Fish Aqua Sci 13(1), 12-17, 2010

KOSFAS FISHERIES AND AQUATIC SCIENCES
The Korean Society of Fisheries and Aquatic Science

Improvement of the Functional Qualities of Sea Tangle Extract through Fermentation by *Aspergillus oryzae*

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This study was conducted to evaluate the potential of a microbial fermentation procedure to improve the functional qualities of seaweeds. *Aspergillus oryzae*, which has been used in traditional Korean fermented foods, was inoculated and cultivated in an aqueous extract of sea tangle (*Laminaria japonica*). Fermentation of the sea tangle extract by *A. oryzae* for 4 days resulted in a 3-fold increase in γ-aminobutyric acid (GABA) content. GABA is known to be a bioactive compound. Fungal fermentation of the extract also enhanced its antioxidant activity and increased its total content of phenolic compounds. It was assumed that these changes stemmed from the biodegradation of active compounds of the sea tangle packaged within its rigid structural matrix or occurred as result of fungal fermentation. These results suggested that the application of microbial fermentation to the processing of seaweeds will help in the development of processed foods to meet consumer demands.

Key words: γ-Aminobutyric acid, Antioxidant activity, *Aspergillus oryzae*, Fermentation, Phenolic compound, Sea tangle

Introduction

Human consumption of marine algae has been documented since 600 BC. This group of organisms has received much attention in field of marine natural products over the last 25 years (Chapman and Chapman, 1980). Many published reviews have reported the importance of these organisms as potential sources of pharmaceutical leads (Bugni and Chris, 2004). Seaweed products are suitable as human foods and animal feeds and may also be used as fertilizers, fungicides, herbicides, and phycocolloids (Chapman and Chapman, 1980). Seaweeds are known to contain specific metabolites that have biological activities, including antimicrobial, antioxidant, anticoagulant, anti-inflammatory, and antitumor activities (Usui et al., 1980; Hiroyuki et al., 1990; Colliec et al., 1991; Park et al., 1991; Nishino et al., 1991; Ferial et al., 2000). Production of domestic seaweeds has increased dramatically in the past decade: 811,000 tons were produced in 2007 (Ministry for Food, Agriculture, Forestry and Fisheries, 2008). However, these seaweeds have been only simply processed, for example, for use in, raw, frozen or salted products, and offered no advantages over highly processed seaweeds (Heo and Jeon, 2005). Therefore, the development of various seaeed-based processed foods is required to increase attractiveness to consumers and thus enhance conumption.

Fermentation is a biochemical reaction that meabolizes high-molecular weight organic compounds, yielding relatively simple products. Fermentation enhances the nutrient content of foods through the biosynthesis of vitamins, essential amino acids, and proteins, and by improving protein quality and fiber digestibility. It also enhances micronutrient biovailability and aids in degrading anti-nutritional factors (Achinewhu et al., 1998; Adewusi et al., 1999). The object of this study was to evaluate the potential of a microbial fermentation procedure to improve the nutritional and functional qualities of seaweeds. Thus, aqueous sea tangle extract was fermented by *Aspergillus oryzae*, which has been used in traditional Korean fermented foods. The physiochemical and nutritional properties of the extract were evaluated before and after fermentation.

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Materials and Methods

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH, a free radical compound) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other reagents were of analytical grade and commercially available.

Plant materials and sample preparation

Sea tangle (*Laminaria japonica*) was purchased at Gi-jang market, Busan, Korea, in March 2008. The sample was rinsed with fresh water to eliminate foreign materials including sand and shells. It was then desalinized through soaking in two volumes of water for 1 h. This desalination process was repeated 2-3 times. Desalinized samples were cut to sizes suitable for efficient extraction using a grinder mill. To prepare an aqueous extract of the sea tangle ("sea tangle extract"), three volumes of water were added to the desalinized seaweed, which was then heated at 70°C for 1 hr and centrifuged to remove tissue remnants. After cooling to room temperature, the extract was stored at -70°C until further use.

