Thesis for the Degree of Master of Engineering

Recovery and Characterization of Bioactive Compounds from Brown Seaweed (*Sargassum honer*i) using Sub- and Supercritical Fluids

by

Yin Shipeng

Department of Food Science and Technology

The Graduate School

Pukyong National University

February 2015

Recovery and Characterization of Bioactive Compounds from Brown Seaweed (*Sargassum honer*i) using Sub- and Supercritical Fluids

아임계 및 초임계유체를 이용한 갈조류 (모자반)으로부터 생리활성 물질 회수 및 특성

Advisor: Prof. Byung-Soo Chun

by

Yin Shipeng

A thesis submitted in partial fulfillment of the requirements

for the degree of

Master of Engineering

in Department of Food Science and Technology, The Graduate School,

Pukyong National University

February 2015

Recovery and Characterization of Bioactive Compounds from Brown Seaweed (*Sargassum honeri*) using Sub- and Supercritical Fluids

A dissertation

by

Yin Shipeng

Approved by:

Young-Mog Kim, Ph.D.
(Chairman)

Yang-Bong Lee, Ph.D.
(Member)

Byung-Soo Chun, Ph.D.
(Member)

February 2015

CONTENTS

List of Figures

List of Tables

Recovery and Characterization of Bioactive Compounds from Brown Seaweed (*Sargassum honeri*) using Sub- and Supercritical Fluids

Yin Shipeng

Department of Food Science and Technology

The Graduate School

Pukyong National University

Abstract

Seaweed is a potential renewable resource in the marine environment. It has unexplored bioactive compounds, which could be potentially utlized as functional food for human health. Seaweed provides for an excellent source of bioactive compounds such as carotenoid, dietary fiber, protein, vitamins, essential fatty acid, and minerals. Interestingly, seaweeds are a rich source of phytochemicals having antioxidant and antimicrobial properties. Among the functional effects of the seaweed, nutritional and health-related benefits have been widely studied. Most of seaweed has the anti-tumor, antifungal, anti-inflammatory, antioxidant and a wide range of biological activities. Generally, depolymerization methods have been done by traditional methods, e.g. organic solvents, using hexane, methanol or ethanol. Nowadays, many researchers have been competed to find new green technology methods which prevent the usage of harsh chemical solvents. Sub- and supercritical fluid extractions are environment-friendly technique which uses water as main solvent.

Seaweed (*Sargassum honeri*) was extracted using different solvents (acetone, hexane, methanol, ethanol, hexane mix ethanol and acetone mix methanol) and an environment-friendly solvent supercritical carbon dioxide (SC-CO_2). The SC-CO_2 was maintained at a temperature of 45 ^{o}C under the pressure of 250 bar. The flow rate of CO_2 (26.81 g/min) was

constant during the entire 2 h extraction period, ethanol (96%) was used as a co-solvent. Five different solvents (acetone, hexane, methanol, ethanol, hexane mix ethanol and acetone mix methanol) were used to extract and been run in a 200 mL flask with magnetic stirring overnight under the dark at 25 ℃ and the ratio of material to solvent was 1:10 (w/v). Fucoxanthin and the factty acid composition of the oil were analyzed using high performance liquid chromatography and gas chromatography. Antioxidant properties of *S. honeri* extracted using SC-CO_2 with ethanol and different solvents were showed the different activities. The highest activity belongs to the SC-CO_2 with ethanol extracted oil with DPPH and TFC were 68.38 ± 1.21 % and 5.571 ± 0.015 mg/g, respectively. The ABTS and TPC were shown the highest activity belongs to the hexane extracted oil (92.39 ± 1.25 %) and methanol extracted oil (0.639 ± 0.023 mg/g). Antihypertensive activity was found in different solvents extraction oil.

Depolymerization experiments using subcritical water hydrolysis were conducted on the SC-CO_2 extracted resduies at short residence times to evaluate decomposition products. The experiment conditions were performed between 180 ^{o}C to 260 ^{o}C for the reaction temperatures and 15 bar to 220 bar for the pressures. The ratio of material and water was 1:25 (w/v) and the reaction time of each condition was maintained for 3 min. High pressure pump was applied for flow water from tank to reactor for getting 1 bar initial pressure. An aqueous solution was obtained after hydrolysis process and was prepared for further analysis. The mixture of 1% formic acid and 1% sodium bicarbonate was used as catalyst. The hydrolysis yield was found higher on the treatment with catalyst addition at higher temperature. Antioxidant properties of *S. honeri* at 260 ^{o}C / 200 bar with catalyst addition was showed the best activity than other conditions, with the value of DPPH, ABTS, TPC and TFC of 20.05 ± 0.031 %, 94.02 ± 0.058 %, 200.84 ± 0.024 ug/L and 552.87 ± 0.018 ug/L, respectively. The recoveries of total sugar and reducing sugar at low temperature with catalyst addition were higher than those of other conditions. Hydrolysis treatment at 180 ^{o}C/ 15 bar with catalyst addition was the optimum condition to get high recovery of total organic carbon. Antihypertensive activity was

found higher at 180 ℃/ 15 bar condition. Twenty-one indentified amino acids were found in hydrolyzates in cluding eight essential amino acids. Color and pH also found different value in different condition. Subcritical water hydrolysis has been proved to decompose complex carbohydrate bonds to simpler bonds. Hydrolysate produced by subcritical water can produce bio-potential compounds which can be applied into food and bio industries.

Introduction

Marine macroalgae (seaweeds) are good potential resources of high biotechnological materials and high value interest due to production of a great diversity of their biological activities. They can be classified into three groups based on the basis of their pigmentation: green seaweed (chlorophyta), red seaweed (rhodophyta) and brown seaweed (phaeophyta). The industry of seaweed provides a wide variety of products that have an estimated total annual value of US$ 5.5-6.0 billion. Food products of human consumption contribute about US$ 5 billion of total annual value (McHugh, 2003).

Seaweed is an excellent source of magnesium, iron, sodium, calcium, iodine; an excellent source of vitamin A, B1, C, E, and K; low in fat and very low in calories. The main products of seaweed industry are alginates, agar and carrageenans which have the abilities to increase the viscosity of aquaeous solution to, form gels, and to stabilize properties of water-based products and can be widely applied in medicine industry as wound healer (Perreira and Costa-Lotufo, 2011). The nutrient composition of seaweed is affected by the species, geographic areas and seasons of the year and temperature (Manivannan et al., 2011).

Green seaweed usually appears green because of abundant chlorophyll a and b in the same proportion as the ‘higher’ plants. The other pigments of green seaweed are lutein and β-carotene. Red seaweed is probably well known for their economic values, for example as marine hydrocolloid sources of agars

and carrageenans. Red algae contains a variety of pigments, including cholorophyll A, phycobiliproteins, red phycoerythrin, blue phycocyanin, carotenes, lutein, and zeaxanthin. Most of red seaweed is pink to deep red in color because their plastids contain large amounts of the red accessory pigment of phycoerythrin, which obscures chlorophyll A (Graham, 2009). Phycoerythrin is the most important pigment on red seaweed, this pigment reflects red light and absorbs blue light. Brown seaweed has larger morphology than red and green seaweeds. The brown seaweed size range from the large kelp is around 20 meter (Meinita, 2010).

Brown seaweed produces terpenoids, acetogenins and terpenoid-aromatic compounds of mixed biosynthetic origin as their most common secondary metabolites (Blunt et al., 2011). Most of brown seaweed contains fucoxanthin which is responsible to give greenish-brown color. Moreover, it also has polyphenol i.e. phlorotannins, in higher concentration mainly in species from temperate and polar region (Koivikko, 2008).

Polysaccharides have been widely used as thickener agent and stabilizers in pharmaceuticals, cosmetics and as emulsifier in food products. There is also a large and growing potential for alginate in biotechnological applications (Draget et al., 2005). To obtain monomeric sugar from alginate, it must be prepared by breaking down the polysaccharide structure into simple monosaccharide. Therefore, to produce the appropriate monomeric sugar and other compounds, depolymerization treatment of alginate is needed.

Sargassum honeri belongs to the brown algae family of phaeophyta which

has lots of bioactive compounds. Furthermore, it has lots of unexplored bioactive compounds which have high value on economical aspect and on human health. Brown seaweed abundantly contains the polysaccharide such as alginate, mannitol and glucan (Rioux, 2007). Other important metabolites found in brown seaweeds are polyphloroglucinol of phenolic compounds (Steinberg et al., 1991) and secondary metabolites such as terpenoids, acetogenins, terpenoid-aromatic compounds (Blunt et al., 2011) and carotenoids (e.g. fucoxanthin).

More recently, a growing area of inquiry has focused on seaweed substances as functional foods and possibility of renewable bioenergy production. There are several examples of pure compounds and crude extracts of seaweeds that exhibit biological activities. Therefore, we show examples of some of these activities, such as cytotoxicity, antiviral, antioxidant, antibacterial, neuromodulator, NAPDPH-dependent lipid peroxidation, antifungal, anthelmintic, anti-inflammatory, and anticancer activities (Table 1).

Polysaccharides and other metabolites in brown seaweed have been reported to have antimicrobial activities (Osman et al., 2009; Tuney et al., 2006; Nagayama et al., 2002), antifouling (Cho, 2012), antioxidant (Alstyne, 1994; Peng et al., 2012; Rajauria, 2010), anti-allergic (Samee, 2009), anticoagulant, anti-cancer (Go, 2010), antiviral activities (Pereira, 2011). Also, brown seaweed is known as a new source of biofuel (Wargacki et al., 2012).

Table 1. Different algae bioactive compounds

Seaweed	Bioactive compounds	Activity	Reference
Fucus evanescens	Fucoidan	anti-tumor anti-metastatic	Alekseyenko et al., 2007
Adenocystis utricularis	Galactofuran	inhibitory against HSV 1 and 2	Ponce et al., 2003
Laminaria japonica	Laminarin	anti-apoptotic	Kim et al., 2006
Undaria pinnatifida	Sulfated polysaccharide	anti-viral	Hemmingson et al., 2006
Ecklonia cava	Phlorotannin	inhibitor of HIV-1 RT	Artan et al., 2008
		whitening effect	Ahn et al., 2004
		anti-cancer	Heo et al., 2009
Sargassum vulgare	Alginic acid	antitumor	de Sousa, Torres et al., 2007
Gracilaria edulis	Crude extract	antibacterial	Vallinayagam et al., 2009
Chondria atropupurea	Chondriamide B	anthelmintic	Davyt et al., 1998

The importance of marine algae as sources of functional ingredients has been well recognized due to their valuable effects for heath benefits. Therefore, isolation and investigation of novel bioactive ingredients with biological activities from marine algae have attracted great attention. Among functional ingredients identified from marine algae, fucoxanthin has received particular interest. Fucoxanthin has been attributed with extraordinary potential for protecting the organism against a wide range of diseases and has considerable potential and promising applications in human health. Fucoxanthin has been reported to exhibit various beneficial biological activities such as antioxidant, anticancer, anti-inflammatory, antiobesity, and neuroprotective activities (Kim et al., 2011). Fucoxanthin is a xanthophyll found in brown seaweed. This yellowish brown pigment has recently attracted much attention as a free radical scavenger and as an anticarcinogenic, antiinflammatory and antiobesity agent (Peng et al., 2011; Kim et al., 2011).

Polyphenols of plant origin may act as antioxidants in human and animal diet, thereby reducing the risk of atherosclerosis and coronary heart disease. They can also protect against some forms of cancer (Emmons et al., 2001). It is widely accepted that significant antioxidant activity of food is related to high total phenolic content. Plants and foods contain a large variety of phenolic derivatives, including simple phenols, phenylpropanoids, benzoic acid derivatives, flavonoids, stilbenes, tannins, lignans, and lignins (Shahidi et al., 2004). Polyphenols in marine brown seaweed are called phlorotannins and

known to act as potential antioxidants. Recently much attention has been paid to seaweeds which have been used traditionally as food (Seo et al., 2006).

The use of sub- and supercritical fluid extraction (SFE) offers numerous potential advantages over conventional extraction processes, because of reduced extraction time, reduced organic solvent volume, and more selective extractions. Sub- and supercritical fluids have been gained increasing attention as environment-friendly solvent and attractive reaction medium for a variety of applications. It is cheap, non-toxic, non-flammable, non-explosive, and offers essential advantages compared to other substances, particularly in the field of "green chemistry" (Taylor et al., 1996; Lang et al., 2001; Rogalinski et al., 2008).

Subcritical water hydrolysis (SWH) refers to water above its normal boiling temperature (100 ^{o}C), but below its critical temperature, which is kept in liquid state by applying pressure. It has distinctly different behavior compared to water at ambient conditions due to the dramatic changes in physical properties, namely dielectric strength and ionic product, which in turn can easily be altered by changing temperature and pressure (Alenezi et al., 2009). The ionic product of subcritical water is as much as three orders of magnitude higher than under ambient conditions. Under these conditions, there is a high H_3^+ and OH^- ion concentration. However, the ionic product decreases greatly above the critical point. This fact makes subcritical water an ideal reaction medium for the hydrolysis of organic compounds (Zhu et al., 2010).

Carbon dioxide (CO_2) is an attractive supercritical solventwhich has a

moderate critical temperature (31.1 ^{o}C) and pressure (73.8 bar), because it is non-flammable, non-toxic and inert. Because of these properties, Supercritical CO_2 can be useful when applied to food and pharmaceutical industries. Supercritical carbon dioxide (SC-CO_2) can achieve extraction yields for nature. This technology can be extract economically high purity materials and economical. In recent years, the use of SFE for the removal of organic compounds from different liquid and solid matrices has attracted much attention. This technique has some advantages over more conventional separation techniques, largely due to the unique physical properties of SFs. SFE using CO_2 is a promising process for the extraction and fractionation of edible oils containing labile PUFAs and lipid soluble bioactive compounds (Jose et al., 2008).

Inhibition of ACE-I is considered to be a useful therapeutic approach in the treatment of hypertension (Wijesekaraa et al., 2010). Therefore, in the development of drugs to control high blood pressure, ACE-I inhibition has become an important activity. Many studies have been attempted in the synthesis of ACE-I inhibitors such as captopril, enalapril, alcacepril and lisinopril, which are currently used in the treatment of essential hypertension and heart failure in humans (Ondetti, 1977). However, these synthetic drugs are believed to have certain side effects such as cough, taste disturbances, skin rashes or angioneurotic edema all of which might be intrinsically linked to synthetic ACE-I inhibitors (Kim & Wijesekara, 2010). Therefore, the research and development to find non-toxic and economical ACE-I inhibitors are

necessary for the prevention and remedy of hypertension (Goretta, Ottaviani, & Fraga, 2006). Marine organisms are rich sources of structurally diverse bioactive compounds and a great deal of interest has been developed nowadays to isolate antihypertensive bioactive peptides, which act as ACE-I inhibitors from marine organisms because of their numerous health beneficial effects (Wijesekara & Kim, 2010).

Bioactive peptides usually contain 3–20 amino acid residues per molecule and they are inactive within the sequence of the parent protein molecule. Moreover, bioactive peptides can be liberated by gastrointestinal digestion by proteolytic enzymes or during fermentation process (Korhonen & Pihlanto, 2006). Up to now, large numbers of bioactive peptides with antihypertensive activity have been derived from various marine organisms such as tuna (Hwang, 2010; Je et al., 2009 and Qian et al., 2007), Alaska “pollack” (Byun & Kim, 2001), blue mussel (Je, Park, Byun, Jung, & Kim, 2005), oyster (Je, Park, Jung, & Kim, 2005) sea cucumber (Zhao et al., 2007) and algae (Sheih et al., 2009 and Suetsuna and Chen, 2001). Seaweed pipe fish (Syngnathus schlegeli), a marine teleost fish, is well known for its special medicinal composition and used as one of the most famous and expensive materials in the traditional Chinese medicine (Khan, Qian, Ryu, & Kim, 2009). However, its antihypertensive activity of bioactive peptides has not yet been investigated.

The aim of this study was to recoveruseful materials from *S. honeri* such as fatty acids, fucoxanthin, polyphenols, reducing sugars and totalorganic carbonfrom *S. honeri* using SC-CO_2 extraction and subcritical water

hydrolysisat different experimental conditions and the recovered materials were characterized.

Materials and Methods

1. Materials

The brown seaweed *Sargassum honeri* was collected from Guemil-eup, Wando-gun, Jeollanam, South Korea. High-purity carbon dioxide gas (99%) was supplied by KOSEM (Yangsan, Republic of Korea). Fucoxanthin (≥95 % purity), captopil (≥98% purity), o-phthalaldehyde (OPA), Hipouril Histidine leucine (HHL), 2,2-Diphenyl- 1-picrylhydrazyl (DPPH, 95% purity), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonate) (ABTS, ≈98% purity), gallic acid (98% purity), (+)-catechin hydrate (≥98% purity), trolox (97% purity) and D-glucose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Distilled water was used in these experiments. All reagents used in this study were analytical or high performance liquid chromatography (HPLC) grade.

2. Sample preparation

After washing fresh *S. honeri* samples with fresh water, unused materials, attached salt, and minerals were removed. The samples were cut into small pieces. The small pieces of seaweeds were dried in freeze drier (Eyela FDU-

2100, Tokyo Rikakikai Co., LTD, Japan) equipped by square-type drying chamber (Eyela DRC-1000, Tokyo Rikakikai Co., LTD, Japan) at temperature -80 ℃ for 72 hours. The dried samples of *S. honeri* were collected into sealed plastic bags. After then, the dried samples were finely ground by mechanical blender (PN SMKA-4000 mixer) and sieved by 710 μm stainless steel sieving mesh. The samples which pass though of the sieving mesh were stored at -20 ℃ for one day prior to use.

3. Method

3.1. Measurement of proximate composition

3.1.1. Measurement of moisture content

Proximate compositions such as the moisture content, crude lipid, crude protein and ash content of samples were measured. Moisture content was measured using AOAC (2000) methods with slight modifications. Three grams of samples was heated in oven at temperature 105 ^{o}C for an appropriate period and weighed. Weighing samples was stopped when the sample weight was constant. The moisture content was calculated using an equation:

$$(W_1\text{-}W_2)/W \times 100\% \quad (1)$$

Where W_1 is initial weight of samples and chamber (g), W_2 is final weight of samples and chamber (g) and W is initial weight of samples (g).

3.1.2. Crude lipid analysis

Crude lipids were conducted by AOAC (2000) method with slight modifications using Soxhlet system. Soxhlet boiling flask was dried for a recent time, cooled and balanced without touchingit with fingers. Five grams of samples was put into a thimble and placed in the extraction apparatus. Petroleum ether was loaded into the flask at 2/3 of total volume. Heater was turned on and maintained at the temperature of 105 ^{o}C for a period of times. When the measurement was finished, solvent was evaporated using a rotary evaporator and then weighed. The crude lipid content was calculated using the below equation:

$$(W_1\text{-}W_2)/W \times 100\% \quad (2)$$

Where W_1 is weight of flask with fat (g), W_2 is weight clean dry flask (g) and W is weight of samples (g).

3.1.3. Crude protein analysis

Crude protein was determined based on AOAC (2000) method using Kjedahl system. This method evaluates the total nitrogen content of the sample after it has been digested in sulphuric acid with mercury or selenium catalyst. One gram of samples was loaded into Kjedahl's flask followed the addition of 10 g potassium sulphate, 0.7 g mercuric oxide and 20 ml concentrated

sulphuric acid. The flask was placed at an angle in the digester, brought to boiling point and retained until the solution was clear. The 90 mL of distilled water was added to cool the mixture followed of 25 mL sodium sulphate solution and stirred. One glass bead and 80 mL of 40% sodium hydroxide solution were added and the flask was kept tilted. The flask was rapidly connected to the distillation unit, heated and collected 50 mL of distillate containing ammonia in 50 mL of indicator solution. At the end of distillation, the receptor flask was removed, rinsed and titrated with the standard chlorhydric acid solution.

The crude protein contents were calculated using the below equation:

$$\text{Nitrogen content in sample (\%)} = (A \times B/C \times 0.014) \times 100\% \quad (3)$$

$$\text{Crude protein content in sample} = \text{nitrogen in sample (\%)} \times 6.25 \quad (4)$$

Where A is chlorhydric acid used in titration (ml), B is normality of standard acid and C is weight of sample (g). 6.25 is the nitrogen conversion factor and 0.014 is the molar mass of nitrogen.

3.1.4. Measurement of ash content

The ash determination was done using a method described by AOAC (2000) with slight modifications. Two grams of samples was placed in a crucible which previously weighted until it reached the constant weight. The crucible

was placed in a furnace of 550 ^{o}C for 10-15 hours or until the sample color changed into white. In the last of analysis, crucible with sample was weighted to obtain the ash content. The calculation of ash contents was obtained by the below equation:

$$(W_1\text{-}W_2)/W \times 100\% \quad (5)$$

Where W_1 is initial weight of crucible with sample (g), W_2 is final weight of crucible with sample, W is weight of sample (g).

3.2. SC-CO_2 extraction

The set up of a laboratory scale of supercritical fluid extraction (SFE) process is shown in Fig.1. *S. honeri* sample (100 g) was put into a 500 mL stainless steel extraction vessel, and the volume fraction of solid in the extraction vessel was 75 %. A thin layer of cotton was placed at the bottom of the extraction vessel. Before plugging with cap another layer of cotton was used at the top of the sample. CO_2 was pumped at constant pressure into the extraction vessel by high pressure pump (Milroyal, Milton Roy, USA) up to the desired pressure. A back pressure regulator was used to control the pressure of CO_2. The extraction temperature was maintained by connecting the extraction vessel with water bath. Flow rates and accumulated gas volume passing through the apparatus were measured using a gas flow meter

(Shinagawa, Tokyo, Japan). Ather 10 min intervals, extraction oil was collected on the glass separation vessels and weighted. After SC-CO_2 extraction, the kelp residues remaining in the vessel was stored at -20 ℃ until further use and analysis. *S. honeri* samples were extracted at temperature 45 ℃ and 250 bar pressure for 2 h using SC-CO_2. The flow rate of CO_2 was kept constant at 26.81 g/min for all extraction conditions (Roh et al. 2008). Ethanol (96%) was used as co-solvent with a flow rate of 1 mL/min.

