were identified in the black rice cultivar Kumdoisakhet and BGMSN 11, which were cyanidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside-5-O-rhamnoside, peonidin-3-*O*-glucoside, cyanidin-3-*O*-diglucoside, cyanidin-3-*O*-diglucoside-5-*O*-glucoside, cyanidin-3-*O*-(*p*-coumaroyl)glucoside-5-*O*-glucoside, cyanidin-3-*O*-(feruloyl)glucoside-5-*O*-glucoside, peonidin-3-*O*-diglucoside, mavidin-3-*O*-(*p*-coumaroyl)glucoside-5-*O*-glucoside and peonidin-3-*O*-(*p*-coumaroyl)glucoside-5-*O*-xyloside, and the two tentatively identified anthocyanins; cyanidin-3-*O*-xyloside glucoside, and cyanidin-3-*O*-xyloside glucoside. The monoglucosidic anthocyanin pigments such as cyanidin-3-*O*-glucoside and

peonidin-3-*O*-glucoside were found only in seed of BGMSN 11 rice that has green leaves, but appeared in both leaves and seed of the Khumdoisakhet having purple-black leaf. In rice seed, their relative contents varied significantly from the milk grain, dough, until maturation stage.

The black rice anthocyanins having two and three sugars (or acylate groups) such as cyanidin-3-*O*-xyloside glucoside, cyanidin-3-*O*-xyloside glucoside, cyanidin-3-*O*-diglucoside, cyanidin-3-*O*-(*p*-coumaroyl)glucoside-5-*O*-glucoside and cyanidin-3-*O*-(feruloyl)glucoside-5-*O*-glucoside, were not found in seed of both black rice cultivars. Their contents varied in leaves with similar pattern, increasing from seedling to booting stage and decreasing from milk grain to maturation stage. However, there were two anthocyanins having peonidin as aglycone, peonidin-3-*O*-diglucoside and peonidin-3-*O*-(*p*-coumaroyl)glucoside-5-*O*-xyloside, which were found in both leaves and seeds of the two black rices. Their contents were highest in the rice seeds at the milk grain stage. Overall, the higher molecular weight anthocyanins with two or three sugars (or acylate groups) and possessed cyanidin as aglycone were found only in leaves of the black rices, whereas those having peonidin aglycone were found in both leaves and seed. There was no correlation among the molecular weight of the identified anthocyanins, their relative contents and growth stages of both black rice cultivars.