Microorganism and fermentation

Microbial strain used in this study, *Aspergillus oryzae*, was isolated from Nuruk and maintained at the Laboratory of Food Microbiology, Pukyong National University. The strain was grown aerobically at 30°C in yeast and mold broth (YM, Difco Laboratories, MI, USA). The mycelium was harvested after incubation for 4 days.

Fermentation was performed in a flask containing 500 mL of sea tangle extract that had previously been inoculated with 3% (v/v) *A. oryzae* mycelium. Samples were grown aerobically (with agitation at 120 rpm) at 30°C, and sampling was performed at regular intervals. The growth of *A. oryzae* in the extract was monitored by measuring the dry weight of the mycelium (Yamane et al., 2000).

Physiochemical analysis

During fermentation of the sea tangle extract, the changes in salinity and pH were measured, the latter using a pH meter (Accumet model 15 pH meter, Fisher Sci. Co., Springfield, USA).

Analysis of sea tangle extract composition

Moisture content was measured by oven-drying at 105°C to constant weight. The contents of crude ash, crude protein, and crude lipid in a cell-free extract, prepared by removing the fungal mycelium through filtration, were determined using standard methods (AOAC, 1995). The extract's reducing sugar content was determined by Somogyi's method (Somogyi, 1952). All experiments were performed in triplicate, and mean values are presented.

Analysis of total content of phenolic compounds

Total contents of phenolic compounds (TP) were determined by the Folin-Ciocalteu method (Slimestad et al., 2009) using phloroglucinol (a basic structural unit of phlorotannins) as a standard, and data are expressed as phloroglucinol equivalents (PGE) (Jiménez-Escrig et al., 2001). A 0.1 mL aliquot of the diluted sample was mixed with 0.5 mL of 1 N Folin-Ciocalteu reagent in a 1.5 mL tube. The resulting mixture was allowed to rest for 3 min before 0.4 mL of 20% Na_2CO_3 was added. Samples were incubated in the dark at room temperature for 45 min and centrifuged at 1,600×*g* for 8 min. The resulting supernatant's optical density (OD) at 765 nm was measured using a GENios® microplate reader (Tecan Austria GmbH, Grödig, Austria).

DPPH radical scavenging activity

DPPH radical scavenging activity was measured using the method described by Nanjo et al. (1996). A volume of 1.5 mL of non-fermented or fermented extract was added to 1.5 mL of a 0.1-mM ethanolic solution of DPPH. After mixing vigorously for 30 sec, the resulting solutions were incubated at 37°C for 30 min. The ability of the solutions to scavenge DPPH radicals was measured by recording absorbances at 540 nm using a spectrometer. DPPH radical scavenging activity was calculated using the following equation:

$$\text{Radical scavenging activity} = 1 - \frac{H}{H_0} \times 100$$

where H and H_0 represent the relative heights of the radical peaks produced in the presence and absence of sample, respectively.

Results and Discussion

Fungal cell growth in the sea tangle extract

A. oryzae is a filamentous fungus that has been used to saccharify rice, other grains, and potatoes during the production of alcoholic beverages such as *sake* and *shochu* for at least 2000 years (Rokas, 2009). We decided to evaluate the potential of an *A. oryzae*-based fermentation procedure to improve the nutritional and functional qualities of seaweeds. We first investigated fungal cell growth in sea tangle extract. As shown in Fig. 1, fungal cell numbers gradually increased as fermentation progressed. Dry

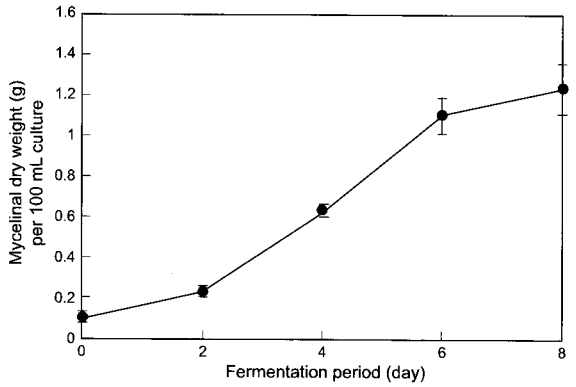

Fig. 1. Fungal cell growth in sea tangle water extract. *Aspergillus oryzae* was inoculated in the extract and incubated at 30° as described in Materials and Methods. Experiments were repeated three times. Lines having different superscript are significantly different ($P<0.05$).

weight of mycelium was calculated to be 1.243 g per 100 mL after 8 days of culture.