3.3. Organic solvent extraction

Dried seaweed (*S. honeri*) was used to test the extraction efficiencies of different organic solvents (acetone, hexane, methanol, ethanol, hexane mix ethanol (9:1) and acetone mixmethanol (5:5 and 7:3)) for the extraction of analysis. Exactly, 20 g of freeze dried raw sample with 200 mL solvent was placed into the beaker and stirred 20 h by a magnetic stirrer at 25 ℃ and 300 rpm. After extraction, the hexane solution was filtered by a filter paper and then evaporated in a rotary vacuum evaporator (EYELA N-1100, Tokyo, Japan) at 40 ℃. The remaining residue was dried using dry oven at 40 ℃ for 6 h and then oils were stored at -20 ℃ until further use and analysis.

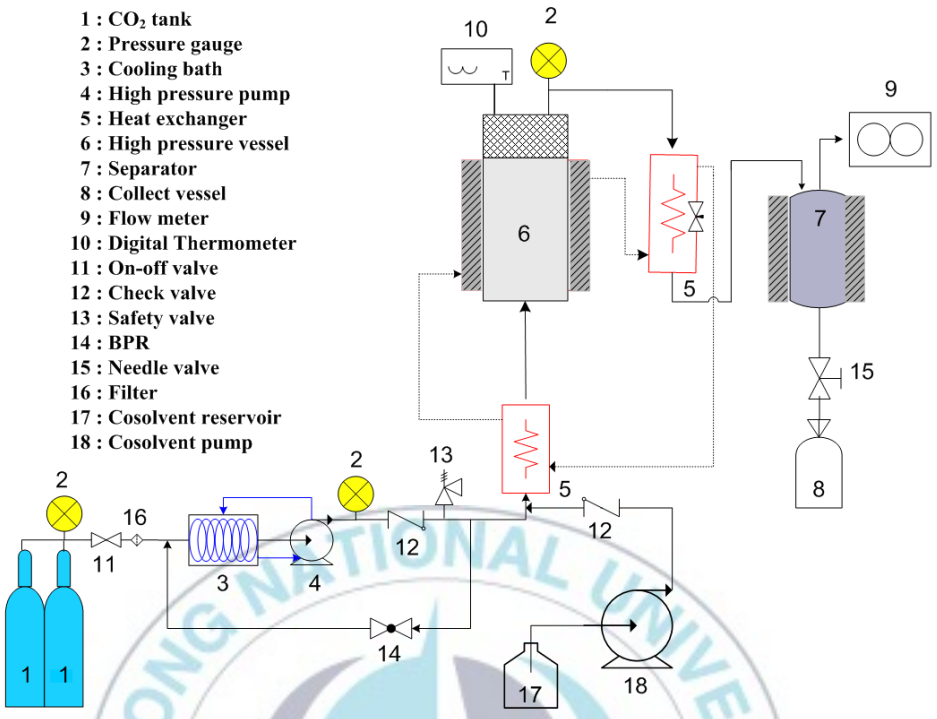


Fig.1. Schematic diagram of supercritical fluid extraction

3.3.1. Analysis of fucoxanthin from *Sargassum honeri*

The methods of Terasakiet al. (2009) and Noviendriet al. (2011) were adopted for fucoxanthin content determination by HPLC. All HPLC analyses were carried out using a Waters 600 E HPLC system (Waters, Milford, USA) equipped with a Tunable absorbance detector (Waters 484). Fucoxanthin content in seaweed extract was determined by reversed-phase HPLC (RP-HPLC) with methanol-acetonitrile (7:3, v/v) as the mobile phase at a flow rate of 1.0 mL/min. All RP-HPLC analyses were carried out at ambient temperature using a RP column (XTerra® MS C_{18}, 5.0 μm particle size, 250 mm × 4.6 mm i.d.; Waters, Milford, USA) protected with a guard column (10 × 4.6 mm i.d.) having the same stationary phase. Briefly, an aliquot of seaweed extract was dissolved in mobile phase, filtered with a 0.22 μm membrane filter, and a detection wavelength was set at 450 nm for detecting fucoxanthin (Fig. 2). A standard curve prepared using authentic standard was used for quantification of fucoxanthin content in seaweed samples. Fucoxanthin content in seaweed sample was expressed as mg/g oil of seaweed sample. The amount of fucoxanthin was quantified from the peak area using a standard curve with commercial fucoxanthin (Sigma-Aldrich, St. Louis, USA).

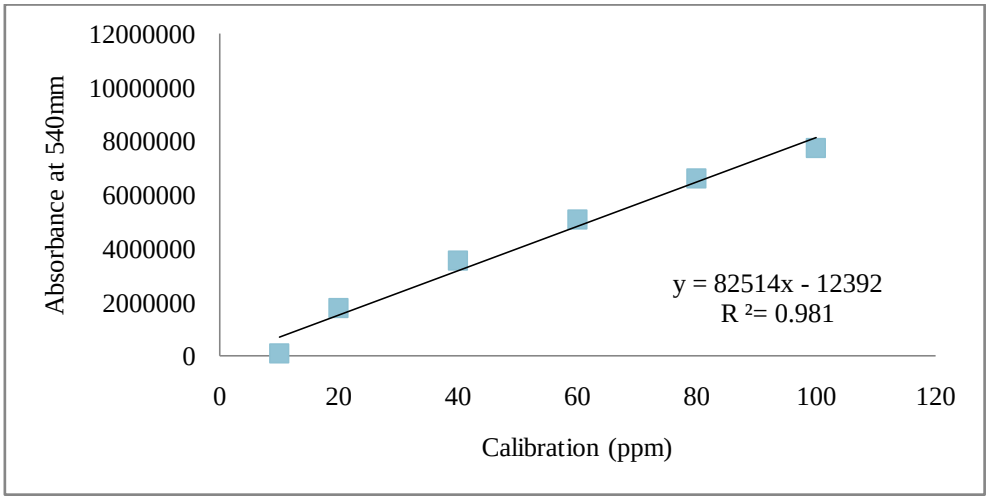


Fig. 2. Calibration curve of fucoxanthin standard

3.3.2. Fatty acid compositions analysis

Fatty acid compositions of *S.honeri* obtained after SC-CO_2 and organic solvent extraction were analyzed by gas chromatography (GC) according to the method of Lee et al. (2012). An 6890 Agilent Technologies (Wilmington, DE, USA) gas chromatograph with a fused silica capillary column (length, 100 m; internal diameter, 0.25 mm; length of film, 0.2 μm), Supelco (Bellefonte, PA, USA) was used. Before running the sample in the GC, the sample was prepared methyl ester according to the official method and recommended practices of the AOCS (Ce 2-66) (1998). The flow rate of helium was 1.0 mL/min as a carrier gas. The oven temperature was programmed starting at a constant temperature of 130 ℃ for 3 min, and then it was increased to 240 ℃ at a rate of 4 ℃/min and hold at 240 ℃ for 10 min. Injector and detector

temperature were 250 ℃. Fatty acid methyl esters were identified by comparison of retention time with standard fatty acid methyl esters mixture (Supelco^{TM}, USA).

3.4. Subcritical water hydrolysis

The subcritical water hydrolysis was carried out in 200 cm^3 of a batch reactor made of 276 Hastelloy with temperature control. Fig. 3 shows a schematic diagram of the subcritical water hydrolysis apparatus. Six gram deoiled samples treated by SC-CO_2 were suspended separately in 150 mL of distilled water (material to water ratio of 1:25) and charged into the reactor. The reactor was then closed and heated by an electric heater to the desired temperaturesat 180 ℃, 200 ℃, 220 ℃, 240 ℃ and 260 ℃. The pressures were estimated based on saturated steam between 15 bar to 220 bar for the temperature range studied. The temperature and pressure in reactor of each experiment was measured by temperature controller and pressure gauge, respectively. The sample was stirred at 150 rpm. The heat-up time for reaching the desired temperature took from 28 to 65 min (heat-up time is the amount of time that a device or system requires going from a cold start to operating temperature). The mixture of 1% formic acid and 1% sodium bicarbonate was used as catalyst. After rapidcooling to room temperature, the hydrolyzed samples from the reactor were collected and filtered using 0.45um Whatman nylon membrane filter 0.45 μm and stored 4 ℃ for future analysis. The liquid

portion called hydrolysate was analyzed for polyphenol, total organic carbon and reducing sugars. All experiments were performed in duplicate.

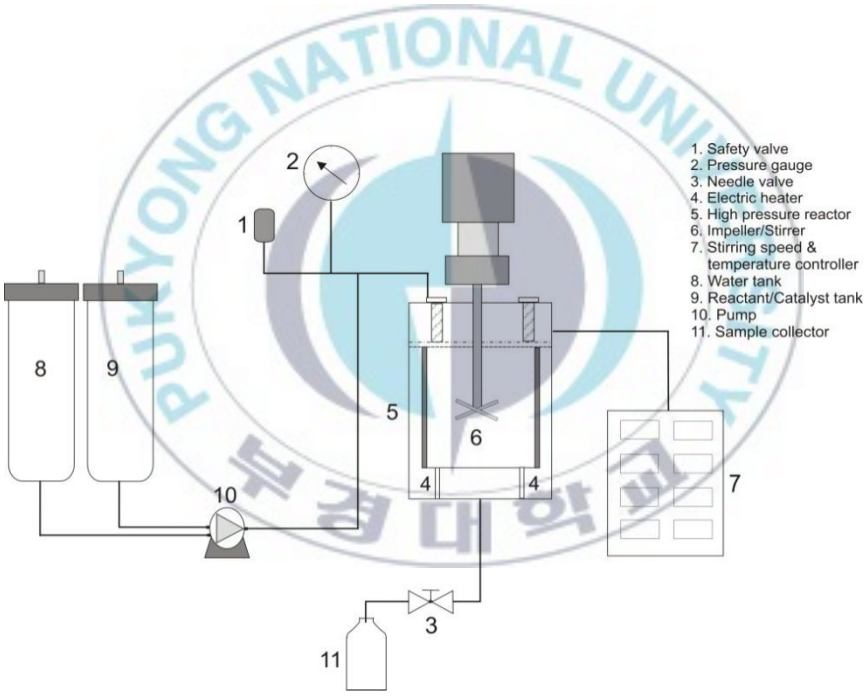


Fig. 3. Photograph and schematicdiagram of pressurized hydrothermal hydrolysis experimental apparatus

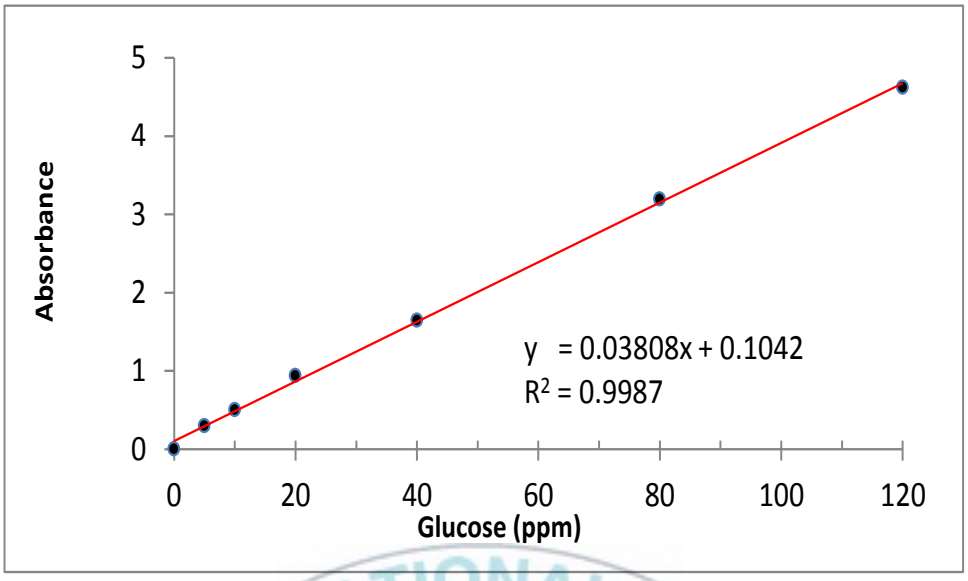
3.4.1. Subcritical water hydrolysis

3.4.1.1. Measurement of total sugar

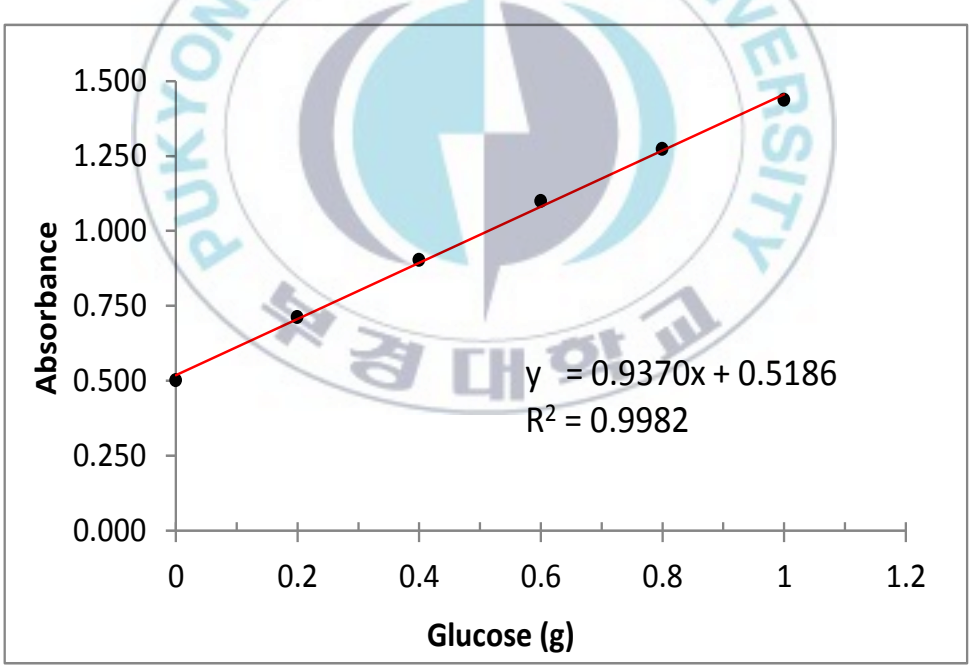
The total glucose measurement was carried out by phenol sulphuric acid method by Dubois et al. (1956). D-glucose was used as standard (Fig. 4) to make calibration curve. The 0.75 mL sample was mixed with 2.25 mL of concentrated sulphuric acid. Then, 0.45 mL of 40 % phenol was added and heated in water bath. The mixture was cooled at room temperature and the absorbance was measured by using a spectrophotometer at 490 nm.

3.4.1.2. Measurement of reducing sugar

Reducing sugar analysis (Fig. 4) was conducted by 3,5-dinitrosalicylic (DNS) acid method by Miller (1959). The reagents were made by mixing 10 g NaOH and 700 mL water until the mixture dissolved perfectly. Then, 300 g of potassium sodium tartrate was added and followed by 10 g DNS acid. After all of the mixtures were fully dissolved, 0.5 g sodium sulfite was added and followed by 2 g phenol. The volume of mixture was adjusted to 1,000 mL and kept away from light. The analysis of reducing sugar was done by mixing 0.5 mL of hydrolysate water and 0.5 mL of reagents. The mixture was heated for 10 minutes and 5 mL of cold water was immediately added. The absorbance was measured at 540 nm.



(A)



(B)

Fig. 4. Calibration curve of (A) Total sugar; (B) Reducing sugar

3.4.1.3. Measurement of total organic carbon

Total organic carbon (TOC) of hydrolysate water was measured using TOC analyzer. The analysis was done using 80 ppm of hydrolysate water of each condition at 180 ^{o}C temperature. The analysis parameters for total organic carbon were described in Table 2.

Table 2. Operating conditions of total organic carbon analyzer

Parameters	Conditions
Instrument	Shimadzu (Japan), TOC-Vcph, SSM
System	TOC-V
Detector	Combustion
Catalyst	Regular sensitivity
Cell length	Long

3.4.1.4. Measurement of amino acids

Hydrolyzates of mackerel skin obtained by pressurized hydrothermal hydrolysis were filtered and loaded onto a S430 (SYKAM) amino acid auto

analyzer for amino acid analysis. Cation separation column LCA K07/Li (4.6 x 150 mm), column temperature (37-74 ^{o}C), buffer pH of 2.90-7.95 were used for amino acid analysis. The mobile phase was 5 mM of *p*-toluenesulfonic acid solution at a flow rate of 0.45 mL/min. A mixture of 5 mM *p*-toluenesulfonic acid, 20 mM of bis-tris and 100 mM of EDTA was used as post column reagent at a flow rate of 0.25 mL/min. Excitation and emission wavelength was kept at 440 and 570 nm for both operational conditions (Asaduzzaman and Chun, 2014).

3.5. Antioxidant activity measurement

3.5.1. Total phenolic content assay

Total phenolic content (TPC) of crude extract was determined by the Folin-Ciocalteau colorimetric method according to Li et al. (2008) and Wong et al. (2006) with slight modification. one mL of 10 times diluted (v/v) crude extract was mixed with 1 mL of 1:10 (v/v, in deionized water) diluted Folin-Ciocalteu reagent (FCR). After 4 min, 800 μL of sodium carbonate solution (7.5%, w/v) was added into the mixture. Then, the mixture was vortexed for 5 sec and stored at room temperature in dark environment for 2 hours. Blank was also prepared by replacing 1 mL of deionized water. The absorbance of mixture was measured at 765 nm against blank using UV-spectrophotometer (UVmii 1240, Shimadzu Co., Japan). The measurements were carried out in triplicate.

Gallic acid was used for calibration of standard curve (Fig. 5.).

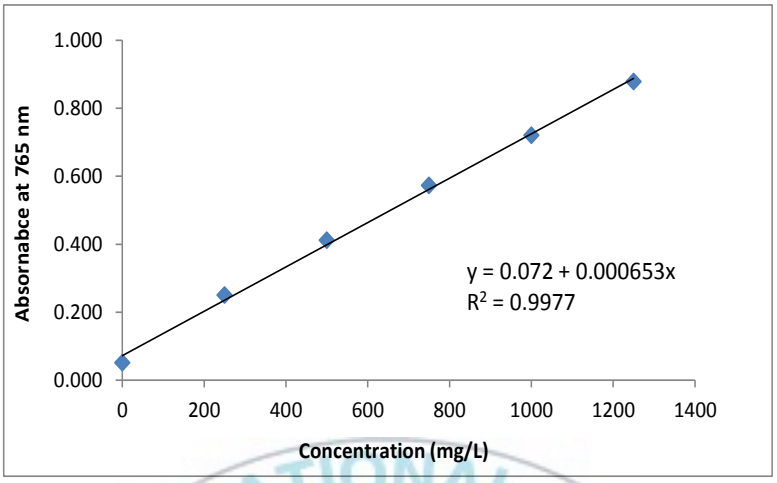


Fig. 5. Calibration curve of gallic acid standard for TPC

3.5.2. Total flavonoid content assay

Total flavonoid content (TFC) of crude extract was estimated using procedures described by Karadenizet al. (2005) and Ozsoyet al. (2007). 1.25 mL of deionized water was added into 0.25 mL of undiluted crude extract, followed by addition of 75 µL of 5% (w/v) sodium nitirite solution. The mixture was allowed to stand for 6 min and 150 µL of 10 % (w/v) aluminium chloride solution was added. The mixture was allowed to stand for another 5 min and 0.5 mL of 1 M sodium hydroxide solution and 275 µL of deionized water were added, accordingly. Subsequently, the mixture was vortexed for 5 sec and its absorbance was determined at 510 nm against blank using UV-

spectrophotometer (UVmii 1240, Shimadzu Co., Japan). The measurements were carried out in triplicate. Blank was prepared by replacing 0.25 mL of undiluted crude extract with 0.25 mL of deionized water. Catechin was used for calibration of standard curve (Fig. 6).

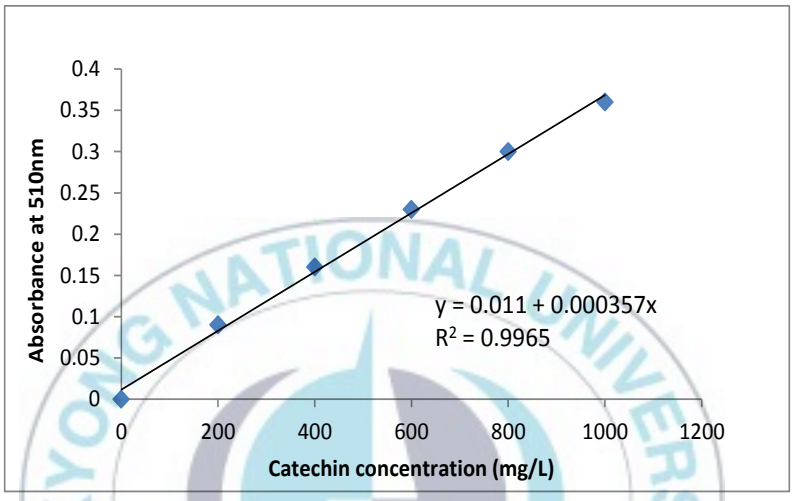


Fig. 6. Calibration curve of catechin standard for TFC

3.5.3. DPPH free radical scavenging assay

The DPPH radical scavenging capacity of crude extract was determined based on the method described by Miliauskaset al. (2004), Sahaet al. (2004) and Caiet al. (2006) with slight modification. The 3.9 mL of ethanolic DPPH (60 μM) was firstly mixed with 0.1 mL of undiluted crude extract or ethanol (as control) and they were stored in dark environment at room temperature for 30 mins. Subsequently, the absorbance of crude extract and control was

measured against ethanol (as blank) at 517 nm using UV-spectrophotometer (UVmii 1240, Shimadzu Co., Japan). The absorbance measurements of crude and control were done in triplicate. The percentage of DPPH free radical scavenging capacity was calculated using this formula:

DPPH free radical scavenging activity (%) = [1-(As/Ac)] ×100% (6)

Where, As is absorbance of crude extract at 517 nm and Ac is absorabance of control at 517 nm. The samples of blank and control (0.01 mg/mL standard trolox) were analyzed as described above.

