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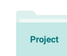

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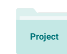

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Enzyme-assisted extraction of *Ecklonia cava* fermented with *Lactobacillus brevis* and isolation of an anti-inflammatory polysaccharide

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Fermentation and enzyme-assisted extraction (EAE) improve nutritional and functional properties of foods by increasing the extraction of active compounds, ingestion rates, and body absorption. In this study, we investigated whether applying the EAE process improves the extraction and isolation efficiency of a polysaccharide from fermented *Ecklonia cava* (FE), which inhibited NO production in lipopolysaccharide (LPS)-activated RAW 264.7 cells. The results showed that the FE using the fungi *Candida utilis* and two different bacteria, namely *Lactobacillus brevis* and *Saccharomyces cerevisiae* increased protein and carbohydrate contents in comparison with those in non-fermented *E. cava* (NE). Aqueous extracts of fermented *E. cava* increased extraction yields and carbohydrate content, compared with the aqueous extract of NE. In addition, treating LPS-stimulated RAW 264.7 cells with aqueous extracts resulted in reduced NO production compared to that in LPS-treated cells. Ten EAEs of *L. brevis*-fermented *E. cava* (LFE) improved NO inhibitory effects in LPS-activated RAW 264.7 cells and the Viscozyme extract (VLFE) from the resulting extracts showed the highest NO inhibitory effect. We found that the >30 kDa fraction of VLFE led to markedly high inhibition of LPS-induced NO production as compared to that in the <30 kDa fraction. The crude polysaccharide isolated from >30 kDa fraction (VLFEP) consisted of fucose and markedly decreased NO production induced by LPS stimulation. VLFEP could be useful as an anti-inflammatory agent to suppress macrophage activation.

Key Words: anti-inflammatory; *Ecklonia cava*; enzyme-assisted extraction (EAE); fermentation; *Lactobacillus brevis*; polysaccharide

INTRODUCTION

The brown seaweed *Ecklonia cava* (*Alariaceae)* contains various biologically active compounds such as fucoidan, alginate, fucan, laminarin, and phlorotannins (Guiry and Blunden 1991, Ahn et al. 2008*a*). Biological activity studies have shown that *E. cava* has anti-oxidant, anticancer, anticoagulant, immunomodulation, anti-inflammation, immune activation and matrix metalloproteinase inhibition effects *in vitro* (Athukorala et al. 2006, 2009, Kim et al. 2006*a*, 2006*b*, Ahn et al. 2007, 2008*a*, 2008*b*, 2011). Additionally, the major compounds of *E. cava* are polysaccharides and phlorotannins, which have anti-inflammatory and / or immune activation capacity

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(Ahn et al. 2008*a*, Jung et al. 2009). Due to these beneficial capacities, food and cosmetic industries have been developed related to the active ingredients in *E. cava*. However, when the active polyphenol and polysaccharide compounds were used in various industries, the industries using polyphenols suffered from non-water solubility, and high costs due to low extraction yields, compared to those using polysaccharides. Thus, isolating polysaccharides and improving their extraction efficiency can play an important role in the development of functional foods or products for the cosmetic industry.

Fermentation is used in plant foods to increase the nutritional quality and remove undesirable compounds (Frias et al. 2005). Several microbial fermentation products are also incorporated into food as additives and supplements such as antioxidants, flavors, colorants, preservatives, and sweeteners (Couto and Sanromán 2006). Microorganism-fermented foods play an important role, particularly in Asian countries, where the production process for many foods includes fungal fermentation (Geisen and Färber 2002). Microorganisms play a central role in the production of a wide range of primary and secondary metabolites.

In this study, we used enzyme-assisted extraction (EAE) followed by fermentation to improve extraction efficiency of the active polysaccharides in *E. cava*. Applying EAE and fermentation improves nutritional and functional properties of foods by increasing extraction of active compounds such as polysaccharides and peptides. Indeed, previous reports have demonstrated that, the extraction yield of active compounds increase after EAE and fermentation, leading to antioxidant and anti-inflammatory effects *in vitro* and *in vivo* (Link-Amster et al. 1994, Chen et al. 2008, Fernandez-Orozco et al. 2008, Matsushita et al. 2008).