This result suggests that sea tangle extract contains abundant quantities of the nutrients necessary to support fungal cell growth. Therefore, the sea tangle extract was fermented by *A. oryzae*, and the physiochemical and nutritional changes of the extract were investigated before and after fermentation.

Changes in the physiochemical properties of the sea tangle extract following fungal fermentation

During fermentation of the sea tangle extract by *A. oryzae*, changes in pH and salinity were monitored. The pH of sea tangle extract gradually increased as its fermentation progressed (Table 1). However, the reason for this was unclear, as *A. oryzae* has previously been shown to generate organic acid and thus cause acidification during the fermentation of carbohydrates. We assume that *A. oryzae* may generate basic compounds during the fermentation of sea tangle extract.

Salinity of the sea tangle extract was not influenced by fungal fermentation and remained at 9.0‰ after fermentation (Table 1). This salt concentration provided a suitable environmental condition for fungal growth because *Aspergillus* spp. grow well in salt solutions with a_w values >0.7. The a_w value of the extracts were calculated to be 0.97.

Table 1. Physiochemical change of sea tangle water extract by the fermentation of *Aspergillus oryzae*

	Fermentation period (day)				
	0	2	4	6	8
pH	5.78	6.75	7.51	7.73	7.83
Salinity (‰)	9.00	9.00	9.00	9.00	9.00

Fermentation was carried out at 30°C.

Changes in sea tangle extract composition resulting from fungal fermentation

It is well known that microbial fermentation changes the properties of foods. Therefore, it was expected that a change in the properties of the sea tangle extract would occur during fermentation by *A. oryzae*. We determined the extract's moisture, crude protein, crude lipid, and crude ash content before and during fermentation. As shown in Table 2, the initial moisture content of the sea tangle extract was almost 96%. Moisture content was maintained at a near-constant level during fermentation (increasing less than 1%), and the extract's crude ash content was also not affected by fungal fermentation. By contrast, its crude protein content decreased dramatically as the fermentation progressed, with no crude protein detected after 6 days of fermentation, suggesting that *A. oryzae* may have used the protein as a nitrogen source (Table 2). Levels of crude lipid and reducing sugar also decreased during the fermentation period, suggesting that lipid and sugar also would be utilized as substrates for fungal growth.

Changes in sea tangle-extract free amino acid contents caused by fungal fermentation

Changes in free amino acid (FAA) contents during fermentation of the sea tangle extract by *A. oryzae* were also investigated (Table 3). A total of 20 species of FAA were detected in the raw sea tangle extract by the FAA analysis. Among them, aspartic acid and glutamic acid were the most abundant. Levels of some FAAs were changed by *A. oryzae* fermentation. Levels of aspartic acid, proline and alanine decreased dramatically during the course of fermentation. However, glutamic acid known to be a major source of flavor in the sea tangle extract, changed little during the first 6 days of fermentation.

During the fermentation process, the most interesting change was the increase in γ-aminobutyric acid (GABA) content. GABA contents of the sea tangle extract increased approximately 3-fold after 6 days of fermentation. GABA is a non-protein amino acid that is primarily produced from the α-decarboxylation of L-glutamic acid, a process catalyzed by the enzyme glutamate decarboxylase (Oh, 2003; Fainesale et al., 2005). Therefore, we assume that the observed increase in GABA levels stemmed from the metabolism of sea tangle-extract glutamic acid by enzymes secreted by *A. oryzae* using abundant.

