Study on extraction and enzymatic inhibitor activity of bioactive compounds from brown seaweed Undaria pinnatifida stem

Abstract

Undaria pinnatifida, commonly known as wakame in Japan, is a type of brown seaweed broadly farmed in the Sea of Japan, China, and Korea. It contains valuable bioactive compounds such as carotenoid, dietary fiber, protein, vitamins, essential fatty acid, and minerals, and has been a traditional and healthy food in many countries. However, most of *Undaria pinnatifida* that do not meet product standards are normally discarded as wastes or returned to the sea, which sometimes causes an environmental concern. The aims of this study were to investigate the yields of various bioactive compounds in *Undaria pinnatifida* stems, one of typical unused part. Furthermore, enzymatic (α-amylase and glucoamylase) inhibitor activities of the stem extracts were examined, some of which have been little investigated in earlier studies.

In Chapter 1, introduction about seaweed sustainability, relevance of seaweeds, type of seaweed, and the experimental techniques used for extraction the bioactive compounds from the seaweed were described.

In Chapter 2, extraction of bioactive compounds from stems of *Undaria pinnatifida* was conducted by several extraction methods were used by conventional liquid solvent extractions (water, ethanol, methanol, acetone and acetone mixed with methanol) and supercritical carbon dioxide (SC-CO_2) fluid extraction. And the contents of total phenol, total flavonoid, fucoxanthin, epicatechin, and gallic acid in the extracts were measured. The SC-CO_2 with ethanol extraction produced high amounts of phenolic (51.79 ± 0.51 mg/g dried crude extract) and flavonoid (32.48 ± 0.31 mg/g dried crude extract) compounds, fucoxanthin (178.33 ± 2.98 μg/g freeze dried *Undaria pinnatifida*), epicatechin (319.36 ± 2.14 μg/g freeze dried *Undaria pinnatifida*), and gallic acid (11.12 ± 0.08 μg/g freeze dried *Undaria pinnatifida*). These results suggested that the SC-CO_2 with ethanol extraction was useful for extracting these compounds and *Undaria pinnatifida* stems have value as a raw material to extract these bioactive substances.

In Chapter 3, the response surface methodology (RMS) was used to optimize extraction conditions of bioactive components from *Undaria pinnatifida* stems for SC-CO_2 with ethanol extraction. The Box-Bhenken design was used for the optimization of extraction parameters in terms of total phenolic contents, total flavonoid contents, fucoxanthin, epicatechin, and gallic acid contents. The statistical analysis of the experiments indicated that individual factors such as extract time, pressure, temperature and sample particle size, and interactive factors such as pressure with temperature and time had significant effects on bioactive compounds yield. Result suggested the optimal conditions obtained by the RSM from *Undaria pinnatifida* stems were as follows: extraction time 135 - 240 min, extraction pressure 6000 psi (41.37 MPa), extraction temperature 50 - 80 °C, sample particle size 100 μm, CO_2 flow 20 mL/min and entrainer 5 ml. In the optimal conditions, the experimental total phenolic contents were 49.92 ± 0.12 mg/g dried crude extract, total

flavonoid contents were 32.15 ± 0.11 mg/g dried crude extract, epicatechin contents were 41.99 ± 0.10 mg/g dried crude extract and gallic acid contents were 1.44 ± 0.06 mg/g dried crude extract, specially fucoxanthin contents were 21.93 ± 0.20 mg/g dried crude extract. From the this data, it can be summarize the following three points about optimizing the SC-CO_2 extraction process: first, the extraction time, pressure, temperature and sample particle size had the positive effects on all the bioactive compounds; secondly, the highest bioactive compounds were found in the mid-term with time, pressure and temperature increasing; finally, in the early period of time, pressure and temperature had a great influence on the extracts amount, with the time increasing, the influence of pressure and temperature on the extracts amount tends to be stable.

In Chapter 4, the enzymatic (α-amylase and glucoamylase) inhibitor activities of the extracts obtained by the conventional liquid solvent extraction, the SC-CO_2 extraction were investigated. The bioactive compounds (fucoxanthin, epicatechin and gallic acid) included in the extracts were also examined. The extracts obtained by SC-CO_2 with ethanol exhibited the strongest inhibitor activity among the extracts studied. Since among the compound examined fucoxanthin showed the strongest inhibition and the fucoxanthin content in the extract obtained by SC-CO2 with ethanol was the highest, it was suggested that suggest contributions of fucoxanthin to the inhibition of α-amylase and glucoamylase in the presence of stem extracts. Further research will be necessary to confirm the contribution of fucoxanthin and understand the reaction mechanisms of enzyme inhibition by the extracts from *Undaria pinnatifida* stems. The inhibition activity of the extracts by SC-CO_2 with ethanol was comparable to the acarbose, a practically used medicine to treat diabetes mellitus.

In conclusions, it was shown that stems of *Undaria pinnatifida* was rich in bioactive substances, such as phenolic compounds, flavonoids, epicatechin, and fucoxanthin. The SC-CO_2 extraction with ethanol was a promising method to obtain these compounds from *Undaria pinnatifida* stems. Furthermore, the extracts obtained from *Undaria pinnatifida* stems had significant α-amylase and glucoamylase inhibitor activities; thereby potentially it can retard glucose liberation from starches and alleviation of postprandial hyperglycemia. As the *Undaria pinnatifida* stems are very cheap and available, they would have a potential to be used for new health supplements.