Murine macrophage cells such as RAW 264.7 cells play a central role in the inflammatory response and serve as an essential interface between innate and adaptive immunity (Iontcheva et al. 2004). During the inflammatory process, large amounts of the proinflammatory mediator nitric oxide (NO) are generated by inducible isoforms of NO synthase (iNOS) and cyclooxygenase-2 (COX-2) (Vane et al. 1994). Additionally, abnormal NO production can be deleterious and has been implicated in the pathogenesis of various inflammatory diseases (Yun et al. 1996). For these reasons, inhibiting NO production is important to reduce the pathogenesis of various inflammatory diseases.

Therefore, the present study documented whether co-applying EAE and fermentation would improve the extraction yield of polysaccharide contents from *E. cava* and to determine whether the isolated polysaccharides inhibited NO production in lipopolysaccharide (LPS)-activated RAW 264.7 cells.

MATERIALS AND METHODS

Preparation of fermented *E. cava* (FE) samples

E. cava was collected from the coast of Jeju Island, South Korea, washed with fresh water, freeze-dried, and pulverized into powder with a grinder. Then, the freeze-dried *E. cava* was applied for fermentation using three kinds of microorganisms, namely *Lactobacillus brevis* (LFE), *Saccharomyces cerevisiae* (SFE), and *Candida utilis* (CFE) for 24 h. The fermentation process was carried out according to the method described by Đorđević et al. (2010) with some modifications. Each 15 grams of LFE, SFE, CFE, and non-fermented *E. cava* (NE) were homogenized with 300 mL of distilled water in a 1 L flask. Then, the mixture was autoclaved and allowed to cool before adding the microorganisms. The mixture was inoculated with 1% (v/v) of each microorganism separately, and the samples were allowed to ferment at 1,200 rpm and 30°C for 24 h. After the fermentation, the samples were freeze-dried and used for experiments.

Chemical composition analysis

The chemical composition of all samples was analyzed by measuring the contents of carbohydrate, protein, ash, and / or lipid from the weight difference after drying samples, according to the methods described by Association of Official Analytical Chemists.

Preparation of aqueous extracts from NE, LFE, SFE, and CFE

Aqueous extracts were prepared from NE, LFE, SFE, and CFE. One gram of freeze-dried sample was homogenized in distilled water (100 mL). After 24 h, the samples were obtained and kept at -20°C for further experiments.

Preparation of the LFE enzymatic extracts

LFE was used for EAE with several enzymes according to a previously reported method (Heo et al. 2005). Fifty grams of LFE was homogenized in distilled water (2 L) with 500 μL of 10 kinds of enzymes separately. Each re-

actant was adjusted to the optimum pH and temperature range of the respective enzyme, and enzymatic reactions were performed for 24 h. Following digestion, the digest was boiled for 10 min at 100°C to inactivate the enzymes. After centrifugation (3,000 rpm for 20 min at 4°C), the supernatant was adjusted to pH 7.0. The samples were kept at -20°C for further experiments.

Preparation of molecular weight fractions from the Viscozyme extract of LFE (VLFE)

Extracts were applied to a lab-scale tangential flow filtration system (Millipore, Billerica, MA, USA) using an ultra-filtration membrane (30 kDa) to prepare different molecular weight fractions Then, all fractions (whole extract, <30 kDa fraction, and >30 kDa fraction) were separately evaluated for their NO inhibitory effects.

