CHAPTER 4

CONCLUSION

The proposed HPLC method and solvent extraction conditions were found to be applicable to the analysis of phenolic compounds in various seaweed samples. HPLC-UV conditions for the separation and simultaneous determination of phenolic compounds were optimized using a Phenomenex Luna C_{18} column.

Solvent extraction has been used commonly method for extraction of natural substances. In the first part of the study, brown seaweed sample was used as sample for the selection of a suitable solvent for extraction and for optimization of HPLC conditions for the separation of components in the seaweed extracts. Four kinds of solvent were used to examine the effects of extraction solvent on total phenolic compound content and antioxidant activity evaluation. It was found that methanol: water: hydrochloric acid (75:20:5 v/v) yielded higher extraction efficiency activities (total phenolic content and DPPH scavenging) than methanol, methanol: water (75:25 v/v) and methanol: water: formic acid (75:20:5 v/v) in the extraction of phenolic compounds from the seaweed samples.

The optimum separation of phenolic compounds, namely gallic acid, catechin, epicatechin, caffeic acid, rutin and quercetin, were obtained by appropriately adjusting the composition of mobile phase, type and the concentration of common acids used. The emphasis was placed on the use of low amount of acids, in order to avoid column damage.

The HPLC method for the simultaneous determination of phenolic compounds was obtained by adjusting the composition of the mobile phase containing phosphoric

acid and acetic acid. The optimization was performed by increasing the concentration of acidic solutions from 0.1-0.5 % (v/v). The optimum HPLC condition was achieved with the use of the mixture of acetonitrile and 0.1% (v/v) acetic acid solution in gradient elution program at the flow rate of 0.6 ml min^{-1} and the detection wavelength at 275 nm. This method could be used to separate the six phenolic compounds with resolution greater than 1.5 in approximately 30 min under gradient condition at room temperature.

The accuracy expressed in terms of percentage recovery of this method for gallic acid, catechin, epicatechin, caffeic acid, rutin and quercetin was found to be between 95-113, 90-106, 88-97, 95-109, 97-113 and 87-108, respectively. The repeatability of retention time and peak area of each compound expressed as %R.S.D. were found to be 0.0-0.5% and 0.4-2.5%, respectively. The reproducibility of the retention time and peak area obtained were 0.2-1.2% and 0.8-2.3%, respectively. The limit of detection for each phenolic compound was calculated as three times the background standard deviation. The detection limits of gallic acid, catechin, epicatechin, caffeic acid, rutin and quercetin were 0.04, 0.08, 0.08, 0.16, 0.02 and 0.10 ppm, respectively.

Prior to HPLC analysis of phenolic compounds, seaweed samples were subjected to extraction using methanol: water: hydrochloric acid (75:20:5 v/v) as extraction solvent. No phenolic compound was detected in natural and commercial dry seaweed samples (S1, S3, S4 and S7-S9). The concentrations of gallic acid, epicatechin and quercetin in natural red seaweeds collected from Chon Buri province (S5) were found to be 488, 31 and 19 μg/g DW, respectively. For commercial dry seaweeds for cooking band B (S2), the concentrations of epicatechin and quercetin

were found to be 2 and 9 μg/g DW, respectively. Concentrations of catechin, epicatechin, rutin and quercetin detected in natural green seaweeds collected from Trat province (S6) were 62, 4, 5 and 10 μg/g DW, respectively. In natural brown seaweeds collected from Narathiwat province (S10), the concentrations of rutin and quercetin were found to be 18 and 19 μg/g DW, respectively.

The confirmation of phenolic compounds was performed using the negative-ion electrospray-mass spectrometry (ES-MS). The high intensity of the pseudomolecular ions, corresponding to $[M-H]^-$ was observed at m/z 169, 289, 289, 179, 609 and 301 for gallic acid, catechin, epicatechin, caffeic acid, rutin and quercetin, respectively.

The method successfully developed for simultaneous determination of phenolic compounds in this study has been demonstrated to offer superior performance characteristics [56], *i.e.* a simple method, significant improvement in resolution, short analysis time and low amounts of common acids under gradient condition. In addition, the use of low amounts of acid also increases the column's life and it only requires very short re-equilibration time between each injection. The method developed in this study is thus suitable for both the quantification and identification of individual of phenolic compound.