GABA functions in animals as a major inhibitory

Table 2. Changes of sea tangle extract compositions by *Aspergillus oryzae* fermentation

	Fermentation period (day)				
	0	2	4	6	8
Moisture (%)	96.02 ± 0.02	96.52 ± 0.03	96.58 ± 0.02	96.79 ± 0.01	97.20 ± 0.01
Crude protein (%)	0.19 ± 1.22	0.08 ± 1.26	0.03 ± 0.93	ND*	ND
Crude lipid (%)	0.80 ± 0.57	0.79 ± 0.73	0.46 ± 0.79	0.45 ± 0.71	0.37 ± 0.74
Crude Ash (%)	1.07 ± 0.02	1.14 ± 0.01	1.14 ± 0.01	1.15 ± 0.01	1.03 ± 0.05
Reducing sugar (%)	0.027 ± 0.0006	0.014 ± 0.0007	0.012 ± 0.0003	0.012 ± 0.0003	0.012 ± 0.0007

Fermentation was carried out at 30°C; *, not determined.

Table 3. Changes of free amino acid contents by *Aspergillus oryzae* fermentation in sea tangle extract

(unit : μmol/100 g)

Free amino acid	Fermentation period (day)				
	0	2	4	6	8
Aspartic acid	2,731.47	2,470.22	144.53	145.52	547.47
Serine	13.55	-*	28.39	-	-
Glutamic acid	2,207.05	2,084.93	2,175.05	1,915.60	605.71
Proline	275.35	-	-	-	-
Glycine	68.45	166.35	28.70	73.98	-
Alanine	615.48	300.33	84.90	122.88	11.08
Valine	42.07	38.75	401.28	40.25	-
Methionine	-	-	7.87	22.03	-
DL-Allocystathionine	17.54	11.00	-	-	-
Isoleucine	12.93	12.12	25.42	31.80	-
Leucine	15.20	23.80	34.78	54.88	-
Tyrosine	12.09	13.02	17.79	25.01	-
Phenylalanine	20.91	21.08	21.81	25.32	-
g-Aminobutyric acid	14.19	34.95	44.02	18.10	9.11
Ethanolamine	46.20	41.08	73.23	144.40	-
Ammonium chloride	551.13	695.70	973.70	155.13	928.15
Lysine	18.28	18.98	31.80	21.85	7.46
Histidine	-	12.17	5.37	12.95	6.45
Arginine	21.14	13.34	35.29	52.66	-

Fermentation was carried out at 30°C; *, not determined.

neurotransmitter (Mody et al., 1994; Oh, 2003). For example, owing to its activities in blood pressure regulation and the recovery of liver cell damage induced by alcohol, GABA has recently been studied as a bioactive ingredient in foods (Nakagawa and Onota, 1996; Oh, 2003). The results presented in Table 3 suggest that it may be possible to improve the quality of the sea tangle extract by microbial fermentation, even if the amount of GABA bioconverted by *A. oryzae* is relatively low.

Changes in sea tangle extract DPPH radical scavenging activity caused by fungal fermentation

Previous studies have reported that aqueous sea tangle extracts possess antioxidant and free radical scavenging activities (Han et al., 2002). Thus, the DPPH radical scavenging activity of the sea tangle extract was compared before and after fermentation by *A. oryzae*. The raw sea tangle extract (control) exhibited 62% scavenging activity against DPPH (Fig 2). Scavenging activity increased as fermentation progressed, peaking 4 days after its commencement. The DPPH-scavenging activity of the fermented extract was superior to that of the control extract for the first 6 days of fermentation (Fig. 2). We assume that the enhancement of DPPH-scavenging activity in the fermented extract resulted from the biodegradation of active compounds from the sea tangle packaged within its rigid structural matrix or from the production of active compounds as a result of fungal fermentation (Park and Han, 2006).