Isolation of a polysaccharide from the >30 kDa fraction of VLFE (VLFEP)

VLFEP was isolated from a >30 kDa VLFEP by ethanol precipitation according to a slightly revised method from a previous study (Athukorala et al. 2009). The >30 kDa VLFE fraction (1 L) was mixed with 2 L of 99.5% ethanol for 24 h at 4°C. After centrifugation at 10,000 rpm for 20 min at 4°C, crude polysaccharides were collected from the precipitant. Then, the crude VLFEP was freeze-dried and used for subsequent experiments.

Analysis of mono-sugar and sulfate group contents

VLFE, and the >30 kDa and <30 kDa fractions as well as VLFEP were hydrolyzed in a sealed glass tube with 4 M trifluoroacetic acid for 4 h at 100°C to analyze neutral sugar content. The samples were digested using 6 N of HCl for 4 h to analyze mono-sugars. Then, the samples were separately applied to a CarboPac PA1 column (4.5 × 250 mm; Dionex, Sunnyvale, CA, USA) with a CarboPac PA1 cartridge (4.5 × 50 mm), respectively. The column was eluted using 16 mM NaOH at a 1.0 mL min^{-1} flow rate. Each sugar was detected using an ED50 Dionex electrochemical detector, and data were analyzed using Peak Net on-line software.

Nitrite assay

RAW 264.7 cells (murine macrophage cell line) were plated at a density of 1×10^5 cells $well^{-1}$ in 96-well plates for 16 h. The cells were pre-treated with aqueous and 10 enzymatic extracts of LFE, <30 kDa, and >30 kDa fractions of VLFE and VLFEP for 2 h and then stimulated with LPS (1 μg mL^{-1}) for 24 h. After the incubation, the culture media (100 μL $well^{-1}$) was mixed with 100 μL Griess reagent, an indicator of NO production (1% sulfanilamide in 2.5% phosphoric acid and 0.1% naphthylenediamine dihydrochloride in distilled water), for 10 min, and the absorbance of the mixture was measured at 540 nm using a microplate reader (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The nitrite levels were obtained from a sodium nitrite standard curve. The experimental results represented one of three experiments and were expressed as the mean of triplicate determinations.

Statistical analysis

Data were analyzed using SPSS for Windows version 10 (SPSS Inc., Chicago, IL, USA). Values are expressed as means ± standard errors. A p-value < 0.05 was considered significant.

RESULTS

The three FEs showed plentiful carbohydrate and protein content after fermentation (Table 1). In contrast, we identified three FE samples in which the carbohydrate content increased slightly, but their protein content increased markedly, compared to that of NE. Increased extraction yields were observed for aqueous extract of LFE (ALFE), aqueous extract of SFE (ASFE), and aqueous extract of CFE (ACFE) (Table 2). Additionally, carbohydrate content increased markedly following fermentation. Among them, ALFE showed the highest carbohydrate content and extraction yield. Further study showed that ALFE, ASFE, and ACFE led to higher NO production in-

Table 1. Chemical composition of NE, LFE, SFE, and CFE

	NE	LFE	SFE	CFE
Carbohydrate	33.55 ± 0.97	45.04 ± 0.41	41.03 ± 1.34	44.05 ± 1.01
Crude fiber	30.80 ± 0.46	5.80 ± 0.11	8.50 ± 0.87	6.10 ± 0.31
Crude protein	11.02 ± 1.13	29.56 ± 0.34	31.87 ± 0.45	30.75 ± 0.81
Moisture	9.36 ± 0.02	6.46 ± 0.34	3.45 ± 0.10	4.62 ± 0.21
Crude ash	14.00 ± 1.41	10.50 ± 0.71	12.00 ± 0.52	11.50 ± 0.71
Crude lipid	1.27 ± 0.70	2.64 ± 0.30	3.15 ± 0.47	2.98 ± 0.30

Values are presented as percentage.
NE, non-fermented *Ecklonia cava*; LFE, *Lactobacillus brevis*-fermented *E. cava*; SFE, *Saccharomyces cerevisiae*-fermented *E. cava*; CFE, *Candida utilis*-fermented *E. cava*.

