World Academy of Science, Engineering and Technology
International Journal of Agricultural and Biosystems Engineering
Vol:3, No:12, 2009

The Effection of Different Culturing Proportion of Deep Sea Water(DSW) to Surface Sea Water(SSW) in Reductive Ability and Phenolic Compositions of *Sargassum Cristaefolium*

H. L. Ku, K. C. Yang, S. Y. Jhou, S. C. Lee, C. S. Lin

***Abstract*—**Characterized as rich mineral substances, low temperature, few bacteria, and stability with numerous implementation aspects on aquaculture, food, drinking, and leisure, the deep sea water (DSW) development has become a new industry in the world. It has been report that marine algae contain various biologically active compounds. This research focued on the affections in cultivating *Sagrassum cristaefolium* with different concentration of deep sea water(DSW) and surface sea water(SSW). After two and four weeks, the total phenolic contents were compared in *Sagrassum cristaefolium* culturing with different ways, and the reductive activity of them was also be tried with potassium ferricyanide. Those fresh seaweeds were dried with oven and were ground to powder. Progressively, the marine algae we cultured was extracted by water under the condition with heating them at 90℃ for 1hr.The total phenolic contents were be executed using Folin–Ciocalteu method. The results were explaining as follows: the highest total phenolic contents and the best reductive ability of all could be observed on the 1/4 proportion of DSW to SSW culturing in two weeks. Furthermore, the 1/2 proportion of DSW to SSW also showed good reductive ability and plentiful phenolic compositions. Finally, we confirmed that difference proportion of DSW and SSW is the major point relating to ether the total phenolic components or the reductive ability in the *Sagrassum cristaefolium*. In the future, we will use this way to mass production the marine algae or other micro algae on industry applications.

***Keywords*—**deep sea water(DSW), surface sea water(SSW), phenolic contents, reductive ability.

I. Introduction

CURRENTLY, the utilization of deep sea water (DSW) is receiving much attention due to its high productivity, large quantity, and potential for recycling energy. Deep sea water, accounting for 95% of all sea water, generally refers to sea water from a depth of more than 200 m. character of Deep sea water has cold temperature, abundant nutrients, and good water quality that is pathogen-free and stable (Fig.1. 2009). The research emphasis on many national organizations, prefectures, universities, and private companies is shifting from basic research to feasibility studies or practical applications of deep sea water. With the worldwide population explosion contributing to an ever-increasing consumption of animal protein, the focus on aquaculture will undoubtedly also intensify. The high productivity of DSW as a renewable energy source may increase the role of aquaculture to cultivate food for the expanding human population.〔1〕

A major advantage of using deep sea water for aquaculture is the ability to culture coldwater organisms and deep-ocean organisms in tropical areas. Another is the ease at which water temperature can be controlled by mixing surface water with deep sea water. A third advantage is disease control, as there are few viruses and pathogenic bacteria in deep sea water. A disadvantage of using surface sea water is the maintenance required to keep the water intake pipes free of organisms that cling to the pipes and foul the water. However, when DSW is used for aquaculture purposes, maintenance of the pipes to remove harmful bacteria and other organisms is not necessary.〔2〕

Variables	SSW	DSW
Temperature(℃)	18.9	9.8
pH	7.97	7.54
salte(ppt)	33.7	33.8
Carbon(mg/L)	-------	0.9
Nitrate(mg/L)	0.406	0.787
Nitrite(uM)	0.207	<0.01
Phosphate(uM)	1.545	2.423
Silicate(uM)	13.247	78.702
Ammonia(mg/L)	-------	0.05
Bromine(mg/L)	-------	67.9
Fluorine(mg/L)	-------	0.76
Zinc(mg/L)	0.0031	0.0039
Copper(mg/L)	0.0008	0.0006
Manganese(mg/L)	0.0001	0.0002
E. coli(CFU/100mL)	<1	<1
colony(CFU/mL)	--------	<0.04

Fig .1 The character of deep sea water(DSW) and surface sea water(SSW) (618m, 2009).

H. L. Ku is assistant researcher in Stone & Resource Industry R&D Center, Taiwan(email: ygu_33@srdc.org.tw)
K. C. Yang is assistant researcher in Stone & Resource Industry R&D Center, Taiwan(email: winwing@srdc.org.tw)
S. Y. Jhou is assistant researcher in Stone & Resource Industry R&D Center, Taiwan(email: teresa@srdc.org.tw)
S. C. Lee is assistant manager in Stone & Resource Industry R&D Center, Taiwan(email: hylaman@srdc.org.tw)
C. S. Lin is manager in Stone & Resource Industry R&D Center, Taiwan(email: cslin@srdc.org.tw)

To alleviate some of the anticipated problems, a cascade system of using deep sea water has been proposed (Fig. 2)〔3〕. In this system, intake water is used for airconditioning and then used for aquaculture. After it is used for aquaculture, the DSW is discharged into the ocean for environmental restoration. This system can decrease the negative environmental impact of discharging cold water while maintaining the positive attributes of deep sea water.