3.5.4. ABTS radical scavenging capacity assay

ABTS radical scavenging capacity assay was carried out according to the procedures described by Caiet al. (2006), Wetwitayaklunget al. (2006), Guimarãset al. (2007) and Surveswaranet al. (2007). ABTS radical solution was firstly prepared by mixing 10 mL of 7 mM ABTS solution with 10 mL of 2.45 mM potassium persulphate solution in a amber bottle. Subsequently, the ABTS radical solution was allowed to stand in a dark environment at room temperature for 12-16 hours to give a dark blue solution. The ABTS radical solution was diluted with denatured ethanol until its absorbance was equilibrated to 0.70 ± 0.02 at 734 nm before usage.

The 3.9 mL of ABTS radical solution was firstly mixed with 0.1 mL of undiluted crude extract or ethanol (as control) and they were allowed to store in dark environment at room temperature for 6 min. Subsequently, the absorbance of crude extract and control was measured against ethanol (as blank) at 734 nm using UV-spectrophotometer (UVmii 1240, Shimadzu Co., Japan). The absorbance measurements of crude and control were done in triplicate. The percentage of ABTS free radical scavenging activity was calculated using this formula:

$$\text{ABTS free radical scavenging activity (\%)} = [1- (As/Ac)] \times 100\% \quad (7)$$

Where, As is absorbance of crude extract at 734 nm and Ac is absorabance of control at 734 nm. The samples of blank and control (1.0 mg/mL standard trolox) were analyzedas described above.

3.6. Biogicalactivity measurement

3.6.1. Antihypertensive activity

ACE inhibitory activity assay was performed using the method of Cha et al. (2006) with slight modifications. Buffer A (pH 8.3 with HCl) contained 20 mM sodium borate and 0.3 M NaCl. Buffer B (pH 12.0 with NaOH) was composed of 0.1 M sodium borate and 0.2 M NaOH. OPA reagent (Fluoraldehyde Reagent Solution) was prepared at least 1 hrs before the

experiment by mixing 1.5 ml of OPA solution (10 mg/ml in ethanol) and 1.5 ml of 2-mercaptoethanol solution (5 μl/ml in ethanol) in 100 ml of Buffer B. The final concentrations of OPA and 2-mercaptoethanol in the OPA reagent were both about 1 mM. ACE solution and HHL solutions were freshly prepared, respectively, by dissolving ACE (40 mU/ml) and HHL (15 mM) with Buffer A.

Sample solutions were diluted to different extents with Buffer A for the ACE-inhibitory assays. The ACE catalyzed reactions (37 ℃ × 2 h) were performed in test tubes containing 100 μl of sample solution, 100 μl of ACE solution, and 100 μl of HHL solution (Mixture 1). Another mixture containing 100 μl of sample solution and 200 μl of Buffer A (Mixture 2) was used to obtain the background absorbance of the sample solutions for the colorimetric method. The third mixture containing 100 μl of Buffer A, 100 μl of ACE solution, and 100 μl of HHL solution (Mixture 3) was used to obtain the data for 100 % reaction. The fourth mixture containing 300 μl of Buffer A (Mixture 4) was used to obtain the background absorbance of the OPA reagent. The enzymatic reactions (working pH 5-10) were terminated by adding either 3 ml of the alkaline (pH 12.0). All the mixtures were measured at 390nm after 20 min of 25 ℃ incubation. The inhibitory ratios were calculated by the following equation.

$$I\,(\%) = [1-(M1- M2) / (M3- M4)] \times 100 \qquad (8)$$

3.7. Physical and sensory propertis of *Sargassum honeri*

3.7.1. Oil stability measurement

3.7.1.1. Acid value

The acid value (AV) was assessed according to the method described previously byPing et al. (2008). 1g of sample was dissolved in 100 mL of ether : ethanol (1:1, v/v) by shaking, after which phenolphthalein, as an indicator was added by drops. AV of oil was analyzed by titration with a 0.1 N KOH-ethanol solutions until the pink color persisted for at least 30 s, it was calculated using the following equation:

$$\text{Acid value (AV)} = 56.11 \times A \times F/S \qquad (9)$$

Where, A is volume of the KOH-ethanol solution of the titration (mL), F is concentration of the KOH-ethanol factor, S is mass of oil (g), and 56.11 is molecular weight of KOH in mg.

3.7.1.2. Perxide value

The peroxide value (POV) was determined according to the AOCS method Cd 8-53 (American Oil Chemists's Society, 1998) using modified amount of sample. 1 g of sample oil was dissolved in 6 mL of a 3:2 acetic acid : chloroform solution. Then, 0.1 mL of saturated potassium iodine (KI) solution was added to the mixture and allowed to stand with occasional shaking for 1

min. Distilled water (6 mL) was immediately added to the solution allowedto stand. The solution was titrated with 0.1N of sodium thiosulfate until the yellow iodine color almost disappeared. Next, 0.4 mL of a starch indicator solution was added by shaking to extract iodine from chloroform layer, and again titrated until the blue color disappeared. A blank determination was performed with the same procedure. POVs wereexpressed as milliequivalents of a peroxide/1,000 g sample:

$$\text{Peroxide value (POV)} = \frac{(A - B) \times N \times 1000}{W} \quad (10)$$

Where, A is volume of titrant of sample (mL), B is volume of titrant of blank (mL), N is normality of sodium thiosulfate solution, W is mass of sample (g).

3.7.2. Viscosity measurement

A Brookfield DVII + Pro viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA 02346 USA) was used to measure the viscosity from *S. honeri* hydrolysate samples according to the method described by Ogawa et al. (2004) with slight modifications. 8 mL of 0.1% (w/v) sample in 0.1 M acetic acidwasincubated at 10 ℃ for 20 min, and then placed in a vessel. Spindle SC4-18 with agitation at 150 rpm was thenused to measure viscosity, which was expressed as centipose (cP).

3.7.3. pH measurement

pH of hydrolysate water was measured by Mettler Toledo Five Easy Plus pH meter at 20 ^{o}C. The adjustment of pH meter was done before measurement by technical buffer solution at pH 4.01 ± 0.02, 7.00 ± 0.02 and 9.21 ± 0.02.

3.7.4. Color compounds mesaurement

The CIE L*a*b* (or CIELAB) color model was used for determination of the crumb and crust color. The three parameters of such model represent the lightness of color (L*) which ranges from 0 to 100 (black to white), its position between red and green (a*, negative values in dicategreen while positive values indicate red) and its position between yellow and blue (b*, negative values indicate blue and positive values indicate yellow) (Sciarini et al., 2010).

3.8. Statistical analysis

All experiments were performed in triplicate. Data were analyzed using analysis of variance (ANOVA) and the differences between means were evaluated using Duncan's multiple range test. The SPSS statistics program

(SPSS version 15.0 for Windows, SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results and Discussion

1. Proximate composition of *Sargassum honeri*

Generally, the chemical composition of brown algae varies considerably between species and habitants throughout the year. Brown algae exposed to seasonal changes usually accumulate mannitol and laminaran in the light season (spring to autumn), and consume the carbohydrates in the dark season (Hung et al., 1954). Proximate analysis was done to measure the general composition of *S. honeri*. The proximate analysis is the vital information of a research to identify and analyze the relationship between proximate compositions and other compounds inside. The proximate analysis of *S. honeri* was shown in Table 3. The proximate compositions of *S. honeri* were 55.51 ± 0.29 % for carbohydrate, 28.93 ± 0.05 % for ash, 7.32 ± 0.17 % for moisture, 21.43 ± 0.07 % for ash, 6.87 ± 0.04 % for protein and 1.37 ± 0.09 % for lipid. The proximate composition of *S. honeri* used in this study was similar to that reported by Jang et al. (2012).

Table 3. Proximate analysis of *Sargassum honeri* samples

Component	Amount (%)
Carbohydrate	55.51 ± 0.29 [1)]
Crude lipid	1.37 ± 0.09
Crude protein	6.87 ± 0.04
Ash	28.93 ± 0.05
Moisture	7.32 ± 0.17

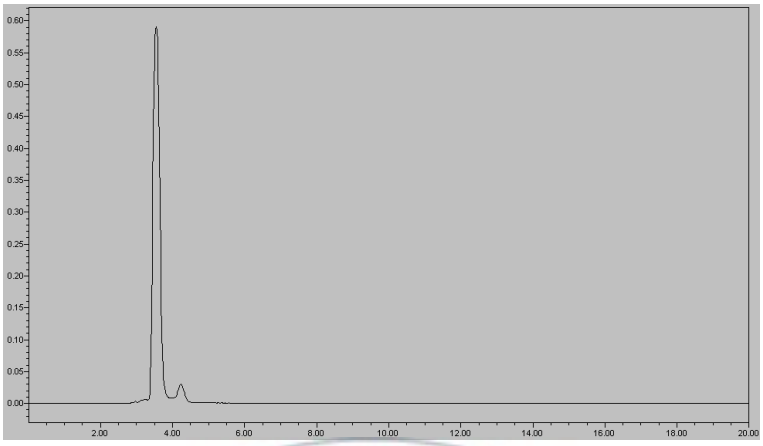
[1)] Error bars represent standard deviation with three replicates

2. Analysis of fucoxanthin from *Sargassum honeri*

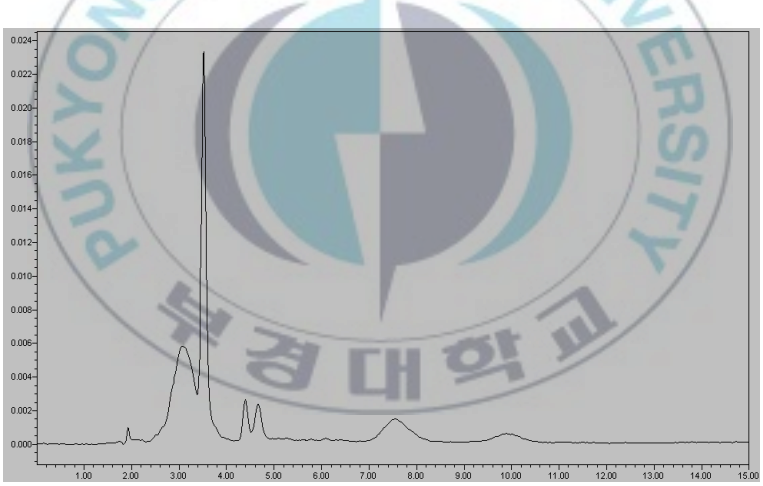
Carotenoids are widely present in marine biota, and their occurrence is species-dependent. Fucoxanthin is a dominant carotenoid characteristic of brown algae and in diatom (Dembitsky et al., 2007). Fucoxanthin is the main carotenoid in various edible brown seaweeds, such as *undaria pinnatifida* and *Hisikia fusiforme* (Haugan et al., 1992; Yan et al., 1999; Mori et al., 2004). However, information on the content of fucoxathin in the other (edible or otherwise) brown seaweeds is very limited. Hence, an effort was made to quantify fucoxanthin in *S. honeri*.

The HPLC chromatograms of fucoxanthin from fucoxanthin standard and *S. honeri* by SC-CO_2 with ethanol as co-solvent shows only one major peak with a retenition time of about 3.5 min (Fig.7).

The optimum method for the extraction of fucoxanthin from powder of *S. honeri* was investigated. The use of acetone, hexane, methanol, ethanol, hexane mix ethanol (9:1), acetone mix methanol (5:5 and 7:3) and SC-CO_2 with ethanol as co-solvent for their efficiency to extract fucoxanthin from dried powder of *S. honeri* was compared. As shown in Fig. 8, maxiumun amount 0.779 ± 0.012 mg of fucoxanthin was found at SC-CO_2 with co-solvent extraction of ethanol from 1 g oil. Other solvent extractions were less efficient than SC-CO_2 with ethanol. These results show that extraction using SC-CO_2 with ethanol as co-solvent is the optimum method for fucoxanthin.

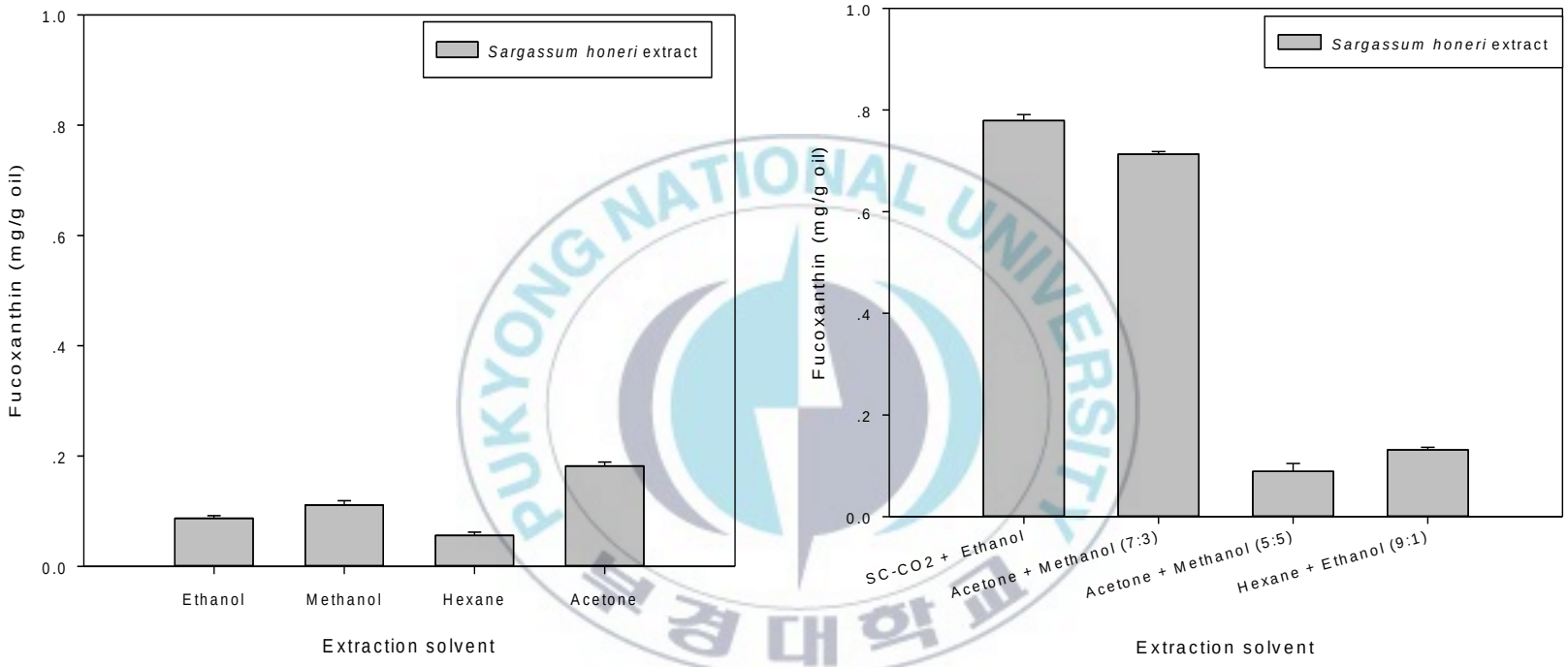


(A)



(B)

Fig.7. HPLC chromatograms of fucoxanthin from *Sargassum honeri* for (A) standard; (B) SC-CO_2 with ethanol as co-solvent



(A) **(B)**

Fig. 8. Fucoxanthin content of *Sargassum honeri* (A) single solvent extraction and (B) mix solvent extraction. Error bars represent standard deviation with three replicates

3. Fatty acid compositions

Although seaweeds are not a conventional source of energy (their total lipid content is low), their polyunsaturated fatty acids contents can be as high as those terrestrial vegetables (Darcy-Vrillon, 1993). The comparison of the fatty acid composition of the oil obtained by SC-CO_2 with ethanol and different organic solvents conditions (acetone, hexane, methanol, ethanol, Acetone mix Methanol ((7:3) and (5:5) and hexane mix ethanol (9:1)) is shown in Table 4.

Within saturated fatty acids (SFAs), palmitic acid (C16:0) was present in the highest concentration, ranging from 13.04 ± 0.08 % to 21.48 ± 0.34% of total identified fatty acids. This result was comparable to other brown seaweed, such as *Hormosirabanksii*, *Ralfsia* sp., *Dictyotadichomota* (Johns et al., 1979), *Stilophorarhizodes, Entonemaparasiticum, Pylaiellalittoralis, Corynophlaea umbellate, Cystoseriacrinita* (Demibitsky et al., 1990), *Saccorhiza polyscides, H. elongate, and L. ochroleuca* (Sánchez-Machado et al., 2004), *S. marginatum* (Bhaskar et al., 2004), *Laminraia sp*. (Dawczynski et al., 2007), *S. binderi, S. duplicatum* (Noviendri et al., 2011) where the single most abundant fatty acids was palmitic acid.

The percentages of the total monounsaturated fatty acids (MUFAs) were high in all extracts. Among the unsaturated fatty acids, oleic acidwas present in higher amounts in all extracts. The important PUFA was eicosapentaenoic acid (EPA, C20:5) ranging from 1.49 ± 0.02 % to 8.08 ± 0.09 % of total fatty acids. Interestingly, docosahexaenoic acid (DHA, C22:6) was noly found in

the analyzed *S. honeri* by SC-CO_2 with ethanol and not found in the analyzed *S. honeri* by other rganic solvents. These results were also found in other studies (Fleurence et al., 1994; Takagi et al., 1985).

The results shown that palmitic acid, heicosanoic acid, EPA, oleic acid, myristic acid were the primary fatty acids in *S. honeri*. The fatty acid content varied strongly within algal strains and, therefore, a unique fatty acid distribution for any given algal strain can't be easily produced because lipid composition changes, depending on various factors, such as temperature, characteristics, intensity of light, levels of minerals, nitrogen compounds, and the period in the life cycle of the algae (Takagi et al., 1985).

Seaweed products represent an important source of long-chain PUFAs (C15-C24, ω-3 and ω-6), that are fundamental for the formation of important structural lipids and elements of cell membranes. In addition, these long-chain PUFAs are precursors of eicosanoids, which influence inflammation processes and immune reactions (Calder et al., 2002; De Pablo et al., 2000). The two classes of PUFAs have opposing physiological functions and their balance is important for normal growth and development. These fatty acids are beneficial for the prevention of cardiovascular diseases and other chronic diseases, such as diabetes, hypertension, and autoimmune diseases. The European Nutritional Societies have recommended that human diet with a ω-6 / ω-3 ratio of 5:1 is health-promoting (D-A-C-H 2000). At present, the most traditional European food products possess a ω-6 / ω-3 ratio of approximately 15-17:1. This would suggest that Western diets are deficient in ω-3 fatty acids and high in ω-6 fatty

acids (Simopoulos, 2002). The results of this study show that the percentage of ω-6 fatty acid is relatively low in *S. honeri*.

The consumption of seaweed products can contribute to the improvement of the dietary supply of ω-3 Fatty acid. The intake of food rich in ω-3 long chain PUFAs can have a positive influence on the composition of blood lipids and can therefore be used for the prevention of arteriosclerosis (Dawczynski et al., 2007).