hibitory effects in LPS stimulated RAW 264.7 cells compared to those treated with aqueous extract of NE (ANE) (Fig. 1A). However, the NO inhibitory effects were similar together. Therefore, we selected LFE for the next experiments, because it showed the highest extraction yield and carbohydrate content. In the next experiment, 10 LFE enzymatic extracts were prepared using five carbohydrates and five proteases. Markedly increased extraction yields

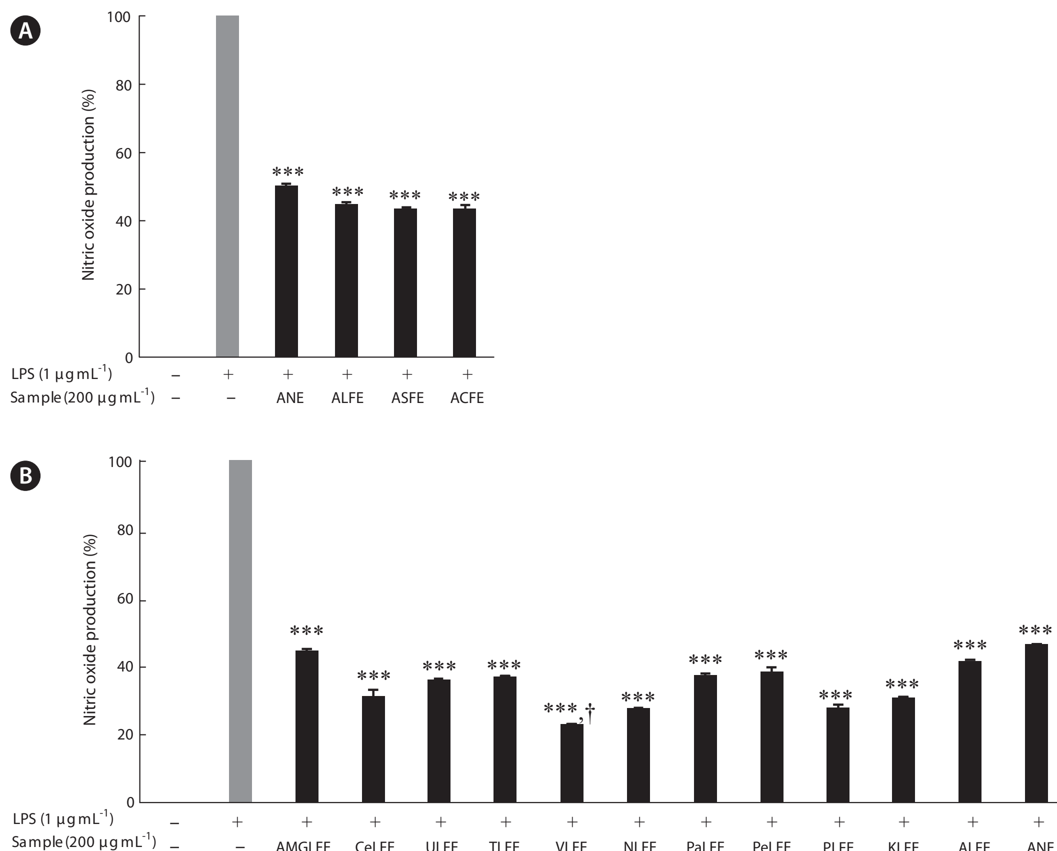

Fig. 1. Effects of aqueous extracts (A) those prepared from FE and NE, and 10 LFE enzymatic extracts (B) on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW 264.7 cells. (A) After 24 h stimulation with LPS, the NO production inhibitory effects of ANE, ALFE, ASFE, and ACFE were identified by NO assay. (B) After 24 h of LPS stimulation, NO production inhibitory effects of 10 LFE enzymatic extracts were measured by NO assay. Experiments were performed in triplicate, and data are expressed as mean ± standard error. FE, fermented *Ecklonia cava*; NE, non-fermented *E. cava*; LFE, *Lactobacillus brevis*-fermented *E. cava*; ANE, aqueous extract of NE; ALFE, aqueous extract of LFE; ASFE, aqueous extract of *Saccharomyces cerevisiae*-fermented *E. cava*; ACFE, aqueous extract of *Candida utilis*-fermented *E. cava*; AMGLFE, AMG extract of LFE; CeLFE, Celluclast extract of LFE; ULFE, Ultraflo extract of LFE; TLFE, termamyl extract of LFE; VLFE, Viscozyme extract of LFE; NLFE, Neutrase extract of LFE; PaLFE, papain extract prepared from LFE; PeLFE, pepsin of LFE; PLFE, Protamex extract of LFE; KLFE, Kojizyme extract of LFE. $^{***}p < 0.005$ vs. LPS-treated RAW 264.7 cells, $^{\dagger}p < 0.005$ vs. the other enzyme extracts of LFE- and LPS-treated RAW 264.7 cells.

Table 2. Chemical composition and extraction yield of ANE, ALFE, ASFE, and ACFE

		ANE	ALFE	ASFE	ACFE
Chemical composition	Yields	30.50 ± 0.50	53.67 ± 2.14	51.33 ± 1.00	53.33 ± 2.00
	Carbohydrate	33.30 ± 0.20	38.79 ± 0.25	27.84 ± 0.25	29.80 ± 0.08
	Total phenol	10.31 ± 0.50	8.68 ± 1.35	8.69 ± 0.51	8.57 ± 1.71
	Protein	10.19 ± 1.25	13.30 ± 2.23	16.47 ± 1.21	11.64 ± 0.42