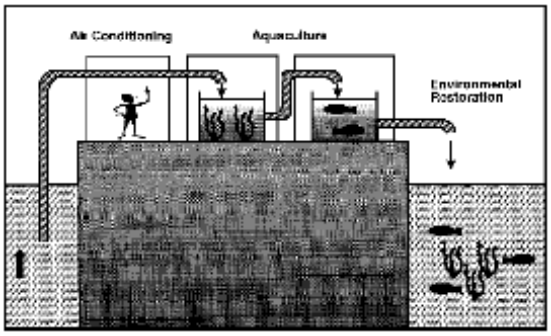

Fig. 2 cascade system of using deep sea water.

Free radicals and other reactive oxygen species which generated in living organism are considered as the main cause for many pathological conditions for their oxidative properties which actively acting as oxygen donor to other compounds in living organism〔4〕. Reactive oxygen species are produced continuously in human body as a consequence of normal metabolic processes. Some reactions would lead to free radical formation. If free radicals are activated, their chemical reactivity can damage all types of cellular macromolecules, including proteins, carbohydrates, lipids and nucleic acids. Many of these effects have been implicated in the causation of degenerative diseases. For example, destructive effects on proteins may play a role in the causation of cataracts, effects on DNA are involved in cancer causation, and effects on lipids apparently contribute to the causation of atherosclerosis. Free radicals and other reactive oxygen species in the human body are derived either from normal, essential metabolic processes or from external sources〔5〕.

Nutrition plays a key role in maintaining the body's enzymatic defenses against free radicals. Several essential minerals including selenium, copper, manganese and zinc are involved in the structure or catalytic activity of these enzymes. If the supply of these minerals is inadequate, enzymatic defenses may be impaired〔6〕. A second line of defense is small-molecular-weight compounds which act as antioxidants; that is, they react with oxidizing chemicals, reducing their capacity for damaging effects (Lillian, 1995).

This research is focused on exploration of marine natural product, especially for *Sagrassum cristaefolium* as a source of abundances nature life from Taiwan which potentially as one a source of antioxidant to defenses from harmful of free radicals.

II. MATERIALS AND METHODS

A. Reagent and Apparatus

Gallic acid (Merck),Folin-ciocalteau reagent (Merck), Sodium carbonate (Merck), glucose (Merck), Sodium phosphate (Merck), potassium ferricyanide (Merck), Trichloroacetic acid (Merck), Ferric chloride (Merck), UV visible spectrophotometer (Aquarious CE9200), water bath (W350, MEMMERT), ultracentrifuge (HIMAC CR22E, HITACHI), oven (DK63, Yamato).

B. Culturing of Sagrassum cristaefolium

Sagrassum cristaefolium was collected from Nawan in Taiwan. Deep sea water (618m depth) and surface sea water were prepared with different concentration for cultivation *Sagrassum cristaefolium*. The different proportions were 0:4, 1:3, 2:2, 3:1, 4:0 of DSW to SSW in volume. After two and four weeks, the totals *Sagrassum cristaefolium* were collected and extracted.

C. Preparation of Sagrassum cristaefolium Extracts

Those fresh *Sagrassum cristaefolium* were dried with oven and were ground to powder. Progressively, the *Sagrassum cristaefolium* was extracted by water under the conditions with heating them at 90℃ for 1hr.

D. Determination of Antioxidant Activity

The antioxidant activity of all extracts was determined according to the total phenolic compounds of Singleton et al., 〔7〕and reductive ability of Oyaizu et al., 〔8〕with some modification.

Determination of Phenolic contents

Sample for testing were prepared at 0.5ml and were mixed with 7ml distil water and 0.5ml Folin-Ciocalteau reagent to stand for 3 minutes at rt. Addition of 2ml Na_2CO_3(20%) into sample and water bath at 100℃ 1 minute, to stand in dark space until the mixture cold. The total phenolic contents was measured at 685nm using spectrophotometer UV-visible Lambda.

Determination of reductive ability

Sample for testing were prepared at 10ml and were mixed with 2.5ml phosphate buffer (0.2M, pH6.6) and 2.5ml potassium ferricyanide (1%). The mixture was been water bath 20 minute at 50℃, and add 2.5ml TCA (10%) into mixture. Then the 5ml mixture was mixed with 5ml distil water and 1ml $FeCl_3$(0.1%), stand for 10 minutes in dark at rt. the reductive ability was measured at 700nm using spectrophotometer UV-visible Lambda.