Table 4. Identification and percentage of fatty acids from *Sargassum honeri* oil extracted by SC-CO_2 at 45 ℃ / 250 bar and different organic solvents

Fatty acid compositions	Area (%)							
	SC-CO_2 + Ethanol	Acetone + Methanol (7:3)	Acetone + Methanol (5:5)	Hexane + Ethanol (9:1)	Acetone	Hexane	Ethanol	Methanol
C10:0	4.91 ±0.07[1)]	5.14 ±0.05	5.01 ±0.03	13.24 ±0.12	ND[2)]	21.17 ±0.31	ND	30.19 ±0.45
C11:0	ND	0.85 ±0.03	0.78 ±0.02	ND	3.83 ±0.06	ND	25.01 ±0.35	ND
C12:0	1.74 ±0.01	2.01 ±0.02	1.89 ±0.02	1.85 ±0.03	1.86 ±0.03	2.79 ±0.03	ND	ND
C13:0	3.43 ±0.05	2.99 ±0.02	2.65 ±0.03	3.11 ±0.04	1.80 ±0.02	2.30 ±0.05	ND	ND
C14:0	7.55 ±0.08	6.58 ±0.04	7.21 ±0.05	8.11 ±0.05	19.01 ±0.18	1.30 ±0.03	3.86 ±0.04	1.90 ±0.02
C14:1	1.66 ±0.02	2.14 ±0.03	2.31 ±0.03	2.45 ±0.04	3.87 ±0.03	2.11 ±0.02	5.64 ±0.05	3.82 ±0.04
C15:0	4.16 ±0.05	5.24 ±0.04	4.52 ±0.02	4.16 ±0.03	1.11 ±0.02	2.12 ±0.04	1.63 ±0.03	1.72 ±0.04
C15:1	1.13 ±0.02	1.59 ±0.03	1.41 ±0.04	1.74 ±0.04	ND	2.84 ±0.05	ND	ND
C16:0	21.48 ±0.34	18.54 ±0.24	19.89 ±0.15	20.85 ±0.23	31.83 ±0.51	18.07 ±0.18	23.32 ±0.29	13.04 ±0.08
C16:1	2.69 ±0.04	2.31 ±0.02	3.01 ±0.03	3.19 ±0.02	3.82 ±0.04	1.31 ±0.03	5.60 ±0.08	7.03 ±0.07
C17:0	ND	2.01 ±0.01	1.81 ±0.01	ND	ND	2.25 ±0.06	ND	ND
C17:1	0.62 ±0.01	2.96 ±0.04	2.56 ±0.02	3.02 ±0.05	3.56 ±0.03	3.79 ±0.07	ND	ND
C18:0	1.71 ±0.02	1.95 ±0.02	1.87 ±0.02	2.13 ±0.03	1.10 ±0.02	2.60 ±0.04	ND	2.41 ±0.02
C18:1n9c	12.29 ±0.05	11.24 ±0.07	10.25 ±0.04	9.89 ±0.06	10.60 ±0.09	8.98 ±0.07	8.37 ±0.11	3.89 ±0.03

C18:1n9t	1.69 ±0.02	1.85 ±0.05	1.74 ±0.03	1.45 ±0.03	1.17 ±0.04	2.34 ±0.03	ND	3.16 ±0.02
C18:2n6t	6.43 ±0.05	7.01 ±0.04	6.98 ±0.05	5.11 ±0.05	ND	2.06 ±0.04	ND	2.67 ±0.04
C18:2n6c	0.73 ±0.01	1.25 ±0.01	1.57 ±0.02	1.85 ±0.02	3.99 ±0.05	1.17 ±0.02	3.86 ±0.04	4.67 ±0.04
C20:0	0.85 ±0.01	1.21 ±0.02	1.34 ±0.03	0.98 ±0.03	0.56 ±0.02	0.86 ±0.01	2.98 ±0.04	4.19 ±0.05
C18:3n6	2.46 ±0.03	1.69 ±0.05	1.22 ±0.06	1.25 ±0.02	0.93 ±0.01	0.95 ±0.01	ND	4.62 ±0.05
C20:2	1.05 ±0.01	1.87 ±0.03	1.71 ±0.03	1.65 ±0.05	1.27 ±0.03	1.06 ±0.02	2.20 ±0.04	3.39 ±0.04
C18:3n3	ND	0.45 ±0.01	0.54 ±0.02	0.51 ±0.01	0.70 ±0.01	0.51 ±0.02	3.02 ±0.06	1.43 ±0.02
C21:0	6.23 ±0.08	6.11 ±0.06	6.01 ±0.05	5.41 ±0.04	2.54 ±0.03	4.43 ±0.04	3.93 ±0.05	3.73 ±0.03
C23:0	ND	0.76 ±0.02	0.65 ±0.01	ND	0.93 ±0.01	ND	1.13 ±0.04	1.13 ±0.01
C20:3n6	6.58 ±0.09	6.45 ±0.05	5.59 ±0.04	5.99 ±0.07	4.04 ±0.04	6.67 ±0.07	6.01 ±0.08	3.36 ±0.05
C22:1n9	ND	0.83 ±0.02	0.74 ±0.03	ND	ND	ND	ND	1.93 ±0.03
C22:2	1.39 ±0.03	0.95 ±0.04	1.15 ±0.05	1.24 ±0.03	ND	0.80 ±0.01	ND	ND
C20:5nc (EPA)	8.08 ±0.09	5.47 ±0.12	4.48 ±0.09	6.13 ±0.14	1.49 ±0.02	7.52 ±0.08	3.44 ±0.05	1.73 ±0.02
C22:6nc (DHA)	1.12 ±0.02	ND	ND	ND	ND	ND	ND	ND

[2] ND, no detect.

[1] Error bars represent standard deviation with three replicates

4. Yield of Subcritical water hydrolysis

The hydrolysis efficiencies of *Sargassum honeri* at different temperatures and pressures after SWH were shown in Fig. 9. The residual *S. honeri* powder recovered after SWH was dried and weighed [W (g)], Conversion yield of *S. honer* as:

$$(W_0\text{-}W)/W_0 \times 100\% \quad (10)$$

Where W_0 is the total amount of *S. honeri* (g) introduced in reactor.

It was found that the hydrolysis yield increased with the increase in temperature and pressure in the vessel. Hydrolysis yield was measured to analyze the experiments conditions which resulted high effectiveness of hydrolysate water production. The highest hydrolysis yield in *S. honeri* was 98.541 % at 260 ℃ / 220 bar without adding catalysts. Similar results were reported by Watchararuji et al. (2008) for subcritical water hydrolysis of rice bran and soybean meal.

Obviously, at relatively higher temperatures and pressures, the amount of solid was reduced by increasing the hydrolysis and decomposition of the sample. The high yield produced by subcritical hydrolysis was obtained because of the difference on the density and polarity of water as a main solvent in this system. Water becomes less polar and shows dramatic increase in solubility as temperature and pressure rises (King, 2000). In fact, because of its strong aggregation through hydrophobic interactions, protein usually has

low solubility in water at ambient temperature. However, the solubility of new materials such as protein in water increases at higher temperature and pressure. In addition, at high temperature and pressure, the hydrolysis yield increased due to the increased rate of hydrolysis caused by the raise in water ionization constant.

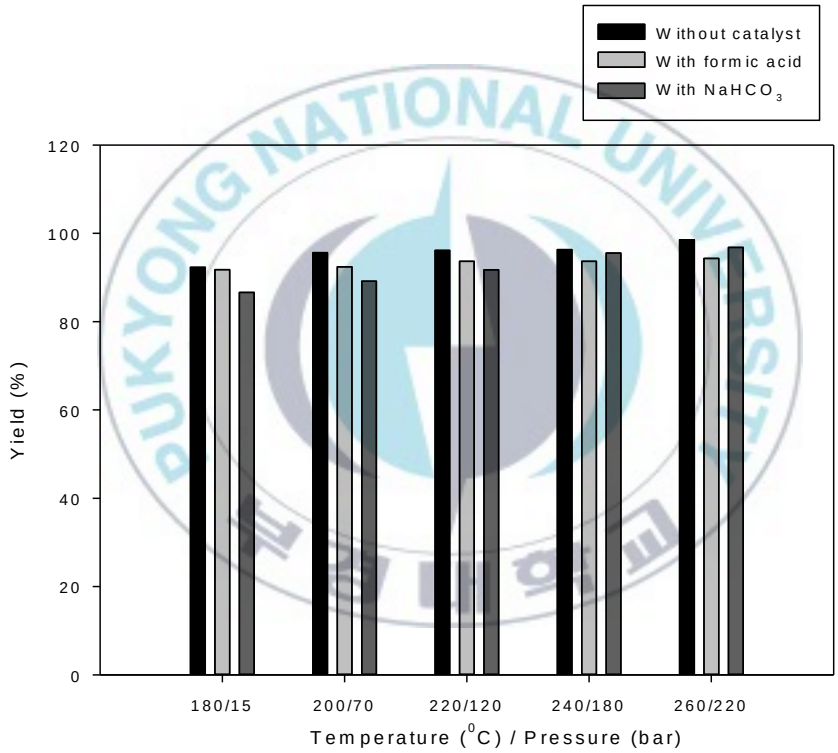


Fig. 9. Hydrolysis yield on different conditions

5. Analysis of *Sargassum honeri* extract

5.1. Antioxidant activity

Antioxidant properties of hydrolysate water was conducted using some antioxidant assays, i.e. DPPH radical-scavenging activity and ABTS radical-scavenging activity, total phenolic content (TPC), total flavonoid content (TFC). DPPH and ABTS radicalscavenging activity measures the ability of a substance to prevent free radical molecules. TPC and TFC describe the total amount of phenolic and flavonoid, respectively, contained in extracted oils.

The DPPH method measures free radical scavenging activity of antioxidants directly from the seaweed extracts. Free radicals oxidize lipids. The ability of a seaweed extract to scavenge the reactive metabolites will inhibit the formation of primary and second dary oxidation products. Devi et al. (2008) reported that DPPH radical scavenging ability differed greatly between the different varieties and ranged between 5 % and 72.5 %. The SC-CO_2 with ethanol and hexane extracted oil demonstrated the highest DPPH radical scavenging activity, with 68.38 ± 1.21 % and 48.90 % ± 1.01%, respectively (Fig. 2). This was significantly ($p < 0.05$) greater than that of the other solvent extracted. The hexane extracted oil (48.90 ± 1.21 %) demonstrated an activity similar to that of acetone mix methanol (7:3) extracted oil (41.87 ± 1.54 %). The acetone extracted oil, hexane mix ethanol (9:1) extracted oil and ethanol extracted oil demonstrated relatively poor radical scavenging activity with 33.23 ± 1.46 % , 32.54 ± 0.89 % and 23.03 ±

1.21 %, respectively.

The ABTS radical cation decolourisation test is another method widely used to assess antioxidant activity. Reduction in colour indicates reduction of ABTS radical (Adedapo et al., 2008). The SC-CO_2 with ethanol, methanol and hexane extracted oil demonstrated the highest ABTS radical scavenging activity, with 83.52 ± 1.25 %, 82.40 ± 1.32% and 81.31 ± 1.41%, respectively (Fig. 2). This was significantly ($p < 0.05$) greater than that of the other solvent extracted. The hexane mix ethanol (9:1) extracted oil (56.45 ± 1.32 %) demonstrated an activity similar to that of acetone extracted oil (56.21 ± 1.36 %) and ethanol extracted oil (49.97 ± 1.05 %). The acetone mix methanol (7:3) extracted oil 44.10 ± 1.61 % demonstrated relatively poor radical scavenging activity.

Previous studies have shown that phenolic compounds are the main contributors to the antioxidant activity of various seaweeds (Jiménez-Escrig et al., 2001; Wang et al., 2009; Zhang et al., 2007). The total phenolic content of the six seaweed extracts was calculated by a modified Folin–Ciocalteu method (Fig. 2). Due to solubility issues of the different extracts in the Folin–Ciocalteu reagent we examined both the lipid and aqueous soluble seaweed fractions. The SC-CO_2 with ethanol extracted oil (0.64 ± 0.02 mg/g) demonstrated a higher total phenolic content than all other seaweed extracted oil. The methanol extracted oil and acetone extracted oil both demonstrated the high level of phenolic from lipid extraction with 0.51 ± 0.02 mg/g and 0.50 ± 0.01 mg/g, respectively. A positive correlation has been found between total

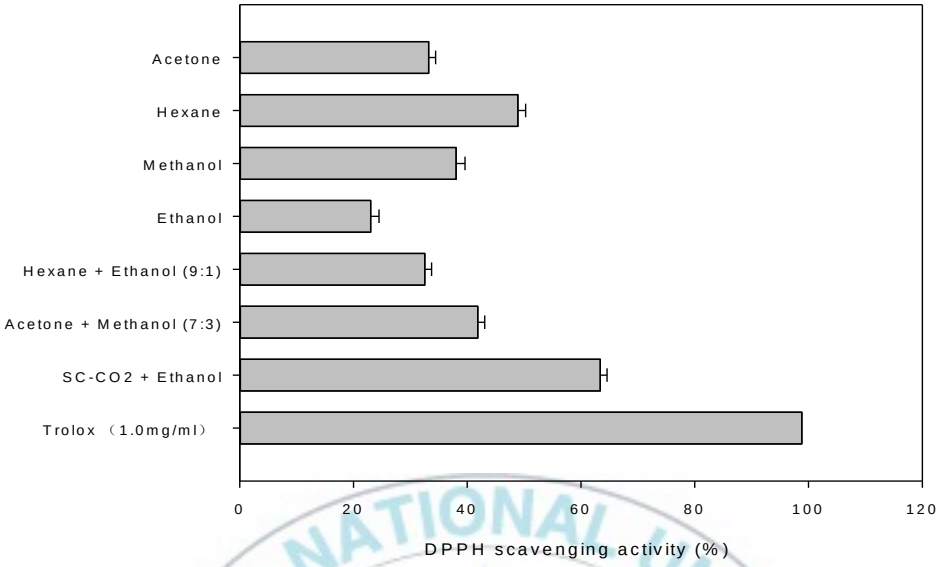
phenolic content and antioxidant activity of different solvent seaweed extracts in previous studies (Jiménez-Escrig et al., 2001).

The TFC of the extracted oils were reported in Fig. 2. The TFC were be grouped into four levels. The first and the highest level with the highest value belong to the SC-CO_2 with ethanol extracted oil (5.57 ± 0.05 mg/g). The second level with the middle TFC included the acetone mix methanol (7:3) extracted oil (4.87 ± 0.02 mg/g) and the acetone extracted oil (4.71 ± 0.02 mg/g). The final level with the lowest TFC was the methanol extracted oil (3.91 ± 0.03 mg/g). It was observed that the effect of solvents on TFC is similar to that on TPC. The same things were described by Do et al., (2014). A correlation analysis was performed on polyphenolic contents (TPC and TFC) of *S. honeri* extract. The correlation between TPC and TFC assay was found to be similar. This indicates that flavonoid is the dominating phenolic group in *S. honeri*. The result is similar to the extraction of phenolic from guava and pisang mas, a variety of banana (Alothman et al., 2009). Some flavonoid that was isolated from *L.aromatica* have been identified by Krisnan et al. (1999) and Bui et al. (2004).

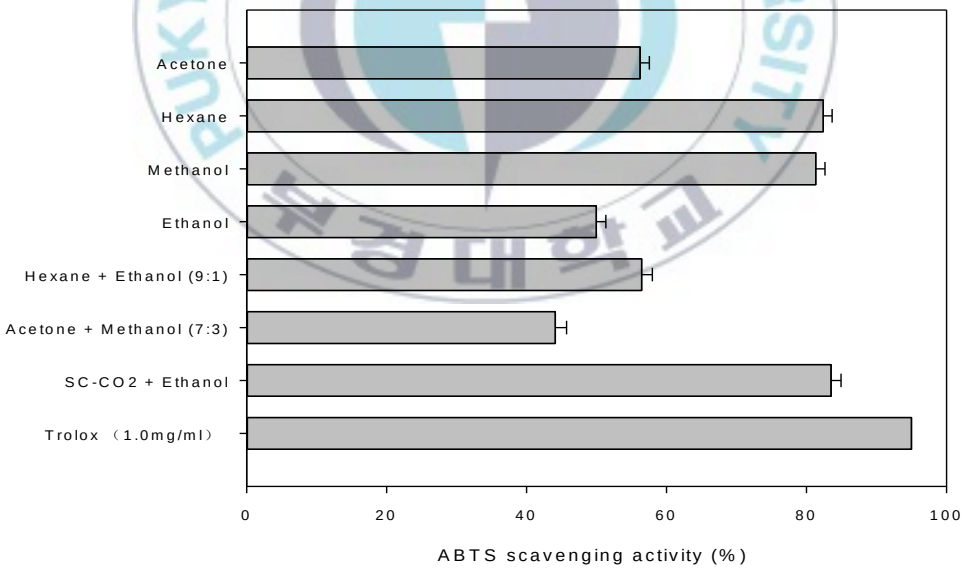
This result demonstrated that these brown algae extracts have significantly enhanced radical scavenging capacity over the commercial synthetic antioxidants. A group of phenolic compounds identified in brown seaweed, have been associated with antioxidant activity (Koivikko et al., 2005). Other compounds in brown algae, such as fucoxanthin and sterols have been demonstrated to work synergistically as radical scavengers during the DPPH

antioxidant assay (Wang et al., 2009).

Antioxidant properties of extracted oil were affected by bioactive compounds such as phenolic, flavonoid, minerals and others. The condition of growth environment, harvest time and storage condition also give huge effects to the amount of those compounds (Roh et al., 2008). Most of antioxidant compounds found in brown seaweed belong to the group of polar compounds. As we know the general principle in solvent extraction “like dissolves like”, which means that suitable solvents only dissolve suitable substances which have similar polarity with the solvents used. Hence, those antioxidant compounds were dissolved in a presence of water as a main solvent.



(A)



(B)

Fig. 10. Antioxidant properties in *Sargassum honeri* of different solvents extraction (A) DPPH radical scavenging activity ; (B) ABTS radical scavenging activity. Error bars represent standard deviation with three replicates

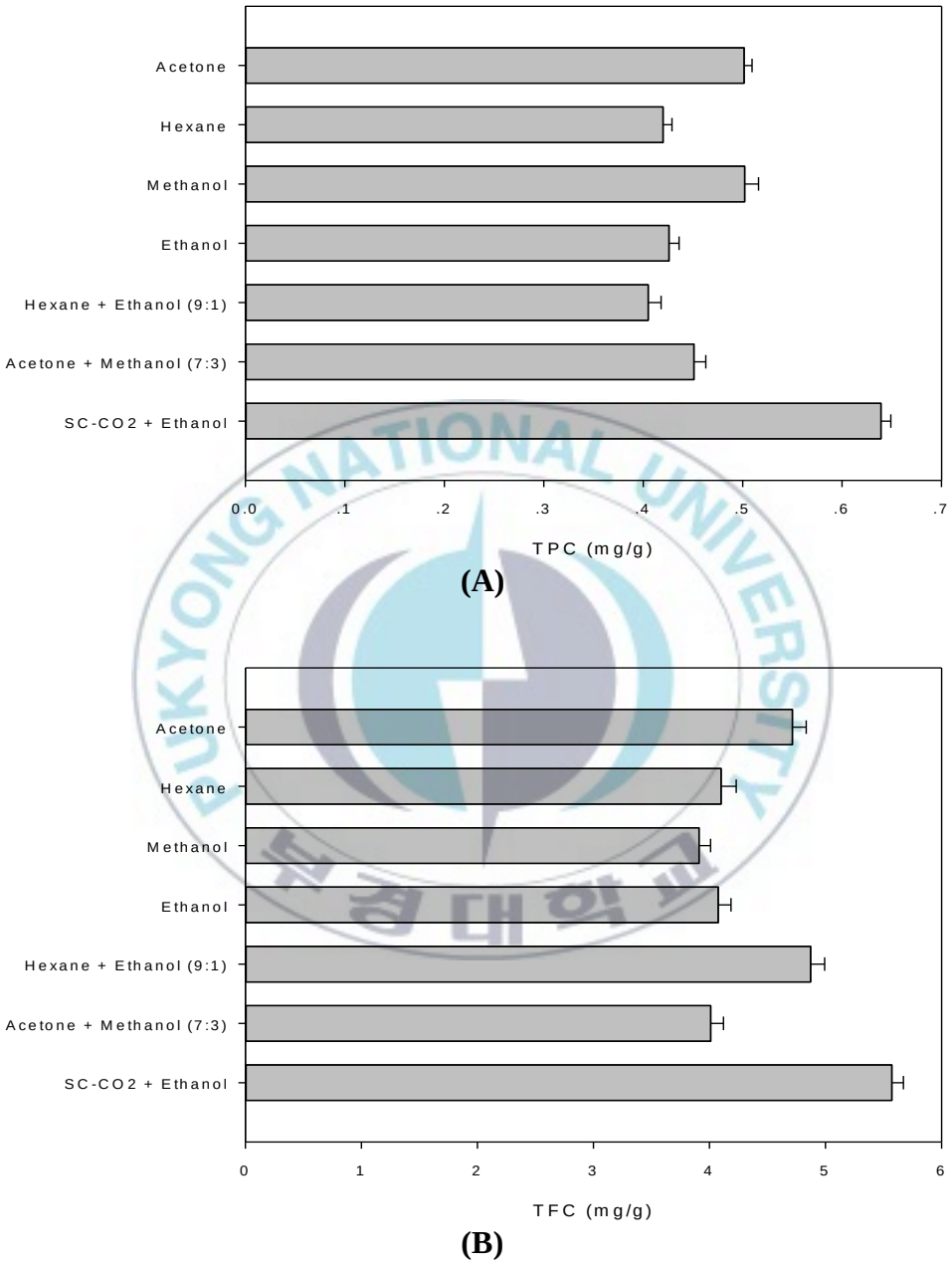


Fig. 11. Antioxidant properties in *Sargassum honeri* of different solvents extraction (A) Total phenolic content (TPC); (B) Total flavonoid content (TFC). Error bars represent standard deviation with three replicates

5.2. Acid value and peroxide value

The quality of oil deteriorates at different rates depending on the productionand storage conditions (Kamal-Eldin and Yanishlieva, 2002). The AV and POV content of oil extracted using SC-CO_2 with ethanol, other solvents and after particle formation are shown in Table 5. The amount of acid value and peroxide value content were higher in hexane extracted oil than in other solvents and SC-CO_2 with ethanol extracted oil. Because the hexane extraction systemis open, samples are exposed to increased amounts of oxygenduring the extraction explaining the increased oxidation. Incontrast, the exposure to low levels of oxygen only during SC-CO_2 extraction caused minimal oxidation. The AV was calculated to determine the acidity of oil and a low AV is indicative of a high oxidative stability (Essien et al., 2012). In contrast, the POV of an oil or fat is a measurement of rancidity which is a result of autoxidation. The AV and POV of *S. honeri* oil obtained using different solvents conditions ranged from 23.83 ± 0.11 to 26.81 ± 0.22 mg KOH/g and 7.01 ± 0.02 to 8.78 ± 0.05 meq/1000 g, and the SC-CO_2 extractioncondition was 20.45 ± 0.09 mg KOH/g and 6.76 ± 0.03 meq/1000 g.

Table 5. Acid value and peroxide value content of *S.honeri* oil obtained by SC-CO_2 (45 ^{o}C / 250 bar) and different solvent extraction

Sample name	Acid value (mg KOH/g)	Peroxide value (meq/1000g)
Hexane extracted oil	26.81 ±0.22^{a} [1)]	8.78 ±0.05^{a}
Acetone extracted oil	25.32 ±0.12^{b}	7.64 ±0.04^{b}
Methanol extracted oil	25.59 ±0.15^{b}	7.12 ±0.08^{b}
Ethanolextracted oil	23.83 ±0.11^{b}	7.01 ±0.02^{b}
Hexane + Ethanol (9:1)	24.83 ±0.14	7.15 ±0.03^{a}
Acetone + Methanol (7:3)	24.91 ±0.18	7.26 ±0.04^{b}
SC-CO_2 + Ethanol extracted oil	20.45 ±0.09^{c}	6.76 ±0.03^{c}

[1)] Error bars represent standard deviation with three replicates. Different lowercase letters in each column indicate significant differences ($P < 0.05$)

5.3. Antihypertensive activity

Numerous in vitro techniques, based on either spectrophotometric or HPLC assay, have been developed for the detection of ACE inhibitory activity of bioactive peptides. In vitro ACE inhibitory activity is generally measured by monitoring the conversion of an appropriate substrate by ACE in the presence and absence of inhibitors. The ACE inhibitory activities are shown in Fig. 12. The SC-CO_2 with ethanol was found best ACE inhibitory activity with 81.75 ± 0.12 % than other solvent extraction. And the other different solvents extraction had the similar ACE inhibitory activity with 69.59 ± 0.32 %, 67.54 ± 0.17 %, 68.21 ± 0.08 %, 70.14 ± 0.24 %, 69.81 ± 0.13 % and 72.54 ± 0.15 % by acetone mix methanol (7:3), hexane mix ethanol (9:1), ethanol, methanol, hexane and acetone, respectively. This confirms that *S. honeri* provides compounds which improve antihypertensive properties of extruded product and they are bio-accessible.