	Other	46.20 ± 1.44	39.23 ± 0.64	47.00 ± 2.11	49.99 ± 0.79

Values are presented as percentage.
ANE, aqueous extract of NE; ALFE, aqueous extract of LFE; ASFE, aqueous extract of SFE; ACFE, aqueous extract of CFE.

were obtained from all enzymatic extract treatments (Table 3). In addition, LFE enzyme extracts strongly inhibited the NO production induced by LPS stimulation in RAW 264.7 cells (***$p < 0.005$ vs. LPS-treated RAW 264.7 cells) (Fig. 1B). Among them, celluclast extract of LFE, VLFE, neutrase extract of LFE, protamex extract of LFE, and kojizyme extract of LFE showed higher inhibitory effects on NO production induced by LPS stimulation in RAW 264.7 cells compared to those of ANE and ALFE (Fig. 1B). In particular, VLFE showed the highest inhibitory effect on NO production in LPS-treated RAW 264.7 cells (†$p < 0.005$ vs. the other LFE enzyme extracts and LPS-treated RAW 264.7 cells). Moreover, VLFE dose-dependently decreased NO production at all concentrations from 25-200 μg mL^{-1} in comparison with that in LPS-treated cells (***$p < 0.005$ vs. LPS-treated RAW 264.7 cells) (Fig. 2A).

As shown in Fig. 2B, both fractions (<30 kDa fraction and >30 kDa fraction) decreased the NO production induced by LPS stimulation and, particularly, the >30 kDa fraction led to a markedly high inhibitory effect on LPS-induced NO production, as compared to that of the <30 kDa fraction at all concentrations (***$p < 0.005$ vs. LPS-treated RAW 264.7 cells).

The crude polysaccharide isolated from VLFE (VLFEP) showed the highest carbohydrate content among the others (Table 4). Additionally, fucose and mannose contents increased with the increase in carbohydrate content in VLFE. In particular, VLFEP showed the highest fucose

Table 3. Extraction yields of ANE and 10 enzymatic extracts prepared from LFE

Enzymatic extracts		Yields
Carbohydrases	AMGLFE	56.00 ± 1.12
	CeLFE	62.00 ± 0.97
	ULFE	58.50 ± 1.25
	TLFE	62.00 ± 1.41
	VLFE	57.00 ± 1.69
Proteases	NLFE	63.50 ± 1.02
	PaLFE	62.50 ± 1.36
	PeLFE	87.50 ± 1.44
	PLFE	66.50 ± 0.88
	KLFE	68.50 ± 1.01
	ALFE	53.70 ± 0.93
	ANE	30.50 ± 0.30