Changes of TP contents by fungal fermentation in sea tangle extract

As shown in Fig. 2, the antioxidant activity of the sea tangle extract was enhanced by fermentation. It has been reported that phenolic compounds possess various biological activities including antifungal, antibacterial, antiviral antioxidant, and antitumor activities (Jiménez-Escrig et al., 2001). These findings,

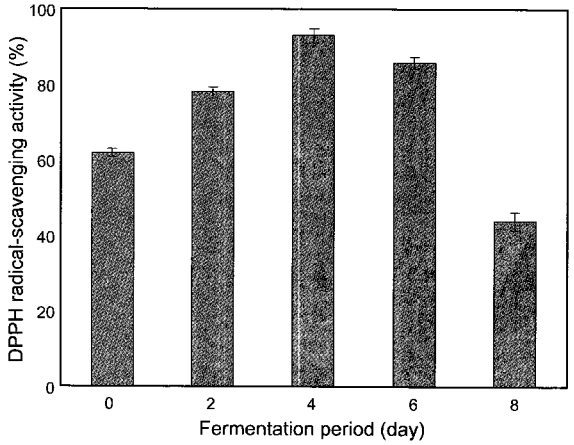

Fig. 2 Changes of DPPH radical scavenging activity by *Aspergillus oryzae* fermentation in sea tangle water extract. Experiments were repeated three times. Columns with different superscript are significantly different ($P<0.05$).

together with our own results, led us to suspect that fungal fermentation might change the content of the sea tangle extract TP content. We therefore measured the extract's TP content before and after fermentation by *A. oryzae*. As shown in Fig. 3, TP content increased during the first 4 days of fermentation before subsequently decreasing. These results were consistent with the findings of the DPPH radical scavenging assay. We suggest that the enhancement of DPPH scavenging activity and increase in TP content resulting from fermentation by *A. oryzae* may be connected.

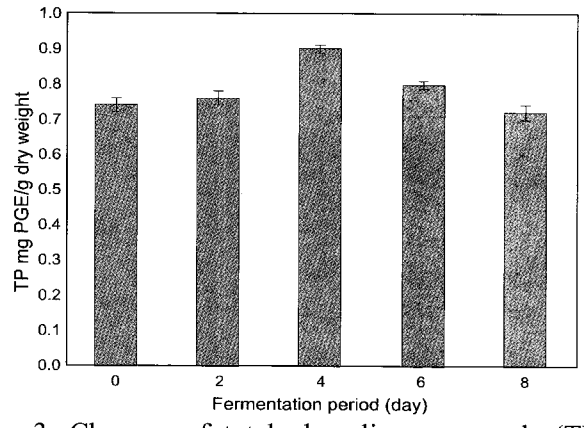

Fig. 3. Changes of total phenolic compounds (TP) contents by *Aspergillus oryzae* fermentation in sea tangle water extract. Experiments were repeated three times. Columns with different superscript are significantly different ($P<0.05$).

As described above, fungal fermentation of the sea tangle extract resulted in an improvement in its quality, as measured by the enhancement of antioxidant activity and increased content of the bioactive compound GABA. These findings suggest that the application of microbial fermentation to seaweed processing may help in the development of various processed foods to meet consumer demands.

Acknowledgments

This work was supported by a grant from the Small & Medium Business Administration (08-1-018). We thank the Brain Busan 21 Project in 2009 for graduate support.

References

Achinewhu SC, Barber LI and Ijeoma IO. 1998. Physicochemical properties and garification (gari yield) of selected cassava cultivars in Rivers State, Nigeria. Plant Foods Hum Nutr 52,133-140.

Adewusi SR, Ojumu TV, Falade OS. 1999. The effect of processing on total organic acids content and mineral availability of simulated cassava-vegetable diets. Plant Foods Hum Nutr 53, 367-380.

AOAC. 1995. Official methods of analysis. 16th ed. Association of Official Analytical Chemists, Washington D.C., U.S.A., 49-59.

Bugni TS and Chris M. 2004. Marine-derived fungi: a chemically and biologically diverse group of microorganism. Nat Prod Rep 21, 143-163.