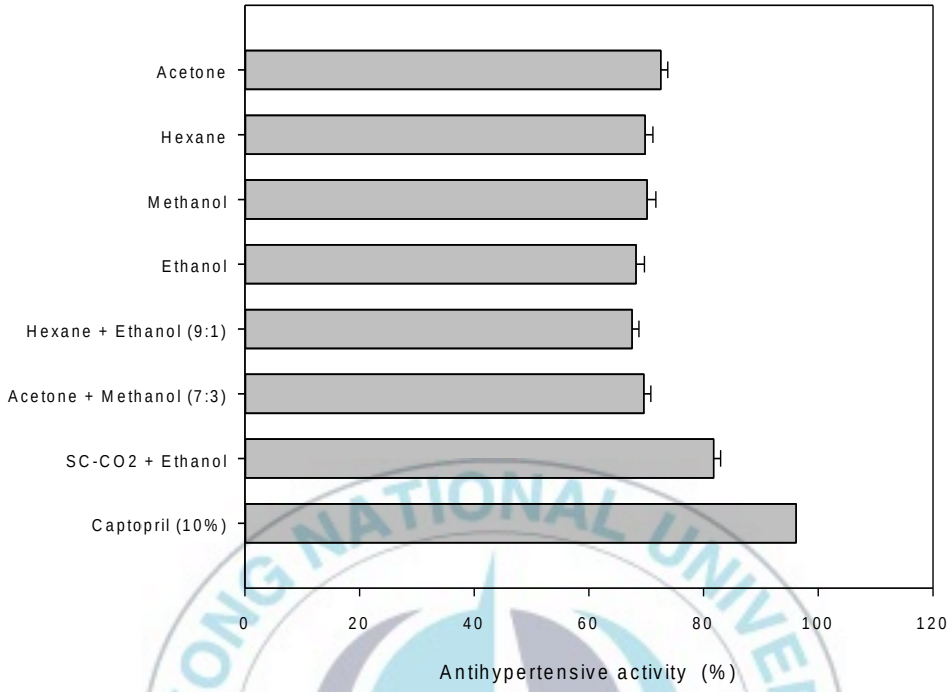


Fig. 12. Antihypertensive activity in *Sargassum honeri* of different solvents extraction. Error bars represent standard deviation with three replicates

6. Analysis of *Sargassum honeri* hydrolyzates

6.1. Total sugar and reducing sugar

Efforts at the discovery of sustainable bioenergy are a big concern of most countries during this time. Our dependency on fossil fuels for energy production is problematic. Biomass (biofuel, biogas, bioethanol, *etc*.), one of renewable energies, is considered to be carbon-neutral and it is the only organic matter among renewable energies. In other words, fuels and chemicals can be produced from biomass in addition to electricity and heat. Bioethanol production from marine products is one of the most potential renewable energies. Bioethanol will be attained from the fermentation of monosaccharide in marine products by yeast.

In this work, total glucose of hydrolysate water was analyzed using phenol sulphuric acid method with slight modifications, to check the possibility of sugar which will be converted to ethanol. Total sugar in the hydrolysate water was calculated using the D-glucose as a standard on different concentrations from 0 to 120 ppm and the absorbance was measured at 490 nm.

The subcritical water technique has a wide range of applications such as extraction and wet oxidation of organic compounds (Kruse et al., 2007). This technique uses hot water with temperature ranging from 100 to 374 ^{o}C andhigh pressure to maintain water in the liquid state. Under these conditions, the characteristics of water change. This leads to a lower solvent viscosity, surface tension and dielectric constant.

The carbohydrate content in samples could be converted into a wide range of water-soluble sugars (such as poly-, oligo-, and mono-saccharides) by hydrolysis under subcritical water conditions. Fig. 13 shows the amount of total sugar as a function of temperature. The highest total sugar was achieved in 180 ^{o}C / 15 barhydrolysis temperature and pressurewithcatalyst of 879.86 ± 0.15 mg/L. Differentiation of total soluble sugar in hydrolysate water is caused by the change of water physical properties during subcritical state (Daneshvar et al., 2012). Higher temperature and pressure applied in this work shown the decreases of total sugar in all conditions. Obviously, water-soluble sugars are one of attractive or important factors for fermentation reactions. Therefore, subcritical water treatment could play an important role as a pretreatment of marine bioethanol production.

Reducing sugar was examined using DNS method (Miller, 1959) with slight modifications. D-glucose was used as a standard and diluted with distilled water on different concentrations. The absorbance of the mixture was measured using a spectrophotometer at 540 nm.

Reducing sugar contains aldehyde groups that are oxidized to carboxylic acids. It has an open chain form with an aldehyde groups or a free hemiacetal group. The kinds of sugar which has aldehyde group in the chain are glucose, mannose, galactose, arabinose, maltose and glucose. *S. honeri* comprises of 60 % carbohydrates. Typical carbohydrates in *S. honeri* consist of fucoidan, laminaran (β-1.3-glucan), cellulose, sugar and alginates. The decomposition of carbohydrate into a reducing sugar, monosaccharide and polysaccharide offers

economic value of the material itself.

Many works have been done to convert carbohydrate of marine macroalgae into monosaccharides and other compounds.Meinita (2010) has been done to produce bio-ethanol by fermentation of the commercial yeast of *Saccharomyces cerevisiae*. Fitton et al. (2007) made a new invention to produce marine cosmetics using fucoidan fractions and phloroglucinol. Glucose, mannose and galactose in brown seaweed belong to reducing sugar groups and they are very essential compounds that can be converted into valuable intermediate products.

The highest reducingsugar was achieved in 180 ^{o}C / 15 barhydrolysis temperature and pressurewithcatalyst of 524.14 ± 0.11 mg/L. *S. honeri* is composes of oligosaccharide, fat, protein, mineral and other compounds inside. Moreover, the presence of other components in subcritical water hydrolysis affects the decomposition rate of carbohydrate (Kruse et al., 2007).

Reducing sugar at 180 ^{o}C / 15 bar with formic acid shown higher values than other experiment conditions (0.12g / L). When the higher temperature and pressure was applied into this process, the recovery of reducing sugar in hydrolysate water shown a decreasing trend. It mean that the reducing sugar substances from hydrolysate water were not stable in the high temperature.

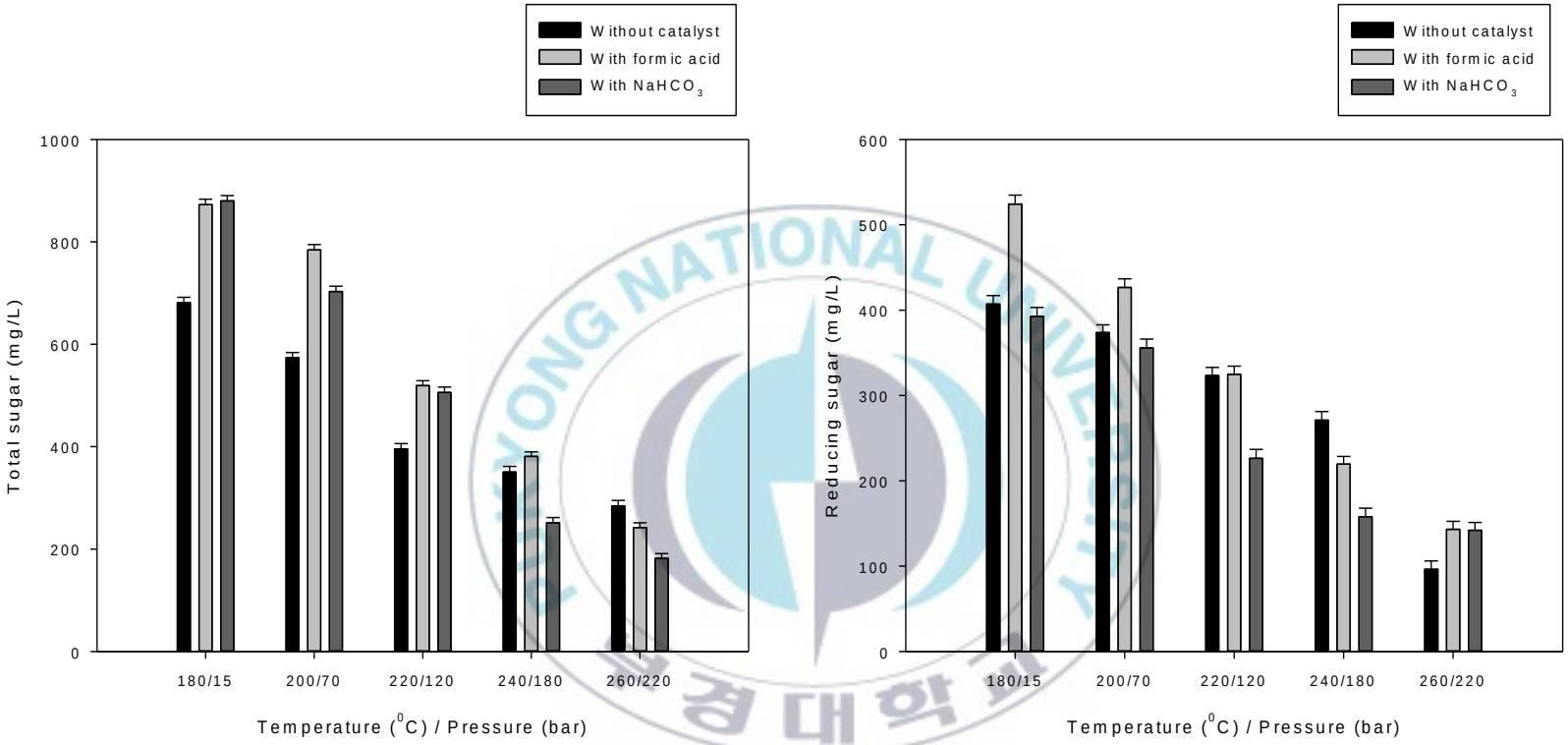


Fig. 13. Sugar content (mg/L) of *Sargassum honeri* hydrolysate samples on different conditions (A) Total sugar; (B) Reducing sugar. Error bars represent standard deviation with three replicates

6.2. Total organic carbon

Total organic carbon in the hydrolysate water was determined to evaluate carbon recovery in the liquid phase. The decrease of carbon recovery indicated that hydrothermal treatment produced carbon products that were not soluble in liquid phase. Higher temperature and pressurewas used, lower total organic carbon was obtained. Based on the data described on Table 6, the addition of catalyst in this process gives the significant results on carbon recovery in liquid phase.

Amount of total carbon in each condition describe rate of decomposition of oligosaccharides in *S. honeri*. When the lowest total carbon was obtained in a condition, it means that the highest decomposition rate was reached in the condition.In this study, the lowest total carbon was obtained at 180 ^{o}C / 15 bar hydrolysis temperature and pressure, and it shown the decreasing trend when higher hydrolysis temperature and pressure were applied. Decomposition of carbohydrate into oligosaccharides and monosaccharides were found to be increased in the hydrolysis condition with temperature and pressure more than 240 ^{o}C / 180 bar.

Table 6. The total amount of organic carbon contained of *Sargassum honeri* hydrolysate samples on different conditions

(Unit: mg/L)

Reaction condition	**Catalyst**		
Temperature (°C)/ Pressure (bar)	**Without catalyst**	**Formic acid**	**$NaHCO_3$**
180/15	61.52 ± 0.05[1)]	101.31 ± 0.03	75.12 ± 0.02
200/70	58.02 ± 0.04	92.47 ± 0.02	74.44 ± 0.05
220/120	56.72 ± 0.05	91.45 ± 0.04	72.33 ± 0.03
240/180	46.89 ± 0.03	83.15 ± 0.05	68.09 ± 0.05
260/220	42.76 ± 0.02	73.19 ± 0.03	66.31 ± 0.02

[1)] Error bars represent standard deviation with three replicates

6.3. Amino acids

Protein in the *S. honeri* was decomposed in hydrolysate water conveted to water-soluble amino acids. Amino acid in hydrolysate at different temperatures and pressures are shown in Table 7, 8 and 9.

In this study, twenty-one indentified amino acids were found in hydrolyzates. Alanine (6.093 ± 0.011mg/100g) was the highest yield among the indentified amino acids without catalyst at 220 ^{o}C / 120 bar, and the same condition also were found hightest yield among indentified amino acids 9.522 ± 0.021 and 21.377 ± 0.023 mg / 100g with formic acid and $NaHCO_3$, respectively. The highest total content of amino acids was 55.097 ± 0.017 mg / 100g at 260 ℃ / 220 bar with $NaHCO_3$ than other experimental operational conditions, and with catalyst total contents generally were reduced with increasing temperature. This was considered due to decomposition of amino acids at high temperature and with catalyst. The use of catalyst enhanced the hydrolysis power and shown lower recovery yield of amino acids at same temperature. This study has shown the feasibility of recovering amino acids from *S. honeri* using subcritical water hydrolysis.

Seven essential amino acids were found in hydrolyzates when the hydrolyzate without catalyst. Among them valine was highest with 1.849 ± 0.011 mg / 100g at 260 ^{o}C / 220 bar. All essential amino acids concentration was decreased due to increase the temperature and pressure except valine, phenylalanine and lysine.

Eight essential amino acids were found in hydrolyzates when the hydrolyzate with formic acid. Among them valine was highest with 3.627 ± 0.017 mg / 100 g at 220 ^{o}C / 120 bar. All essential amino acids concentration was decreased due to increase the temperature and pressure except tryptophan, and lysine. Nonetheless, all the eight essential amino acids were found highest amount at 220 ^{o}C /120 bar.

Eight essential amino acids were found in hydrolyzates when the hydrolyzate with $NaHCO_3$. Among them valine was highest with 5.188 ± 0.018 mg / 100g at 260 ^{o}C / 220 bar. All essential amino acids concentration was increased due to increase the temperature and pressure and all the eight essential amino acids were found highest amount at 260 ^{o}C /220 bar without histidine.

Essential amino acids can not be produced by the body and it should be derived from food. Without these, body can not maintain proper metabolism as a result cause many serious physiological problem and in some extreme cases, cause death. *S. honeri* hydrolyzate contains eight essential amino acids which can meet nutritional value for human body metabolism. Most of the essential amino acids content was found at temperature 180 ^{o}C and pressure 15 bar without catalyst and with formic acid compare to other experimental operational conditions. By this condition, protein break down was almost completely occurred and produced more amino acids. Cheng et al. (2008) and Uddin et al. (2010) reported that most of amino acids gave maximum yield at the reaction temperature range of 180 ^{o}C to 220 ^{o}C and 200 ^{o}C to 290 ^{o}C. It

may be differ due to the different sample sources, processing and experimental operational conditions.

However, many of the essential amino acids content were found higher amount at temperature 260 ^{o}C and pressure 220 bar with $NaHCO_3$. Some other works have been carried out in which thermal degradation of amino acids occured at temperature above 250 to 300 ^{o}C depending on the raw protein and corresponding contact time (Yoshida et al., 2003).

Amino acids and protein hydrolyzates are useful as additives in food industries. We had applied short reaction time for pressurized hydrothermal hydrolysis in order to decrease the decomposition of amino acids and got highest amount of amino acid by applied high temperature and pressure (Asaduzzaman et al., 2014). Cheng et al. (2008) reported the same increase of amino acids yield with the increase in temperature to a certain degree.

Protein usually has a low solubility in water at ambient temperature and pressure, and strong aggregation occurs through hydrophobic interactions. However, the solubility of new material, such as protein in water, increases at higher temperatures and pressures. In addition, at high temperatures and pressures, the hydrolysis yields increasesdue to an increase in the water ionization constant. A similar result was reported by Park et al. (2012) for subcritical water hydrolysis of *Laminaria japonica* (Asaduzzaman et al., 2014).

Table 7. Amino acids yield of *Sargassum honeri* hydrolysate samples on different conditions without catalyst (mg / 100 g)

Amino acid composition	Temperature (°C)/Pressure (bar)				
	180/15	200/70	220/120	240/180	260/220
Phosphoserine	0.652 ±0.003[a] [1)]	0.488 ±0.004[a]	0.318 ±0.003[a]	0.271 ±0.005[b]	0.221 ±0.005[b]
Taurine	0.416 ±0.004[a]	0.384 ±0.003[a]	0.366 ±0.006[a]	0.361 ±0.004[b]	0.358 ±0.004[b]
Aspartic acid	7.298 ±0.009[a]	4.745 ±0.004[a]	2.192 ±0.012[a]	1.531 ±0.007[b]	0.866 ±0.005[b]
Threonine	0.624 ±0.004[a]	0.397 ±0.007[a]	0.138 ±0.004[a]	0.154 ±0.004[a]	0.173 ±0.003[a]
Serine	1.345 ±0.004[a]	1.098 ±0.005[a]	0.851 ±0.003[a]	0.425 ±0.005[b]	0.112 ±0.004[a]
Asparagine	1.023 ±0.006[a]	0.685 ±0.004[a]	0.347 ±0.004[a]	0.174 ±0.006[b]	ND [2)]
Glutamic acid	1.206 ±0.003[a]	0.956 ±0.004[a]	0.652 ±0.004[a]	0.546 ±0.005[b]	0.441 ±0.003[b]
Proline	1.431 ±0.008[b]	1.697 ±0.009[c]	1.961 ±0.011[b]	1.628±0.012[a]	1.295 ±0.007[b]
Glycine	4.578 ±0.014[a]	4.811 ±0.012[b]	5.044 ±0.015[b]	4.485 ±0.015[a]	3.926 ±0.025[b]
Alanine	5.647 ±0.018[a]	5.871 ±0.012[b]	6.093 ±0.011[a]	5.528 ±0.011[a]	4.962 ±0.021[c]
α-aminobutyric acid	0.075 ±0.004[a]	0.168 ±0.003[a]	0.261 ±0.004[a]	0.368 ±0.003[b]	0.474 ±0.005[b]
Valine	1.201 ±0.015[b]	1.434 ±0.014[c]	1.667 ±0.018[c]	1.758 ±0.016[a]	1.849 ±0.011[c]
Methionine	0.312 ±0.003[a]	0.151 ±0.004[b]	ND	ND	ND
Cystathionine	ND	0.781 ±0.006[b]	1.532 ±0.007[b]	2.524 ±0.006[c]	3.517 ±0.004[d]
Leucine	0.508 ±0.004[a]	0.638 ±0.011[c]	0.767 ±0.004[a]	0.384 ±0.015[b]	ND

Tyrosine	1.308 ± 0.016[a]	1.904 ± 0.011[b]	2.496 ± 0.014[a]	2.857 ± 0.008[c]	3.207 ± 0.013[c]
Phenylalanine	0.418 ± 0.007[a]	0.633 ± 0.011[a]	0.847 ± 0.007[a]	0.728 ± 0.010[b]	0.609 ± 0.008[b]
β-alanine	0.332 ± 0.004[a]	0.458 ± 0.005[a]	0.587 ± 0.003[a]	0.531 ± 0.004[a]	0.477 ± 0.003[a]
β-aminoisobutyric acid	0.034 ± 0.004[a]	0.032 ± 0.003[a]	0.031 ± 0.004[a]	0.016 ± 0.003[b]	ND
Histidine	0.047 ± 0.003[a]	0.044 ± 0.003[a]	0.041 ± 0.004[a]	0.036 ± 0.003[a]	0.031 ± 0.003[a]
3-methylhistidine	0.361 ± 0.005[a]	0.482 ± 0.004[a]	0.603 ± 0.007[a]	0.443 ± 0.006[b]	0.281 ± 0.004[b]
Hydroxylysine	0.602 ± 0.003[b]	0.304 ± 0.004[a]	0.013 ± 0.004[b]	0.006 ± 0.001	0.002 ±0.001[a]
Ornithine	0.038 ± 0.005[b]	0.071 ± 0.005[b]	0.103 ± 0.003[a]	0.339 ± 0.006[a]	0.575 ± 0.004[b]
Lysine	0.171 ± 0.004[a]	0.069 ± 0.005[a]	0.103 ± 0.004[a]	0.586 ± 0.007[b]	1.069 ± 0.009[b]
Ethanolamine	0.095 ± 0.005[a]	0.199 ± 0.004[b]	0.303 ± 0.007[a]	0.163 ± 0.004[c]	0.217 ± 0.008[d]
Arginine	0.224 ± 0.004[a]	0.255 ± 0.004[a]	0.286 ± 0.006[a]	0.143 ± 0.003[b]	ND

[2)] ND, no detect. [1)] Error bars represent standard deviation with three replicates. Different small letters in each row indicate significant differences ($P < 0.05$).