Values are presented as percentage.
AMGLFE, AMG extract of LFE; CeLFE, Celluclast extract of LFE; ULFE, Ultraflo extract of LFE; TLFE, Termamyl extract of LFE; VLFE, Viscozyme extract of LFE; NLFE, Neutrase extract of LFE; PaLFE, papain extract prepared from LFE; PeLFE, pepsin of LFE; PLFE, Protamex extract of LFE; KLFE, Kojizyme extract of LFE; ALFE, aqueous of LFE; ANE, aqueous extract of NE.

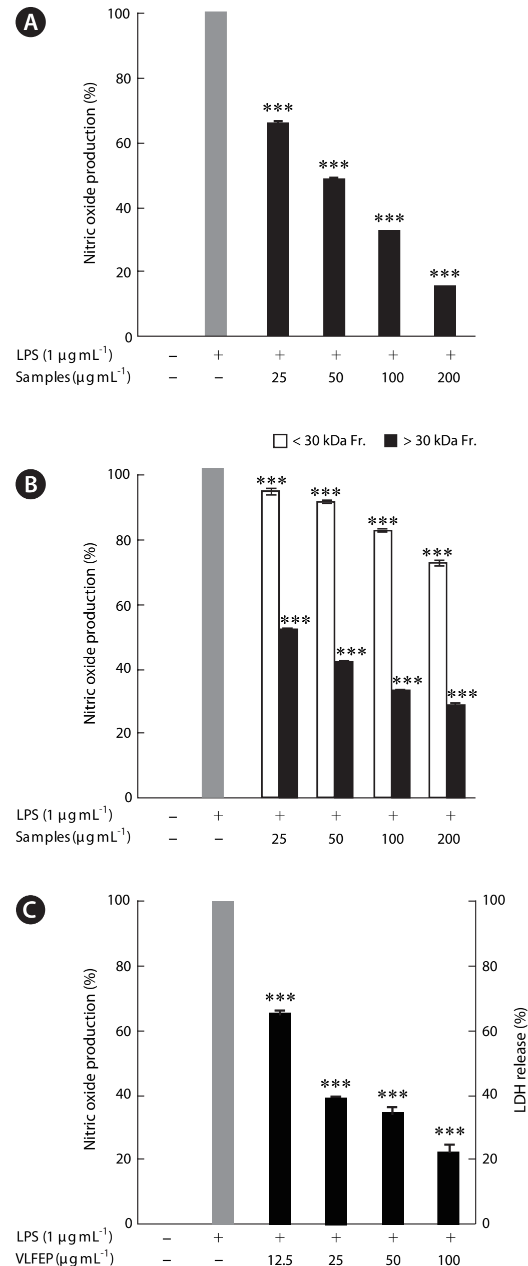

Fig. 2. Effects of VLFE (A), <30 kDa and >30 kDa fractions of VLFE (B), and VLFEP (C) on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW 264.7 cells. After 24 h of LPS stimulation, inhibitory effects of VLFE, the <30 kDa and >30 kDa fractions of VLFE, and VLFEP on NO production were measured by NO assay. Experiments were performed in triplicate, and data are expressed as mean ± standard error. VLFE, Viscozyme extract of LFE; <30 kDa Fr., <30 kDa fraction of VLFE; >30 kDa Fr., >30 kDa fraction of VLFE; VLFEP, crude polysaccharide of VLFE. ***$p < 0.005$ vs. LPS-treated cells.