III. RESULTS AND DISCUSSION

Evaluation of Antioxidant Activity

The total phenolic contents were be executed by using Folin–Ciocalteu method and reductive ability was measured by using potassium ferricyanide. All results were presented in Table I, Table II, Fig. 3 and Fig4. It was observed that DSW in culturing condition (1:3, 2:2, 3:1, 4:0) having higher phenolic

contents (6.03, 5.16, 5.14, 5.51mg/ml) and reductive ability (6.81, 5.75, 5.70, 6.25, OD_{700} value) than SSW culturing condition (phenolic contents 4.23mg/ml and reductive ability 5.24 OD_{700} value) when they were in 14days. In which, the 1:3 had the highest total phenolic contents (6.03mg/ml) and reductive ability .(6.81mg/ml). But it's phenolic contents and reductive ability decreasing (4.40mg/ml and 5.2) on 28 days. The result of 100%DSW culturing condition was the same with 1:3 of DSW to SSW condition. When the *Sagrassum cristaefolium* culturing in difference proportion of DSW to SSW in 14days, the phenolic contents and reductive ability had the same curve trend, the order was 1:3, 4:0, 2:2, 3:1 and 0:4 of DSW to SSW. In 28days, the phenolic contents and reductive ability were increased in all condition except 1:3 and 100%DSW culturing condition.

TABLE I
The total phenolic contents of *Sagrassum cristaefolium* in different culturing condition

Sample (mg/ml)	0:4 DSW:SSW	1:3 DSW:SSW	2:2 DSW:SSW	3:1 DSW:SSW	4:0 DSW:SSW
14days	4.23 ±0.42	6.03 ±1.05	5.16 ±0.59	5.14 ±0.57	5.51 ±0.52
28days	5.30 ±0.92	4.40 ±0.58	5.40 ±0.52	5.30 ±0.49	4.39 ±0.96

Data are mean ± standard deviation (n>3)

TABLE II
The reductive ability of *Sagrassum cristaefolium* in different culturing condition

Sample (OD_{700})	0:4 DSW:SSW	1:3 DSW:SSW	2:2 DSW:SSW	3:1 DSW:SSW	4:0 DSW:SSW
14days	5.24 ±0.32	6.81 ±1.12	5.75 ±0.26	5.70 ±0.46	6.25 ±0.91
28days	5.76 ±0.29	5.20 ±0.30	6.01 ±0.34	5.81 ±0.51	5.19 ±0.79

Data are mean ± standard deviation (n>3)

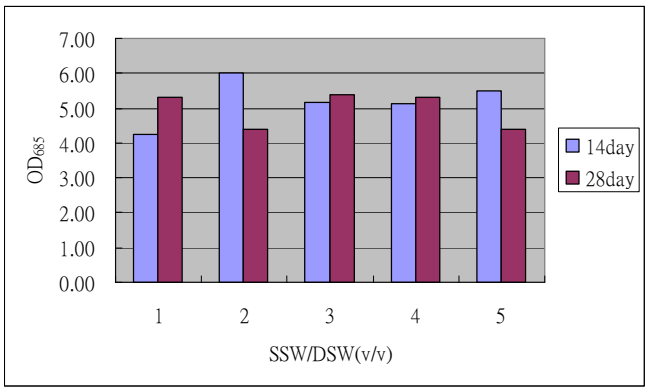

Fig. 3 The total phenolic contents of *Sagrassum cristaefolium* (The 1, 2, 3, 4, 5 mean 0:4, 1:3, 2:2, 3:1, 4:0 of DSW to SSW volume)

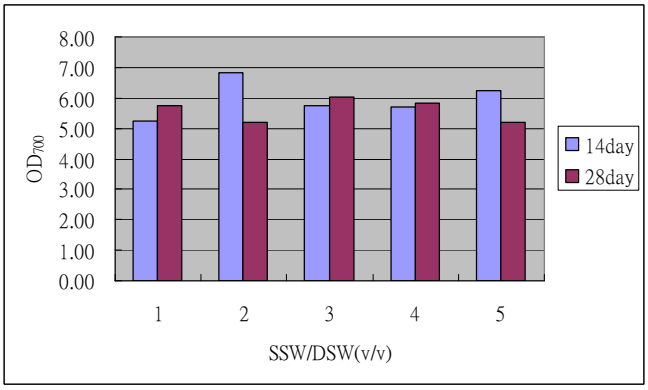

Fig. 4 The reductive ability of *Sagrassum cristaefolium* (The 1, 2, 3, 4, 5 mean 0:4, 1:3, 2:2, 3:1, 4:0 of DSW to SSW volume)

Deep sea water (DSW) has abundant nutrients which is good for marine algae or other aquaculture. Under 200m depth, high pressure, there are rich small organic molecule can be the source of nutrition for marine organism and ease for aquaculture to absorb. Surface sea water (SSW) is more complicated than DSW environment, in this research we though that the culturing of marine algae would be effected by mix of DSW and SSW.

A variety of possible applications of deep sea water are topics of future research. In Japan, using deep sea water for fisheries, agriculture, energy, medical treatment, and environmental purposes are under constant investigation. In Taiwan, it is ease to get deep sea water (DSW) too. There is more and more research show that deep sea water can be applied in many industry.

ACKNOWLEDGMENT

The authors wish to express much appreciation for the financial supports from Department of Industrial Technology (Taiwan) (98-EC-17-A-10-02-0524). We also express our gratitude to Assistant Professor Mr. Chou, Department & Institute of Life Science of Tzu Chi University for his good points of views and experimental and analytical support of this research project.

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