Table 8. Amino acids yield of *Sargassum honeri* hydrolysate samples on different conditions with formic acid (mg / 100 g)

Amino acid composition	Temperature (°C)/Pressure (bar)				
	180/15	200/70	220/120	240/180	260/220
Phosphoserine	ND [1)]	0.176 ± 0.003[a] [2)]	0.352 ± 0.004[a]	0.211 ± 0.004[b]	0.071 ± 0.005[b]
Taurine	2.127 ± 0.008[a]	1.233 ± 0.004[a]	0.292 ± 0.005[a]	0.253 ± 0.005[b]	0.215 ± 0.003[b]
Phosphoethanolamine	0.401 ± 0.005[a]	0.293 ± 0.003[a]	0.185 ± 0.004[a]	0.135 ± 0.004[b]	0.084 ± 0.004[b]
Aspartic acid	7.826 ± 0.011[a]	4.391 ± 0.010[a]	0.954 ± 0.004[a]	0.771 ± 0.005[b]	0.586 ± 0.004[b]
Threonine	1.129 ± 0.099[a]	0.731 ± 0.005[a]	0.333 ± 0.005[a]	0.171 ± 0.003[a]	ND
Serine	1.426 ± 0.011[a]	0.975 ± 0.008[a]	0.523 ± 0.007[a]	0.263 ± 0.005[b]	ND
Aspartic acid	ND	0.175 ± 0.003[a]	0.336 ± 0.004[a]	0.185 ± 0.004[b]	ND
Glutamic acid	2.386 ± 0.018[a]	2.696 ± 0.012[a]	3.005 ± 0.014[a]	2.084 ± 0.015[b]	1.158 ± 0.013[b]
Proline	2.531 ± 0.018[b]	2.535 ± 0.017[c]	2.541 ± 0.015[a]	2.064 ± 0.014[b]	1.588 ± 0.014[b]
Glycine	3.382 ± 0.019[a]	5081 ± 0.015[b]	6.789 ± 0.025[b]	5.355 ± 0.017[a]	3.916 ± 0.018[b]
Alanine	7.975 ± 0.023[a]	8.749 ± 0.019[b]	9.522 ± 0.021[a]	6.587 ± 0.023[a]	3.651 ± 0.017[c]
α-aminobutyric acid	0.139 ± 0.003[a]	0.361 ± 0.005[a]	0.581 ± 0.007[a]	0.438 ± 0.007[b]	0.296 ± 0.006[b]
Valine	1.979 ± 0.012[b]	2.503 ± 0.013[c]	3.627 ± 0.017[c]	1.758 ± 0.016[a]	2.788 ± 0.015[c]
Cysteine	0.077 ± 0.003[a]	0.037 ± 0.003[a]	ND	ND	ND
Methionine	0.658 ± 0.007[a]	0.847 ± 0.003[b]	1.036 ± 0.008[a]	0.721 ± 0.005[a]	0.405 ± 0.006[d]
Cystathionine	ND	1.182 ± 0.011[b]	2.363 ± 0.017[b]	2.197 ± 0.014[c]	2.032 ± 0.014[d]

Leucine	1.269 ± 0.008[a]	1.615 ± 0.012[c]	1.961 ± 0.015[a]	1.295 ± 0.014[b]	0.628 ± 0.005[b]
Tyrosine	2.999 ± 0.018[a]	4.384 ± 0.017[b]	5.768 ± 0.024[a]	4.119 ± 0.017[c]	2.471 ± 0.015[c]
Phenylalanine	0.844 ± 0.005[a]	1.281 ± 0.012[a]	1.717 ± 0.014[a]	1.176 ± 0.011[b]	0.634 ± 0.005[b]
β-alanine	0.581 ± 0.005[a]	0.874 ± 0.007[a]	1.166 ± 0.011[a]	0.828 ± 0.008[a]	0.489 ± 0.007[a]
β-aminoisobutyric acid	0.038 ± 0.003[a]	0.032 ± 0.003[a]	0.027 ± 0.003[a]	0.023 ± 0.001	0.021 ± 0.001
γ-amino-n-butyric acid	ND	0.167 ± 0.003[a]	0.332 ± 0.007[a]	0.149 ± 0.004[a]	ND
Histidine	0.296 ± 0.005[a]	0.309 ± 0.006[a]	0.325 ± 0.008[a]	0.187 ± 0.006[a]	0.054 ± 0.003[a]
3-methylhistidine	1.498 ± 0.011[a]	2.027 ± 0.017[a]	2.555 ± 0.015[a]	1.278 ± 0.012[b]	ND
Tryptophan	0.053 ± 0.004[a]	0.494 ± 0.006[a]	0.934 ± 0.013[a]	0.749 ± 0.014[a]	0.655 ± 0.011[a]
Hydroxylysine	0.011 ± 0.005[a]	0.344 ± 0.005[a]	0.658 ± 0.007[a]	0.698 ± 0.007[a]	0.736 ± 0.008[a]
Ornithine	0.029 ± 0.006[b]	0.051 ± 0.004[b]	0.075 ± 0.004[a]	0.269 ± 0.006[a]	0.464 ± 0.006[b]
Lysine	0.247 ± 0.004[a]	0.069 ± 0.005[a]	0.279 ± 0.005[a]	0.586 ± 0.007[b]	0.918 ± 0.007[b]
Ethanolamine	0.028 ± 0.004[a]	0.074 ± 0.005[b]	0.119 ± 0.005[a]	0.121 ± 0.004[c]	0.122 ± 0.005[d]
Arginine	1.874 ± 0.014[a]	2.215 ± 0.013[a]	2.555 ± 0.016[a]	1.451 ± 0.013[b]	0.339 ± 0.004[a]

[1)] ND, no detect. [2)] Error bars represent standard deviation with three replicates. Different small letters in each row indicate significant differences ($P < 0.05$).

Table 9. Amino acids yield of *Sargassum honeri* hydrolysate samples on different conditions with $NaHCO_3$ (mg / 100 g)

Amino acid composition	Temperature (°C)/Pressure (bar)				
	180/15	200/70	220/120	240/180	260/220
Phosphoserine	1.915 ±0.013[a] [1)]	0.957 ±0.007[a]	ND [2)]	ND	ND
Taurine	0.534 ±0.006[a]	1.416 ±0.013[a]	2.297 ±0.016[a]	1.707 ±0.015[b]	1.117 ±0.012[b]
Urea	8.731 ±0.021[a]	5.811 ±0.018[a]	2.888 ±0.017[a]	1.444 ±0.014[b]	ND
Aspartic acid	0.363 ±0.003[a]	0.695 ±0.007[a]	1.025 ±0.013[a]	0.817 ±0.004[b]	0.609 ±0.006[b]
Threonine	ND	ND	ND	0.491 ±0.005[a]	0.982 ±0.007[a]
Serine	ND	0.046 ±0.003[a]	0.097 ±0.005[a]	0.035 ±0.004[b]	ND
Glutamic acid	ND	0.125 ±0.003[a]	0.251 ±0.006[a]	0.446 ±0.008[b]	0.642 ±0.007[b]
Sarcocine	ND	1.722 ±0.013[a]	3.443 ±0.014[a]	2.381 ±0.016[b]	1.333 ±0.011[b]
Proline	1.022 ±0.016[b]	2.789 ±0.019[c]	4.556 ±0.015[b]	4.784 ±0.019[a]	5.013 ±0.017[b]
Glycine	1.041 ±0.007[a]	0.521 ±0.005[b]	ND	3.265 ±0.018[a]	6.539 ±0.021[b]
Alanine	0.440 ±0.005[a]	10.681 ±0.022[b]	21.377 ±0.023[a]	17.926 ±0.023[a]	14.475 ±0.021[c]
α-aminobutyric acid	ND	0.277 ±0.004[a]	0.455 ±0.004[a]	0.701 ±0.007[b]	0.945 ±0.007[b]
Valine	0.218 ±0.005[b]	1.649 ±0.013[c]	3.081 ±0.018[c]	4.134 ±0.014[a]	5.188 ±0.018[c]
Cysteine	ND	ND	ND	0.041 ±0.003[a]	0.082 ±0.004[b]
Methionine	0.126 ±0.004[a]	0.064 ±0.003[b]	ND	1.127 ±0.013[a]	2.255 ±0.015[a]
Cystathionine	ND	1.311 ±0.012[b]	2.715 ±0.016[b]	1.294 ±0.011[c]	ND

Leucine	0.117 ± 0.003[a]	0.787 ± 0.009[c]	1.478 ± 0.012[a]	1.573 ± 0.011[b]	1.669 ± 0.015[a]
Tyrosine	0.342 ± 0.003[a]	2.665 ± 0.017[b]	5.004 ± 0.014[a]	6.045 ± 0.016[c]	7.034 ± 0.014[c]
Phenylalanine	0.267 ± 0.004[a]	0.784 ± 0.009[a]	1.304 ± 0.011[a]	1.589 ± 0.010[b]	1.893 ± 0.013[b]
β-alanine	0.156 ± 0.003[a]	0.669 ± 0.007[a]	1.182 ± 0.013[a]	1.472 ± 0.012[a]	1.763 ± 0.015[a]
β-aminoisobutyric acid	0.021 ± 0.003[a]	0.027 ± 0.003[a]	0.033 ± 0.004[a]	0.025 ± 0.003[b]	0.023 ± 0.004[a]
γ-amino-n-butyric acid	0.110 ± 0.005[a]	0.055 ± 0.002[a]	ND	ND	ND
Histidine	0.046 ± 0.003[a]	0.073 ± 0.003[a]	0.091 ± 0.004[a]	0.063 ± 0.003[a]	0.043 ± 0.003[a]
3-methylhistidine	0.121 ± 0.005[a]	0.308 ± 0.004[a]	0.495 ± 0.006[a]	0.543 ± 0.005[b]	0.675 ± 0.004[b]
Tryptophan	ND	0.089 ± 0.003[b]	0.175 ± 0.004[a]	0.324 ± 0.004[a]	0.476 ± 0.006[b]
Hydroxylysine	0.051 ± 0.004[b]	0.041 ± 0.003[a]	0.032 ± 0.003[b]	0.035 ± 0.003[a]	0.043 ± 0.004[a]
Ornithine	0.028 ± 0.003[b]	0.198 ± 0.004[b]	0.369 ± 0.005[a]	0.701 ± 0.004[a]	1.088 ± 0.011[b]
Lysine	ND	0.071 ± 0.003[a]	0.152 ± 0.005[a]	1.131 ± 0.006[b]	1.916 ± 0.012[b]
Ethanolamine	ND	0.028 ± 0.004[b]	0.055 ± 0.008[a]	0.073 ± 0.006[c]	0.104 ± 0.008[d]
Arginine	ND	0.191 ± 0.005[a]	0.365 ± 0.006[a]	0.174 ± 0.003[b]	ND

[2] ND, no detect. [1] Error bars represent standard deviation with three replicates. Different small letters in each row indicate significant differences ($P < 0.05$).

6.4. Antioxidant activity

Antioxidant properties of hydrolysate water was conducted using some antioxidant assays, i.e. total phenolic content (TPC), total flavonoid content (TFC), DPPH radical-scavenging activity and ABTS radical-scavenging activity. TPC and TFC describe the total amount of phenolic and flavonoid, respectively, contained in hydrolysate water. DPPH and ABTS radical scavenging activities measure the ability of a substance to prevent free radical molecules.

Based on Fig. 14 and 15, the antioxidant properties were higher in the experiments with catalyst added. The best temperature and pressure to gain the best antioxidant properties was found at 260 ^{o}C / 220 bar. In the treatment of *S. honeri* hydrolysate samples, the highest total phenolic, flavonoid, DPPH and ABTS were found in the treatments with formic acid addition. They were 552.87 ± 0.11 ug/L, 200.84 ± 0.15 ug/L, 20.05 ± 0.05 % and 94.05 ± 0.08 %, respectively.

Antioxidant properties of hydrolysate water were affected by bioactive compounds such as phenolic, flavonoid, minerals and others. The condition of growth environment, harvest time and storage condition also give huge effects to the amount of those compounds (Roh et al., 2008). The addition of formic acid as catalyst had proven to raise the antioxidant properties of hydrolysate water. Addition of acid as catalyst in hydrolysis process produced new properties of water such as variation in density, polarity and solubility. Furthermore, the presences of acid gave more protection of antioxidant

compounds inside from oxidation and damage and also changed the solubility and density of water.

Most of antioxidant compounds found in brown seaweed belong to the group of polar compounds. As we know the general principle in solvent extraction of “like dissolves like” means that suitable solvents only dissolve suitable substances which have similar polarity with the solvents used. Hence, those antioxidant compounds were dissolved in a presence of water as a main solvent. Higher temperature used in this process was increased antioxidant properties of compounds. The antioxidant ability of hydrolysate water was stable at high temperature and pressure.

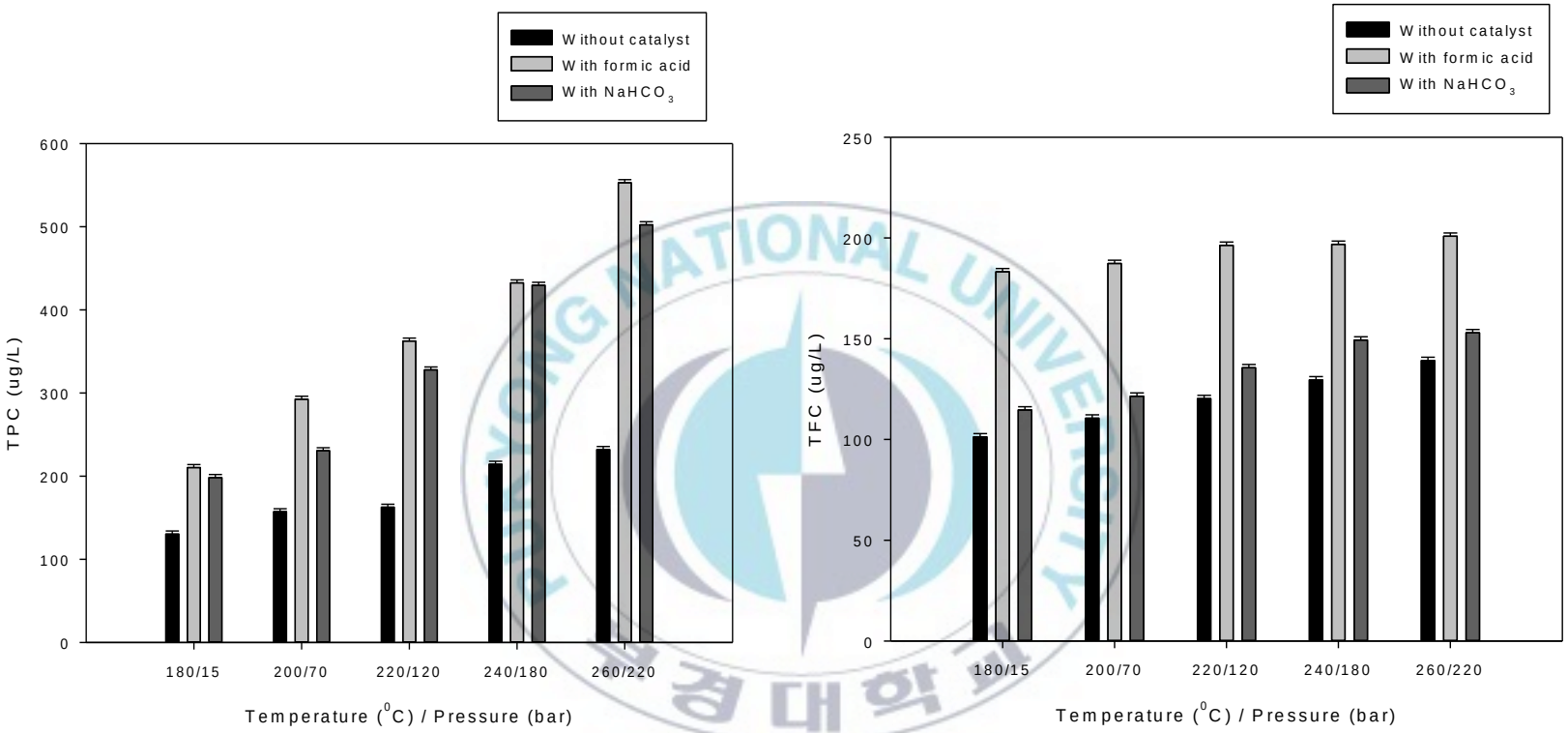


Fig. 14. Antioxidant properties of *Sargassum honeri* hydrolysate samples on different conditions (A) Total phenolic content (TPC); (B) Total flavonoid content (TFC). Error bars represent standard deviation with three replicates

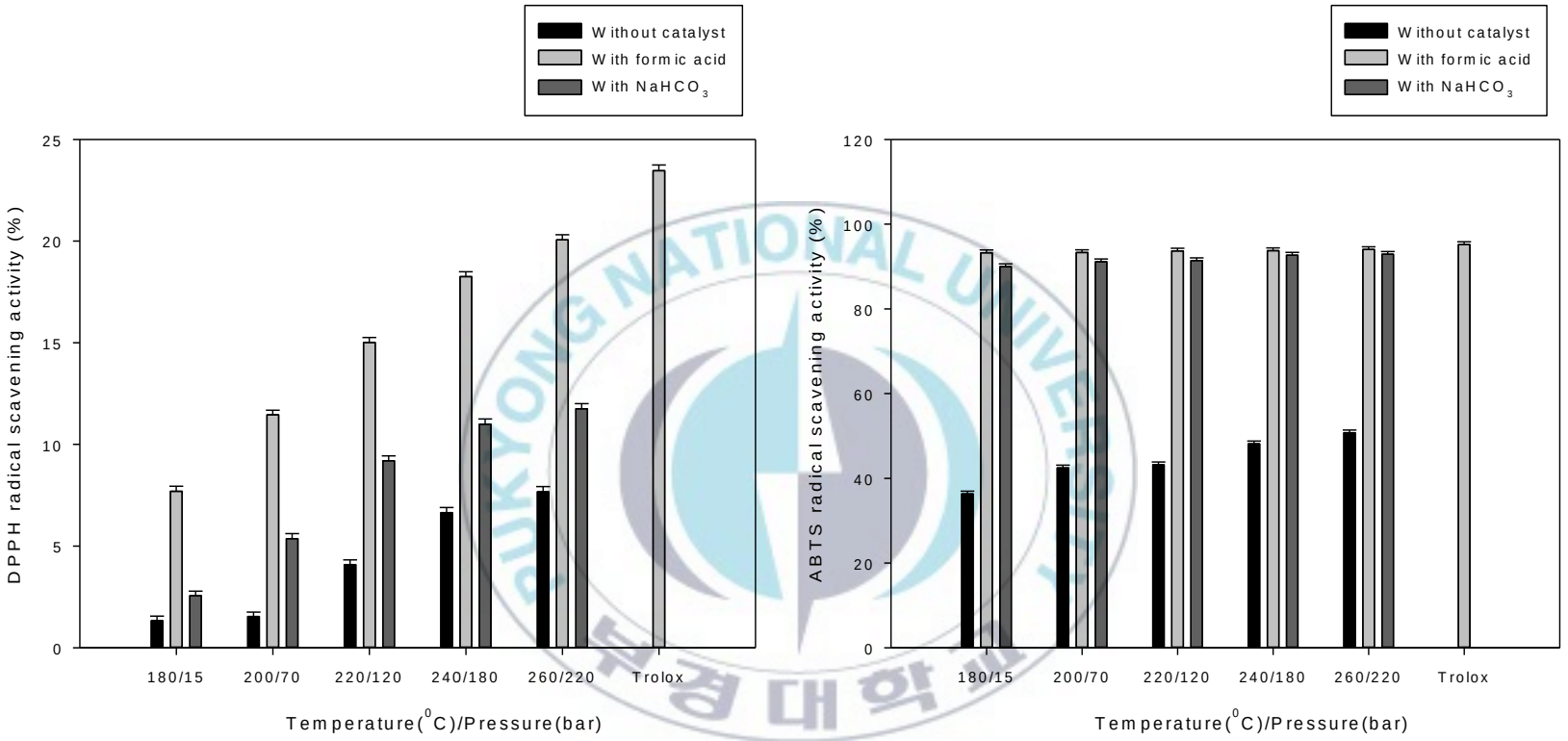


Fig. 15. Antioxidant properties of *Sargassum honeri* hydrolysate samples on different conditions (A) DPPH radical scavenging activity; (B) ABTS radical scavenging activity. Error bars represent standard deviation with three replicates

6.5. Antihypertensive activity

For antihypertensive activity was done according to (Hayakari, 1978) with slight modification. This method is based on the hydrolysis of HHL by ACE to hippuric acid and His-Leu. For this assay, Antihypertensive inhibitory activity is measured through the absorbance of hippuric acid after the reaction of hydrolyzate samples on HHL. The quantity of hippuric acid produced by ACE is measured at 228 nm using a UV-visible spectrophotometer.

ACE inhibitory activity of the *S. honeri* hydrolyzate was measured and calculated as ACE inhibition percentage. The hydrolysate water obtained from *S. honeri* shown inhibition percentage of 58.21 ± 0.14 % at 180 ℃/ 15 bar with formic acid. The ACE inhibitory activity decreased due to increase the temperature and pressure. When compared to other conditions, the 180 ℃/ 15 bar condition shown high inhibitory activity. The standard captopril (10%) showed 95.51 % inhibition percentage showen in Fig. 16. We also confirmed that ACE-I inhibitory activity of the *S. japonica* hydrolyzate to be low activity at 400 ℃ / 400 bar condition report not shown here. So, we conclude subcritical water with lower temperature and pressure condition has high activity. When the *S. honeri* hydrolyzate samples without catalyst changed to with formic acid or with $NaHCO_3$, the ACE inhibitory activity had shown a litte increase without temperature and pressure changing. However the *S. honeri* hydrolyzate samples with formic acid higher than without catalyst and with $NaHCO_3$.