Table 4. Mono-saccharide contents of ANE, VLFE, its >30 kDa fraction and VLFEP

	ANE	ALFE	VLFE	>30 kDa Fr.	VLFEP
Yields	30.50 ± 0.50	53.67 ± 2.14	58.14 ± 1.45	-	-
Protein	10.19 ± 1.25	13.30 ± 2.23	6.58 ± 0.98	8.90 ± 0.47	12.40 ± 1.04
Polyphenol	10.31 ± 0.50	8.68 ± 1.35	4.30 ± 0.43	5.47 ± 0.33	6.04 ± 0.77
Carbohydrate	33.30 ± 0.20	38.79 ± 0.25	39.22 ± 1.48	42.16 ± 1.54	60.98 ± 2.02
Mono-saccharides[a]					
Fucose	48.81 ± 0.45	33.12 ± 1.02	28.98 ± 1.09	56.31 ± 2.01	73.43 ± 3.02
Galactose	25.89 ± 0.23	22.14 ± 0.12	19.38 ± 0.78	0.00 ± 0.44	0.00 ± 0.32
Glucose	9.21 ± 0.03	31.43 ± 1.12	40.10 ± 1.15	26.03 ± 1.02	2.14 ± 0.98
Mannose	1.38 ± 0.02	6.73 ± 0.55	11.54 ± 0.76	17.66 ± 0.85	24.43 ± 1.22
Etc.[b]	14.71 ± 0.13	6.58 ± 0.34	0.00 ± 0.04	0.00 ± 0.22	0.00 ± 0.53
Etc.[c]	46.20 ± 1.44	39.23 ± 0.64	49.90 ± 1.12	43.47 ± 1.60	20.58 ± 1.07

Values are presented as percentage.
ANE, aqueous extract of NE; ALFE, aqueous extract of LFE; VLFE, Viscozyme extract of LFE; VLFEP, crude polysaccharide of VLFE.
[a]Mono-saccharides: The contents of mono-saccharides contained fucose, galactose, glucose, mannose and etc. and were expressed as 100% in carbohydrate contents.
[b]Etc. contained the contents of Arabinose, Rhamnose and Xylose.
[c]Etc. consisted of the fiber, moisture, ash and lipid contents.

content among VLFE and its >30 kDa fraction. Furthermore, VLFEP was not cytotoxic at any concentration and dose-dependently induced a higher inhibitory effect on NO production caused by LPS stimulation in Raw 264.7 cells ($^{***}p < 0.005$ vs. LPS-treated RAW 264.7 cells) (Fig. 2C). Interestingly, the NO inhibitory effect of the samples in LPS-stimulated Raw 264.7 cells corresponded to the increase in fucose content.

DISCUSSION

Many researchers have reported that fermented materials have immune response and oxidative stress activities *in vitro* and *in vivo* (Xia et al. 1990, Fan et al. 2009, Joo et al. 2009, Kuo et al. 2009). In this study, three different microorganisms were used with EAE to improve the extraction efficiency of biologically active polysaccharides from the brown seaweed *E. cava*. However, the increase in relative protein content in the FE might have been caused by proliferation of the fungi or bacteria during fermentation.

Our previous studies reported that *E. cava* enzymatic extracts prepared using the EAE technique contain hydrophilic polysaccharides and protein compounds (Heo et al. 2003, Ahn et al. 2011). Thus, we thought that the active compounds showing an NO inhibitory effect might be hydrophilic components, so we isolated the polysaccharide content from VLFE.

An ultra-filtration system using a 30 kDa molecular weight cut-off membrane is another technique for isolating specific hydrophilic compounds such as saccharides. In this study, we also applied an ultra-filtration process and obtained two molecular weight fractions from VLFE. Polysaccharides from natural sources are a class of macromolecules that can profoundly affect the immune system and, therefore, have the potential to be immunomodulators with wide clinical applications (Tzianabos 2000). Previous studies have indicated that various polysaccharides show beneficial antiinflammatory effects such as NO inhibition (Cui et al. 2010, Jung et al. 2010). Our results suggest that VLFEP might be an active compound exhibiting NO inhibition effects in RAW 264.7 cells.