Chapman VJ and Chapman DJ. 1980. Seaweeds and their uses. Chapman and Hall, London, U.K., 195.

Colliec S, Fischer AM, Tapon-Bretaudiere H, Boisson C, Durand P and Jozefonvicz J. 1991. Anticoagulant of a fucoidan fraction. Thrombosis Res 64, 143-147.

Fainesale S, Keranen T, Daransaari P and Hokaniemi F. 2005, GABA and glutamate transporters are expressed in human. Mol Brain Res 141, 161-165

Ferial HB, Mostafai E, Corinne S and Catherine BV. 2000. Relationship between sulfate groups and biological activities of fucans. Thromb Res 100, 453-459.

Han J, Kang S, Choue R, Kim H, Leem K, Chung S, Kim C and Chung J. 2002. Free radical scavenging effect of *Diospyros kaki, Laminaria japonica* and *Undaria pinatifida.* Fitoterapia 73, 710-712.

Heo SJ and Jeon YJ. 2005. Antioxidant effect and protection effect against cell damage by enzymatic hydrolysates from marine algae. J Food Ind Nutr 10, 31-41.

Hiroyuki N, Hideomi A, Koichi A and Kazutosi N. 1990. Antitumor activity of marine algae. Hydrobiologia 204, 577-584.

Jiménez-Escrig A, Jiménez-Jiménez I, Pulido R and Saura-

Calixto F. 2001. Antioxidant activity of fresh and processed edible seaweeds. J Sci Food Agric 81, 530-534.

Ministry for Food, Agriculture, Forestry and Fisheries, 2008. Major Statistics of Food, Agriculture, Forestry and Fisheries, 412-417.

Mody I, Dekoninck Y, Otis TS and Soltesz I. 1994. Bringing the cleft at GABA synapses in the brain. Trends Neurosci 17, 517-525.

Nanjo F, Goto K, Seto R, Suzuki M, Sakai M and Hara Y. 1996. Scavenging effects of tea catechins and their derivatives on 1,1,-diphenyl- 2-picrylhydrazyl radical. Free Radic Biol Med 21, 895-902.

Nakagawa K and Onota A. 1996. Accumulation of γ-aminobutyric acid (GABA) in the rice germ. Shokuhin Kaihatsu 31, 43-46.

Nishino T, Aizu Y and Nagumo T. 1991. The relationship between the molecular weight and the anticoagulant activity of two types of fucan sulfates from the brown seaweed *Ecklonia kurom*. Agric Biol Chem 55, 791-797.

Oh SH. 2003. Stimulation of gamma-aminobutyric acid synthesis activity in brown rice by a chitosan/glutamic acid germination solution and calcium/calmodulin. J Biochem Mol Biol 36, 319-25.

Park MJ and Han JS. 2006. Radical scavenging and antioxidant activities of fermented *Laminaria japonica* Extracts. J Food Sci Nutr 11, 10-16.

Park JH, Kang KC, Baek SB, Lee YH and Rhee KS. 1991. Separation of antioxidant compounds from edible marine algae. J Kor Food Sci Technol 23, 256-261.

Rokas A. 2009. The effect of domestication on the fungal proteome. TIG 25, 60-63.

Slimestad R, Vangdal E and Brede C. 2009. Analysis of phenolic compounds in six Norwegian plum cultivars (Prunus domestica L.). J Agric Food Chem 57, 1370-1375.

Somogy M. 1952. Notes on sugar determination. J Biol Chem 195, 19-23.

Usui T, Asari K and Mizuno T. 1980. Isolation of highly fucoidan from *Eisenia bicyclis* and its anticoagulant and antitumor activities. Agric Biol Chem 44, 1965-1970.

Yamane YI, Yocshii M, Mikami S, Fukuda H and Kizaki Y. 2000. A solid-state culture system using a cellulose carrier containing defined medium as a useful tool for investigating characteristics of koji culture. J Biosci Bioeng 89, 33-39.

(Received 18 January 2010; Revised 8 February 2010; Accepted 15 March 2010)