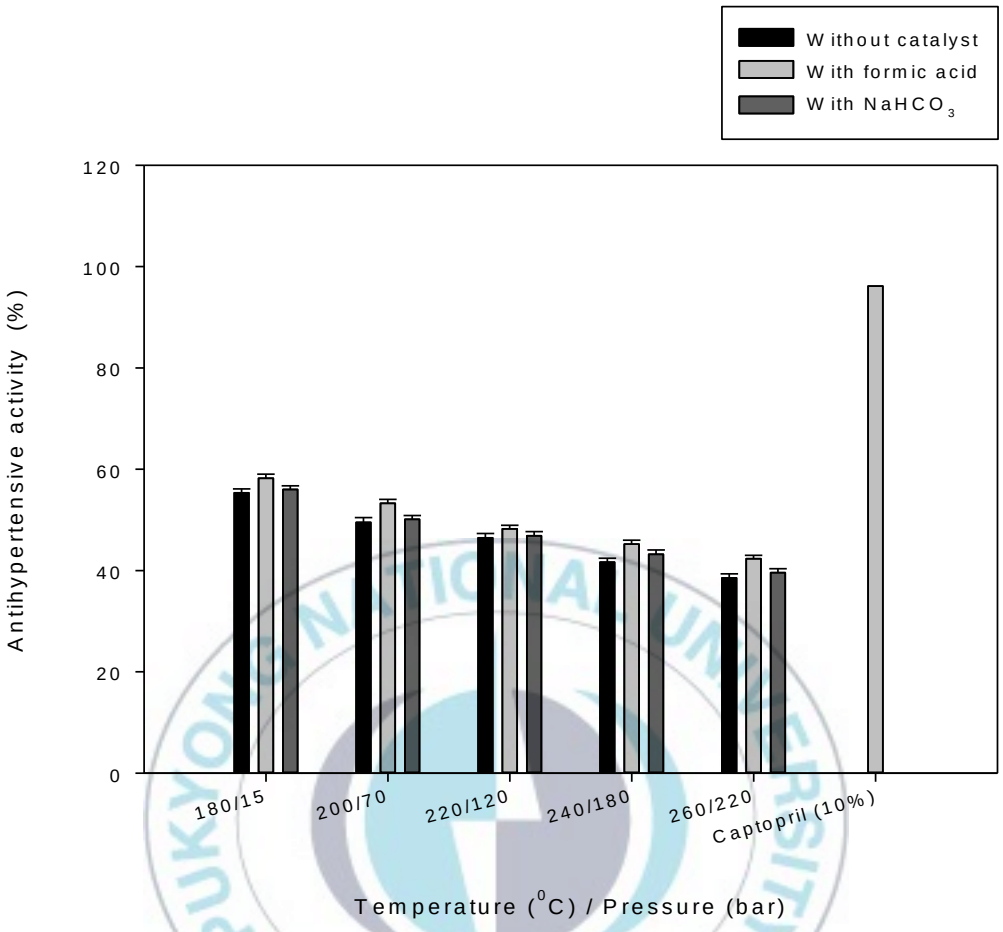


Fig. 16. Antihypertensive activity of *Sargassum honeri* hydrolysate samples on different conditions. Error bars represent standard deviation with three replicates

6.6. Viscosity

Estimation and knowledge of transport properties of fluids are essential for heat and mass flow. Viscosity is one of the important properties which are affected by temperature and pressure (Haj-Kacem et al., 2014). Among the physicochemical properties of binary liquid mixtures required for optimizing and designing industrial processes, it should be mentioned that viscosity is one of the most important factors. In the chemical, cosmetic, food stuff, and pharmaceutical industries etc., viscosity is essential for hydraulic calculations of fluid transport and for energy transference calculations (Ouerfelli et al., 2013 and Herr áez et al., 2008).

The viscosities of *S. honeri* hydrolysate samples on different conditions were shown in Table 10. When the temperature and pressure increase, the viscosities of each condition also increase including with and without catalyst. The highest viscosity was 6.87 ± 0.05 cP with formic acid at 260 ℃ / 220 bar, and high than without catalyst (6.18 ± 0.02 cP) and with $NaHCO_3$ (6.54 ± 0.04 cP) at 260 ℃ / 220 bar.

Table 10. Viscosity of *Sargassum honeri* hydrolysate samples on different conditions

(Unit: cP)

Reaction condition	**Catalyst**		
Temperature (℃)/ Pressure (bar)	**Without catalyst**	**Formic acid**	**$NaHCO_3$**
180/15	3.34 ± 0.02 [1)]	3.71 ± 0.04	3.53 ± 0.02
200/70	4.53 ± 0.03	4.93 ± 0.02	4.86 ± 0.03
220/120	4.74 ± 0.06	5.25 ± 0.03	5.14 ± 0.05
240/180	5.43 ± 0.05	6.16 ± 0.04	5.83 ± 0.03
260/220	6.18 ± 0.02	6.87 ± 0.05	6.54 ± 0.04

[1)] Error bars represent standard deviation with three replicates

6.7. pH

Effect of pH to antioxidant properties (TPC, TFC, DPPH and ABTS) and sugar recovery (total sugar and reducing sugar) were analyzed on hydrolysate water with 1% formic acid and 1% $NaHCO_3$ addition. The pH values of *S. honeri* without catalyst (5.31-6.91) and with 1% formic acid (2.99-4.68) were found to increase as temperature and pressure was elevated, but the pH values of *S. honeri* with 1% $NaHCO_3$ (8.28-7.41) was found to decrease as temperature and pressure was elevated (Fig. 17). Antioxidant properties of *S. honeri* showed the same effect against pH values obtained without catalyst and with 1% formic acid. When pH value was raised, the antioxidant properties showed higher. When the 1% $NaHCO_3$ was added into the hydrolysis, antioxidant properties shown reverse effect against pH values obtained. When pH value was raised, the antioxidant properties showed lower. Lower pH values or higher acidity levels of hydrolysate water were proven to give the best protection for antioxidant compounds inside. The same things were described by Ragan and Glombitza (1986) and Santos-Buelga and Williamson (2003).

Sugar recovery was monitored as a factor affected by pH and temperature. The higher temperature and pressure were applied, lower sugar was obtained. Basically, temperature, pressure and the chemical interaction between a given component and the water molecule determine the solubility of the component in water.In subcritical water state, raising the temperature and pressure causes significant changes in the properties of water. The properties of water, as a

solvent, vary owing to variation of its dielectric constant, conductivity, ionic product and the structure of the hydrogen bond network (Galkin and Lunin, 2005).

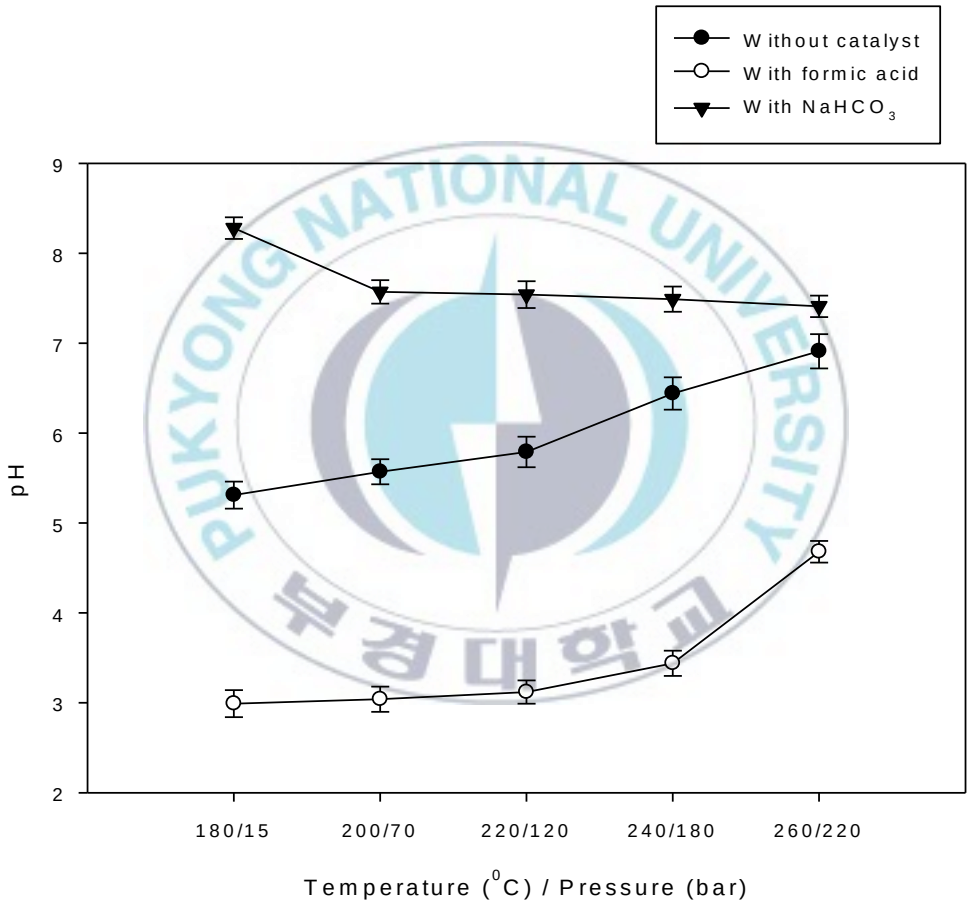


Fig.17. The pH value of *Sargassum honeri* hydrolysate samples on different conditions. Error bars represent standard deviation with three replicates

6.7. Color

Hunter L, a, b and CIE 1976 L*a*b* (CIELAB) are both color scales based on the Opponent-Color Theory. This theory assumes that the receptors in thehuman eye perceive color as the following pair's of opposites.The color of *S. honeri* hydrolysate samples on different conditions shown in Table 11.

In the L* scale, the hydrolysate show the simaler color in the three conditions including without catalyst, with formic acid and with $NaHCO_3$. We found the highest temperature and pressure with 260 ^{o}C / 220 bar shown the lowest L* number, it means more high temperatue and pressure more dark.

In the a* scale, although the hydrolysate samples color were simaler, red color decreased due to increase the temperature and pressure. When the catalyst changed to $NaHCO_3$, red color would become fainter than without catalyst and with formic acid.

In the b* scale, the simaler situation with a* scale, yellow color decreased due to increase the temperature and pressure. The blue color came when the catalyst changed to $NaHCO_3$.

Table 11. Color of *Sargassum honeri* hydrolysate samples on different conditions

Catalyst	Temperature (°C)/ Pressure (bar)	L*	a*	b*
Without catalyst	180/15	29.27 ± 1.02 [1)]	+3.44 ± 0.21	+1.91 ± 0.08
	200/70	32.77 ± 0.91	+3.93 ± 0.16	+2.22 ± 0.07
	220/120	30.68 ± 1.01	+4.00 ± 0.17	+2.34 ± 0.11
	240/180	29.38 ± 0.85	+3.82 ± 0.15	+2.09 ± 0.12
	260/220	28.97 ± 0.92	+3.20 ± 0.11	+1.36 ± 0.09
Formic acid	180/15	31.39 ± 0.74	+3.15 ± 0.18	+4.28 ± 0.11
	200/70	32.61 ± 0.85	+4.14 ± 0.19	+5.67 ± 0.13
	220/120	30.39 ± 0.84	+3.83 ± 0.21	+6.44 ± 0.12
	240/180	27.56 ± 0.93	+3.92 ± 0.18	+5.29 ± 0.11
	260/220	23.17 ± 1.01	+2.48 ± 0.14	+3.86 ± 0.09
$NaHCO_3$	180/15	29.66 ± 1.02	-0.04 ± 0.02	-0.53 ± 0.08
	200/70	30.06 ± 0.82	0.00 ± 0.03	-0.55 ± 0.05
	220/120	28.72 ± 0.61	+0.11 ± 0.09	-0.39 ± 0.07
	240/180	26.21 ± 0.77	+0.62 ± 0.12	-0.38 ± 0.06
	260/220	26.16 ± 0.92	+0.50 ± 0.13	-0.32 ± 0.05

[1)] Error bars represent standard deviation with three replicates

Conclusions

Brown seaweed (*Sargassum honeri)* consist of complex structural bonds. The utilization is in limited field. The certain technology is needed to modify the structure. Subcritical water and supercritical carbon dioxide have gained increasing attention as environment-friendly solvent and widely used to recover useful materials from natural substances in order to break through these limitations. *S. honeri* of natural substances provides an excellent source of bioactive compounds, such as a carotenoid, dietary fiber, protein, vitamins, essential fatty acids, and phenolic compounds which have an important role in human well being.

The aim of this study was to recover useful materials such as fatty acids, fucoxanthin, polyphenols, amino acid, total suagr, reducing sugars and total organic carbon from *S. honeri* using SC-CO_2 extraction and subcritical water hydrolysis. The yields obtained by SC-CO_2 extraction or subcritical water hydrolysis on producing useful materials were also compared to those obtained by organic solvent extraction.

The extraction by SC-CO_2 with ethanol as co-solvent was successfully performed and the content of fucoxanthin, oil stability, antixioidant and antihypertensive activity were higher than that extracted by conventional solvent extraction method. Analysis of fatty acids in *S. honeri* oil obtained by SC-CO_2 extraction also was carried out by GC-FID. The major fatty acids in oil of *S. honeri* components were palmitic acid, heicosanoic acid, EPA, oleic

acidand myristic acid.

The highest yield of hydrolysis of *S. honeri* by subcritical water hydrolysis increased with increasing temperature and pressure. Useful materials such as flavonoid, phenolic compounds, reducing sugars and total organic carbon, amino acid and antihypertensive activity, were successfully recovered.

Total glucose and reducing sugar were measured to give the information about the probability of these comodities which can be changed into renewable bioenergy resources. The highest yields of both total sugars and reducing sugars were found at low temperature and pressure. Moreover, the best condition was reached in low temperature and pressure of 180 ^{o}C / 15 bar.

Total organic carbon shown the potential of hydrolysis treatment to break down complex structure into the simpler one. The decrease of carbon recovery indicated that hydrothermal treatment produced carbon products that were not soluble in liquid phase. Hydrolysis treatment at the elevated temperature was proven to give more influence in depolymerization.

In terms of amino acids, subcritical water hydrolysis process has been successfully applied for production of them from seaweed. The highest yields of amino acids were found at low temperature and pressure. The amounts of amino acids in seaweed tend to decrease with increasing temperature and pressure.

The anti-ACE activities of the extracts from some marine red algae were remarkable, compared to the purified and fractionated peptides from marine organisms (Suetsuna and Nakano, 2000). This suggests that there are excellent

potential ACE like-inhibitors derived from marine red algae. In our previous study, green and brown algae indicated good anti-ACE activity, especially in 70 ME of some brown algae. We concluded marine algae might have good ACE like inhibitors that are associated with not only proteins but also fucoxanthin, phlorotannin and polyphenolic compounds.

These results showed that SC-CO_2 extraction and subcritical water hydrolysis were effective for the production of useful materials from *S. honeri*.

References

Alenezi R., Leeke G.A., Santos R.C.D. and Khan A.R., Hydrolysis of sunflower oil under subcritical water conditions, *Chemical Engineering Research and Design*, 87, p. 867-873, (2009).

Alstyne Van K.L., Comparison of three methods for quantifying brown algal polyphenolic compounds, *Journal of Chemical Ecology*, 21, p. 45-58, (1994).

American Oil Chemists' Society, Official Methods of Analysis. 5th ed. *American Oil Chemists' Society (AOCS)*, Champaign, IL, US, (1998).

AOAC International, *Association of Official Analytical Chemists*, Official Methods of Analysis of AOAC, Washington DC: AOAC International, (2000).

Asaduzzaman A. K. M. andChun B.S., Hydrolyzates produced from mackerel Scomber japonicus skinby the pressurized hydrothermal process contain amino acidswith antioxidant activities and functionalities, *Journal of Food Science and Technology*, 80, p. 369-380, (2014).

Bhaskar N., Hosokawa M. and Miyashita K., Growth inhibition of human pro-myelocyticleukemia (HL-60) cells by lipid extracts of marine algae sargassummarginatum (Fucales, Phaeophyta) harvested off Goa (west coast of indian) with special reference to fatty acid composition, *Indian*

Jouenal of Marine Science., 33 (4), p. 355-360, (2004).

Blunt J.W., Copp B.R., Munro M.H.G., Northcote P.T. and Prinsep M.R., Marine natural products, *Natural Product Reports*, 28, p. 196-268, (2011).

ByunH.G. and KimS.K., Purification and characterization of angiotensin I converting enzyme inhibitory peptides from Alaska pollack (Theragra chalcogramma) skin, *Process Biochemistry*, 36, p. 1155–1162, (2001).

Cai Y.Z., Sun M., Xing J., Luo Q. and Corke H., Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medical plants, *Life Science*, 78(5), p. 2872-2888, (2006).

Calder P.C. and Grimble R.F., Polyunsaturated fatty acids, inflammation and immunity, *Journal of Clinical Nutrition*, 56, p. 14-19, (2002).

Cheng H., Zhu X., Zhu C., Qian J., Zhu N., Zhao L. and Chen J., Hydrolysis technology of biomass waste to produce amino acids in subcritical water, *Journal of Bioresource Technology*, 99, p. 3337-3341, (2008).

Cho J.Y., Antifouling chromanols isolated from brown alga *Sargassum horneri*, *Journal of Applied Phycology*, 25, p. 299-309, (2012).

D-A-C-H, Referenzwertefür die Nährstoffzufuhr. Umschau/Braus GmbH Frankfurtam Main, *Auflage*, 1, (2000).

Daneshvar S., Salak F., Ishii T and Otsuka K., Application of subcritical water for conversion of macroalgaeto value-added materials, *Industrial*

Engineerin Chemistry Research, 51, p. 77–84, (2012).

Darcy-Vrillon B., Nutritional aspects of the developing use of marine macroalgae for the human food industry, *International Journal of Food Sciences and Nutrition*, 44, p. 23-35, (1993).

Dawczynski C., Schubert R. and Jahreis G., Amino acids, fatty acids, and dietary fibre in edible seaweed products, *Food Chemistry*, 103, p. 891-899, (2007).

De Pablo M. and Alvarez de Cienfuegos G., Modulatory effects of dietary lipids on immune system functions, *Immunology and Cell Biology*, 78, p. 31-39, (2000).

Dembitsky V.M., Gloriozova T.A. and Poroikov V.V., Natural peroxy anticancer agents, *Mini - Reviews in Medicinal Chemistry*, 7(6), p. 571-589, (2007).

Demibitsky V.M., Rozentsyet O.A. and Pechenkina E.E., Glycolipids, phospholipids and fatty acids of brown algae species, *Phytochemistry*, 29(11), p. 3417-3421, (1990).

Draget K.I., Smidsrod O. and Skjak-Braek K., Alginates from algae, In polysaccharides and polyamides in the food industry, properties, production and patents., ed. A steinbuchel, SK Rhee:1-30, Weinheim, Wiley-VCH Verlag GmbH & Co. Number of 1-30, (2005).

DuBois M., Gilles K.A., Hamilton J.K., Rebers P.A. and Smith F., Colorimetric method for determination of sugars and related substances, *Analytical Chemistry*, 28 (3), p. 350–356, (1956).

Emmons C.L. and Peterson D.M., Crop breeding, genetics & cytology; antioxidant activity and phenolic content of oats as affected by cultivar and location, *Crop Science Society of America*, 41, p. 1676-1681, (2001).

Fitton J.H., Irhimeh M. and Falk N., Macroalgalfucoidan extracts: new opportunity for marine cosmetics, *Cosmetics and Toiletries Magazine*, 122 (8), p. 55-64, (2007).

Fleurence J., Gutbier G., Mabeau S. and Leray C., Fatty acids from 11 marine macroalgae of the French Brittany coast, *Journal of Applied Phycology*, 6, p. 527-532, (1994).

Galkin A.A. and Lunin V.V., Subcritical and supercritical water: a universal medium for chemical reactions, *Russian Chemical Reviews*, 74(1), p. 21-35, (2005).

Go H., Hwang H.J. and Nam T.J., A glycoprotein from *Laminaria japonica* induces apoptosis in HT-29 colon cancer cells, *Toxicology in Vitro*, 24, p.1546–1553, (2010).

Goretta L.A., Ottaviani J.I. and Fraga C.G., Inhibition of angiotensin converting enzyme activity by flavanol-rich foods, *Journal of Agricultural*

and Food Chemistry, 54, p. 229–234, (2006).

Graham L.E., Graham J.M. and Wilcox L.W., Algae Second Edition, Pearson, (2009).

Guimarães C.M., Gião M.S., Martinez S.S., Pintado A.I., Pintado M.E., Bento L.S. and Malcata F.X., Antioxidant activity of sugar molasses, including protective effect against DNA oxidative damage, *Journal of Food Sciences*, 72(1), p. 39-43, (2007).

Haugan J.A., Akermann T. and Liaaen-Jensen S., Isolation of fucoxanthin and piridinin, In: Packer L (ed) Carotenoids part A: Chemistry, separation, quatitation and antioxidants, Methods in Enzymology, *Academic Press Inc., Orlando*, 213, p. 231-245, (1992).

Herráez J.V., Belda R., Diez O. and Herráez M., An equation for the correlation of viscosities of binary mixtures, *Journal of Solution Chemistry*, 37, p. 233–248, (2008).

Hung A. and Jensen A., Seasonal variations in the chemical compositions of Alariaesculenta, Laminariasaccharina, Laminaria hyperborean and Laminariadigitata from northern Norway, In: Report No. 4, *NorwInst Seaweed Research*, Department of Biotechnology, (1954).

Hwang J.S., Impact of processing on stability of angiotensin I-converting enzyme (ACE) inhibitory peptides obtained from tuna cooking juice, *Food*

Research International, 43, p. 902–906, (2010).

Peng J., Yuan J.P., Wu C.F. and Wang J.H., Fucoxanthin, a marine carotenoid present in seaweeds and diatoms: Metabolism and bioactivities relevant to human health, *Marine Drugs*, 9, p.1806-1828, (2011).

Jang J.S., Cho Y.K., Jeong G.T. and Kim S.K., Optimization of saccharification and ethanol production by simultaneous saccharification and fermentation (SSF) from seaweed, Saccharina japonica, *Bioprocess and Biosystems Engineering*, 35, p. 11-18, (2012).

Je J.Y., Lee K.H., Lee M.H. and Ahn C.B., Antioxidant and antihypertensive protein hydrolysates produced from tuna liver by enzymatic hydrolysis, *Food Research International*, 42, p. 1266–1272, (2009).

Je J.Y., Park P.J., Byun H.G., Jung W.K. and Kim S.K., Angiotensin I converting enzyme (ACE) inhibitory peptide derived from the sauce of fermented blue mussel, Mytilus edulis, *Bioresource Technology*, 96, p. 1624–1629, (2005).

Je Y.J., Park J.Y., Jung W.K. and Kim S.K., Isolation of angiotensin I converting enzyme (ACE) inhibitor from fermented oyster sauce, Crassostrea gigas, *Food Chemistry*, 90, p. 809–814, (2005).

Johns R.B., Nichols P.D. and Perry G.J., Fatty acid composition of ten marine algae from Australia waters, *Phytochemistry*, 18, p. 799-802, (1979).

Jose L. Martinez, Supercritical fluid extraction of nutraceuticals and bioactive compounds, CRC Press, Taylor & Francis Group, Boca Raton, Fla., London, (2008).

Karadeniz F., Burdurlu H.S., Koca N. and Soyer Y., Antioxidant activity of selected fruits and vegetables grown in Turkey, *Turkish Journal of Agriculture and Forestry*, 29, p. 297-303, (2005).

Khan S.B., Qian Z.J., Ryu B.M. and Kim S.K., Isolation and biochemical characterization of collagen from seaweed pipefish, Syngnathus schlegeli, *Biotechnology and Bioprocess Engineering*, 14, p. 436–442, (2009).

Kim S.K. and Pangestuti R., Biological activities and potential health benefits of fucoxanthin derived from marine brown algae, *Advances in Food and Nutrition Research*, 64, p. 111-128, (2011).

Kim S.K. and Wijesekara I., Development and biological activities of marine-derived bioactive peptides: *A review Journal of Functional Foods*, 2, p. 1–9, (2010).

King J.W., Advances in critical fluid technology for food processing, *Food Science and Technology Today*, 14, p. 186-191, (2000).

Koivikko R., Brown algal phlorotannins improving and applying chemical methods, *Dissertation, University of Turku*, (2008).

Korhonen H. and Pihlanto A., Bioactive peptides: Production and functionality,

International Dairy Journal, 16, p. 945–960, (2006).

Kruse A. and Dinjus E., Hot compressed water as reaction medium and reactant: 2. Degradation reactions, *Journal of Supercritical Fluids*, 41 (3), p. 362-380, (2007).

Lang Q. and Wai C.M., Supercritical fluid extraction in herbal and natural product studies—Apractical review, *Talanta*, 53, p. 771–782, (2001).

Lee S.M., Asaduzzaman A.K.M. and Chun B.S., Charecterization of lecithin isolated from anchovy (*Engraulis japonica*) residues deoiled by supercritical carbon dioxide and organic solvent extraction, *Journal of Food Science*, 77, p. 773-778, (2012).

Li H.B., Wong C.C., Cheng K.W. and Chen F., Antioxidant properties in vitro and total phenolic contents in methanol extracts in methanol extracts form medicinal plants, *LWT- Food Science and Technology*, 41(3), p. 385-390, (2008).

Manivannan K., Karthikai D.G., Anantharaman P. and Balasubramanian T., Antimicrobial potential of selected brown seaweeds from Vedalai coastal waters, Gulf of Mannar, *Asian Pacific Journal of Tropical Biomedicine*, 1 (2), p. 114-120, (2011).

McHugh D.J., A guide to the seaweed industry, *FAO Fisheries Technical*, p. 441, (2003).

Meinita M.D.N., Production of bioethanol using red seaweed Kappaphycus alvarezii (cottonii) colected from Indonesia, *Thesis for the degree of doctor engineering, Pukyong National University, South Korea*, (2010).

Miliauskas G., Venskutonis P.R. and Van B.T.A., Screening of radical activity of some medicinal and aromatic plant extracts, *Food Chemistry*, 85 (2), p. 321-327, (2004).

Miller G.L., Use of dinitrosalicylic acid reagent for determination of reducing sugar, *Analytical Chemistry*, 31 (3), p. 426–428, (1959).

Mori K., Ooi T., Hiraoka M., Oka N., Hamada H., Tamura M. and Kusumi T., Fucoxanthin and its metabolites in edible brown algae cultivated in deep seawater, *Marine Drugs*, 2, p. 63-72, (2004).

Nagayama K., Iwamura Y., Shibata T., Hirayama I. and Nakamura T., Bactericidal activity of phlorotannins from the brown alga Eckloniakurome, *Journal of Antimicrobial Chemotherapy*, 50, p. 889-893 (2002).

Noviendri D., Jaswir I., Salleh H.M., Taher M., Miyashita K. and RamLi N., Fucoxanthin extraction and fatty acid analysis of sargassum binderi and s. duplicatum, *Journal of Medicinal Plants Research*, 5(11), p. 2405-2412, (2011).

Ogawa M., Portier R.J., Moody M.W., Bell J., Schexnayder M.A. and Losso

J.N., Biochemical properties of bone and scale collagens isolated from the subtropical fish black drum (*pogonia cromis*) and sheepshead seabream (*Archosargus probatocephalus*), *Food Chemistry*, 88, p. 495-501, (2004).

Ondetti M.A., Design of specific inhibitors of angiotensin-converting enzyme: New class of orally active antihypertensive agents, *Science*, 196, p. 441–444, (1977).

Osman M.E.H., Abushady A.M. and Elshobary M.E., In vitro screening of antimicrobial activity of extracts of some macroalgaecollected from Abu-Qir bay Alexandria, Egypt, *African Journal of Biotechnology*, 12, p. 7203-7208, (2010).

Ouerfelli N.J., Bouaziz M. and Herráez J.V., Treatment of Herráez equation correlating viscosity in binary liquid mixtures exhibiting strictly monotonous distribution, *Physics and Chemistry of Liquids*, 51, p. 55–74, (2013).

Ozsoy N., Can A., Yanardag R. and Akev N., Antioxidant activity of Smilax excels L. leaf extracts, *Food Chemistry*, 110(3), p. 571-583, (2007).

Park J.N., Ali-Nehari A., Woo H.C. and Chun B.S., Thermal studies of polyphenols and fatty acids in Laminaria japonica hydrolyzates produced using subcritical water, *Korean Journal of Chemical Engineering*, 29, p. 1604-1609, (2012).

Peng Z., Liu M., Fang Z. and Zhang Q., In vitro antioxidant effects and

cytotoxicity of polysaccharides extracted from Laminaria japonica, *International Journal of Biological Macromolecules*, 50, p. 1254-1259, (2012).

Pereira R.C. and Costa-Lotufo L.V., Bioprospecting for bioactives from seaweeds: potential, obstacles and alternatives, *Brazilian Journal of Pharmacognosy*, 22 (4), p. 894-905, (2011).

Ping S., Peilong S. and Yanjie Y., Response surface optimiztion of wheat germ oil yield by supercritical carbon dioxide extraction, *Food and Bioproducts Processing*, 86, p. 227-231, (2008).

Qian Z.J., Je J.Y. and Kim S.K., Antihypertensive effect of angiotensin I converting enzyme-inhibitory peptide from hydrolysates of bigeye tuna dark muscle, Thunnus obesus, *Journal of Agricultural and Food Chemistry*, 55, p. 8398–8403, (2007).

Ragan M.A. and Glombitza K.W., Phlorotannins, brown algal polyphenols, In progress in phycological research, Round FE and Chapman DJ (ed). *Biopress Ltd in Bristol*, p. 129-241, (1986).

Rajauria G., Jaiswal A.K., Abu-Ghannam N. and Gupta S., Effect of hydrothermal processing on color, antioxidant and free radical scavenging capacities of edible Irish brown seaweeds, *International Journal of Food Science and Technology*, 45(12), p. 2485-2493, (2010).

Raúl E., Ciana B., María S., Caballeroa, Nora Sabbaga, Rolando J., González, Silvina R. and Dragoa B., Bio-accessibility of bioactive compounds (ACE inhibitors and antioxidants) from extruded maize products added with a red seaweed Porphyra columbina, *Food Science and Technology*, 55(1), p. 51-58, (2014).

Rioux L.E., Turgeon S.L. and Beaulieu M., Characterization of polysaccharides extracted from brown seaweeds, *Carbohydrate Polymers*, 69, p. 530-537, (2007).

Rogalinski T., Liu K., Albrecht T. and Brunner G., Hydrolysis kinetics of biopolymers in subcritical water, *The Journal of Supercritical Fluids*, 46, p. 335-341, (2008).

Roh M.K., Salim Uddin Md. and Chun B.S. Extraction of fucoxanthin and polyphenol from Undaria pinnatifida using supercritical carbon dioxide with co-solvent, *Biotechnology and Bioprocess Engineering*, 13(6), p. 724-729, (2008).

Kim S.K. and Pangestuti R., Biological activities and potential health benefits of fucoxanthin derived from marine brown algae, *Advances in Food and Nutrition Research*, 64, p. 111-128, (2011).

Saha K., Lajis N.H., Israf D.A., Hamzah A.S., Khozirah S., Khamis S. and Syahida A., Evaluation of antioxidant and nitiric oxide inhibitory activities of selected Malaysian medicinal plants, *Journal of Ethnopharmacology*,

92(2-3), p. 263-267, (2004).

Samee H., Li Z.X., Lin H., Khalid J. and Guo Y.C., Anti-allergic effects of ethanol extracts from brown seaweeds, *Journal of Zhejiang University Science B*, 10, p. 147-153, (2009).

Sánchez-Machado D.I., López-Cervantes J., López-Hernández J. and Paseiro-Losada P., Fatty acids, total lipid, protein and ash contents of processed edible seaweeds, *Food chemistry*, 85, p. 439-444, (2004).

Santos-Buelga C. and Williamson G., Methods in polyphenol analysis, *The Royal Society of Chemistry: Cambridge, UK*,(2003).

Sciarini L.S., Ribotta P.D., León A.E. and Pérez G.T., Effect of hydrocolloidson gluten free batter properties and bread quality, *International Journal of Food Science & Technology*, 45(11), p. 2306–2312, (2010).

Seo Y., Kang S.H., Lee H.J., Kim Y.A., Youn H.J., Lee B.J. and Chung H., In vitro screening of seaweed extract on the proliferation of mouse spleen and thymus cell, *Biotechnology and Bioprocess Engineering*, 11, p. 160-163, (2006).

Shahidi F. and Naczk M., Phenolics in food and nutraceuticals phenolics in food and nutraceuticals preface Fereidoon Shahidi, CRC Press, Taylor & Francis Group, Boca Raton, Fla.,London,(2004).

Sheih I.C., Fang T.J. and Wu T.K., Isolation and characterization of a novel angiotensin I-converting enzyme (ACE) inhibitory peptide from the algae protein waste, *Food Chemistry*, 115, p. 279–284, (2009).

Simopoulos A.P., The importance of the ratio of omega-6/omega-3 essential fatty acids, *Biomedicine and Pharmacotherapy*, 56, p. 365-379, (2002).

Steinberg P.D., Edyvane K., Nys R., Birdsey R. and van Altena I.A., Lack of avoidance of phenolic-rich brown algae by tropical herbivorous fishes, *Marine Biology*, 109, p. 335-343, (1991).

Suetsuna K. and Chen J.R., Identification of antihypertensive peptides from peptic digest of two microalgae, Chlorella vulgaris and Spirulina platensis, *Marine Biotechnology*, 3, p. 305–309, (2001).

Suetsuna K. and Nakano T., Identification of an antihypertensive peptide from peptic digest of wakame, *The Journal of nutritional biochemistry*, 11(9), p. 450-454, (2000).

Surveswaran S., Cai Y.Z., Corke H. and Corke M., Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants, *Food Chemistry*, 102 (3), p. 938-953, (2007).

Takagi T., Asahi M. and Itabashi Y., Fatty acid composition of twelve algae from Japanese waters, *Yukagaku*, 34, p. 1008-1012, (1985).

Taylor L.T., Supercritical Fluid Extraction. *John Wiley & Sons.*, Toronto,

(1996).

Terasaki M., Hirose A., Narayan B., Baba Y., Kawagoe C.,Yasui H., Saga N., Hosokawa M. and Miyashita K., Evaluation of recoverable functional lipid components of several brown seaweeds of japan with special reference to fucoxanthin and fucosterol contents, *Journal of Phycology*, 45, p. 974-980, (2009).

Tuney, Cadirci B.H., Unal D. and Sukatar A., Antimicrobial activities of the extracts of marine algae from the Coast of Urla (Izmir, Turkey), *Turkey Journal Biology*, 30, p. 171-175, (2006).

Uddin M.S., Ahn H.M., Kishimura H. and Chun B.S., Production of valued materials from squid viscera by subcritical water hydrolysis, *Journal of Environmental Biology*, 31, p. 675-67, (2010).

Wargacki A.J., Leonard E., Win M.N., Regitsky D.D., Santos C.N.S., Kim P.B., Cooper S.R., Raisner R.M., Herman A., Sivitz A.B., Lakshmanaswamy A., Kashiyama Y., Baker D. and Yoshikuni Y., An engineered microbial platform for direct biofuel production from brown macroalgae, *Science*, 335, p. 308-313, (2012).

Watchararuji K., Goto M., Sasaki M. and Shotipruk A., Value-added subcritical water hydrolysate from rice bran and soybean menal, *Bioresource Technology*, 99, p. 6207-6213, (2008).

Wetwitayaklung P., Phaechamud T., Limmatvapirat C. and Keokitichai S., The study of antioxidant capacity in various parts of Areca catechu L., *Naresuan University Journal*, 14(1), p. 1-14 (2006).

Wijesekara I. and Kim S.K., Angiotensin-I-converting enzyme (ACE) inhibitors from marine resources: Prospects in the pharmaceutical industry, *Marine Drugs*, 8, p. 1080–1093, (2010).

Wijesekaraa I., Qian Z.J., Ryua B.M., Ngoa D.H. and Kim S.K., Purification and identification of antihypertensive peptides from seaweed pipefish (Syngnathus schlegeli) muscle protein hydrolysate, Food Research International, 44, p. 703-707, (2010).

Wong S.P., Leong L.P. and Koh J.H.W., Antioxidant activities of aqueous extracts of selected plants, *Food Chemistry*, 99(4), p. 775-783, (2006).

Yan X., Chuda Y., Suzuki M. and Nagata T., Fucoxanthin as the major antioxidant in Hijikia fusiformis, a common edible seaweed, *Journal of Bioscience and Bioengineering*, 63(3), p. 605-607, (1999).

Yoshida H., Takahashi Y. and Terashima M., A simplified reaction model for production of oil, amino acids and organic acids from fish meat by hydrolysis under subcritical and supercritical conditions, *Journal of Chemical Engineering Japan*, 36, p. 441-448, (2003).

Zhao Y., Li B., Liu Z., Dong S., Zhao X. and Zeng M., Antihypertensive

effect and purification of an ACE inhibitory peptide from sea cucumber gelatin hydrolysate, *Process Biochemistry*, 42, p. 1586–1591, (2007).

Zhu G., Ma Y. and Zhu X., Reactions of cellulose in supercritical water, *Chinese Journal of Organic Chemistry*, 30(1), p. 142-148, (2010).

Zhu G., Zhu X., Fan Q., Liu X., Shen Y. and Jiang J., Study on production of amino acids from bean dregs by hydrolysis in subcritical water, *Chinese Journal of Chemistry*, 28, p. 2033-2038, (2010).

아임계 및 초임계유체를 이용한 갈조류 (모자반)으로부터 생리활성 물질 회수 및 특성

Yin Shipeng

부경대학교 대학원 식품공학과

요약

해조류 는 해양 환경에 잠재적인 신 재생 자원 이다. 그것은 인간의 건강 기능식품으로 활용되어질 수 있는 잠재성이 높은 밝혀지지 않은 생리활성 화합물 중의 하나 이다. 해조류 는 카로티노이드 카로티노이드, 식이 섬유, 단백질, 비타민, 필수 지방산, 미네랄 등 생리 활성 화합물 을 함유한 우수한 바이오 소재 를 제공한다. 또한, 해조류 는 항산화 및 항균 특성을 갖는 생리 활성 물질 이 풍부한 자원이므로 , 해조류 의 기능적 효과 중, 영양 및 건강 관련 이점은 널리 연구되고있다. 대부분 의 해조류 는은 항 암, 항 곰팡이, 항 염증, 항산화 및 넓은 범위의 생물학적 생물학적 활성을 갖는다. 일반적으로 유용물질이 함유된 화합물을 얻기 위해서는 핵산,

메탄올 , 에탄올 등과 같은 유기용매 사용법 을 적용하고 적용하고 있다. 그러나 최근에는, 유기 용매 사용법에서 발생하는 문제점들을 문제점들을 해결하기 위해 친환경적인 방법을 사용하고 있다 .

해조류 (모자반)에 존재하는 유용물질을 유용물질을 회수하기 위하여 다양한 용매 (아세톤, 헥산, 메탄올, 에탄올, 헥산의 혼합 에탄올 및 아세톤의 혼합 메탄올)와 환경 친화적 용매 인 초임계 이산화탄소 이산화탄소 (SC-CO_2)를 사용하여 추출 하였다. SC-CO_2 추출 실험은 온도 45°C 와 압력 250bar 조건 에서 실험 하였다. CO_2 (26.81g / 분)의 유량은 2 시간의 추출기간 동안 일정하 게 유지시켰으며, 에탄올을 보조 용매로 사용하였다. 유기용매 추출은 5 가지 상이한 용매 (아세톤, 헥산, 메탄올, 에탄올, 헥산의 혼합 에탄올 및 아세톤의 혼합 메탄올)를 시료 와 혼합 (10:1 W/V) 시켜 수행 하였고, 25°C 의 빛이 차단된 장소 에서 200ml 플라스크에 마그네틱바로 24 시간 교반하였다. Fucoxanthin 및 오일 의 지방산 조성 은 고성능 액체크로마토그래피 (HPLC) 와 가스크로마토그래피 (GC)를 이용하여

분석 하였다. SC-CO_2 와 에탄올 을 보조용매로 사용하 추출한 모자반의 항산화 특성은 서로 다른 유기용매를 사용하여 추출한 것 보다 높은 활성을 보였다. SC-CO_2 와 에탄올 을 사용한 추출 오일의 DPPH 항산화 활성은 68.38 ± 1.21% 를 나타내었고, TFC 함량은 5.571 ± 0.015mg/g 을 보였다 보였다. ABTS 항산화 활성은 헥산 추출물에서 92.39 ± 1.25% 를 보였고, 메탄올 추출물의 TPC 는 0.639 ± 0.023mg/g 의 함량을 보였다. 항 고혈압제 활성은 SC-CO_2 와 에탄올 추출물에서 크게 나타내었다 나타내었다.

SC-CO_2 추출 후 회수된 잔여물 은 아임계 수를 사용하여 짧은 반응 시간에서 전환시켜 분해된 생성물을 생성물을 회수하였다. 실험은 15-220bar 의 압력 과 180-260℃ 의 반응온도 조건에서 수행 하였다. 시료와 물의 비율은 1:25 이고 각 조건의 반응 시간은 3 분으로 유지하였다. 실고압 펌프는 초기 압력 1 막대를 얻기 위해 반응기에 물 탱크로부터 흐르도록 도포 하였다 하였다. 각 조건에서 수행된 가수 분해 물을 수득하 였고, 전환 물질을 분석 하였다. 포름산과

중탄산 나트륨 1% 를 촉매로 사용하였다. 가수 분해 수율은 높은 온도 와 촉매를 첨가하였을 때 높게나타내었다 게나타내었다. 260°C / 200bar 에서 촉매 를 첨가한 모자반의 항산화 특성은 DPPH, ABTS, TPC 와 TFC 가 각각 20.05 ± 0.031%, 94.02 ± 0.058%, 200.84 ± 0.024ug/L 와 552.87 ± 0.018ug/L 로 다른 조건보다 조건보다 가장 좋은 활성을 보였다. 촉매 를 첨가하였을 때, 낮은 온도에서 온도에서 총 환원당의 함량이 높게 왔다. 180°C / 15 bar 조건에서 촉매 를 첨가하여 가수분해 하였을 때 총 유기탄소 함량이 높았다 . 항 고혈압 활성은 180°C / 15 bar 조건 의 가수분해 반응 에서 높게 나타내었다 나타내었다. 가수분해물에서 8 개의 필수 아미노산을 포함한 21 가지의 가지의 아미노산이 검출 되었다. 또한 색상 및 pH 는 조건에 따라 상이한 상이한 값을 나타내었다. 아임계수를 이용한 가수분해법은 복잡하고 분자량이 큰 탄수화물 결합을 간단 하게 분해하는데 효과적이었다. 아임계 수를 사용하여 생산된 가수분해물질은 유용한 바이오 화합물을 함유하고 있으므로

식품산업과 바이오산업에 적용될 수 있다.

Acknowledgements

I would like to express my greatest gratitude to my advisor Professor Byung-Soo Chun for giving me opportunity to join in research work under his supervision. Without his guidance and persistent help, this thesis would not have been possible. I am very grateful to him for providing the financial assistance and for chance to have experience for study in Korea.

It is my honor to thanks to my thesis evaluation committee, Prof. Young-Mog Kim and Prof. Yang-Bong Lee, for their fruitful evaluations, reviews, criticisms and suggestions to improve my thesis. I would like to express my sincere thanks to all professors and friends in Department of Food Science and Technology, who helped me in numerous ways to complete my mater dissertation a successful event.

I would like to express my cordial thanks to all of my lab members, I have been blessed with a friendly and cheerful environment in lab: A.K.M Assaduzzaman, A.S.M Tanbirul Haque and Periaswamy Sivagnanam Saravana, the help and teach many thing about experiments and papers; Aviannie Meillisa Prathami and Evi Amelia Siahaan, the two Indonesians student in my department, thank you for her advices, helps and times, Kim-Ryong Hee, who always help me, and Adane Tilahun Getachew. Special thanks also for Lee Jaekyung, Jeong Shinyub and Oh Donggeon for helping me with the Korean translation. Great thanks for my Chinese beat friend Han Pengcheng for helps,

times and money. Thanks also for all Korean friends, international students and all Chinese friends for their helping hands and comfortable atmosphere during my study period.

I extend my sincere gratitude to my beloved family, my parents and my older brother.

Finally, I would like to thank everybody who gives their help and support for me. Let me express apologies that I could not mention personally one by one.