Therefore, we first isolated a crude polysaccharide fraction from the >30 kD fraction. Then, we identified the chemical composition including protein, polyphenols, carbohydrates, and the mono-sugar content such as fucose, galactose, glucose, and mannose which are standard components of polysaccharides.

Our results demonstrated that co-applying fermentation, EAE, and an ultra-filtration membrane system improved extraction of fucose and lead to a decrease in NO production in LPS stimulated Raw 264.7 cells. Many studies have reported that NO is generated by iNOS and COX-2 during inflammatory processes, and that abnormal NO production causes the pathogenesis of various inflammatory diseases (Vane et al. 1994, Yun et al. 1996). Hence, inhibiting NO production is important to reduce

the pathogenesis of various inflammatory diseases. NO, COX-2, and iNOS are regulated by a variety of inflammatory cytokines including tumor necrosis factor-α, interleukin (IL)-1β, and IL-6 in LPS-activated RAW 264.7 cells (Chang et al. 2005). Moreover, many researchers have demonstrated that the transcription and secretion of pro-inflammatory mediators and cytokines are mediated by activation of the IκB / nuclear transcription factor kappa-B (NFκB) signal transduction pathway in LPS-activated RAW 264.7 cells (Surh et al. 2001, Lappas et al. 2002). This suggest that the NO inhibition effects of VLFEP might be mediated by reducing pro-inflammatory mediators and cytokines via inhibition of the NFκB signal transduction pathway activated by LPS stimulation in RAW 264.7 cells. Therefore, further study about the biological mechanisms related with the NO inhibitory effect of VLFEP is required.

Taken together, our results suggest that applying fermentation, EAE, and an ultra-filtration system improved the isolation of a polysaccharide component from *E. cava*, which contained plentiful fucose. The isolated polysaccharide showed profound NO inhibitory effects in LPS-stimulated RAW 264.7 cells. Thus, VLFEP might be useful as an anti-inflammatory agent to suppress macrophage activation.

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REFERENCES

Ahn, G., Hwang, I., Park, E., Kim, J., Jeon, Y. -J., Lee, J., Park, J. W. & Jee, Y. 2008*a*. Immunomodulatory effects of an enzymatic extract from *Ecklonia cava* on murine splenocytes. Mar. Biotechnol. 10:278-289.

Ahn, G., Park, E., Kim, D. S., Jeon, Y. -J., Shin, T., Park, J. W., Woo, H. -C., Lee, W. -W. & Jee, Y. 2008*b*. Anti-inflammatory effects of enzymatic extract from *Ecklonia cava* on TPA-induced ear skin edema. Food Sci. Biotechnol. 17:745-750.

Ahn, G., Park, E., Lee, W. -W., Hyun, J. -W., Lee, K. -W., Shin, T., Jeon, Y. -J. & Jee, Y. 2011. Enzymatic extract from Ecklonia cava induces the activation of lymphocytes by IL-2 production through the classical NF-κB pathway. Mar. Biotechnol. 13:66-73.

Ahn, G. -N., Kim, K. -N., Cha, S. -H., Song, C. -B., Lee, J., Heo, M. -S., Yeo, I. -K., Lee, N. -H., Jee, Y. -H., Kim, J. -S., Heu, M. -S. & Jeon, Y. -J. 2007. Antioxidant activities of phlorotannins purified from *Ecklonia cava* on free radical scavenging using ESR and H_2O_2-mediated DNA damage. Eur. Food Res. Technol. 226:71-79.

Athukorala, Y., Ahn, G. N., Jee, Y. -H., Kim, G. -Y., Kim, S. -H., Ha, J. -H., Kang, J. -S., Lee, K. -W. & Jeon, Y. -J. 2009. Antiproliferative activity of sulfated polysaccharide isolated from an enzymatic digest of *Ecklonia cava* on the U-937 cell line. J. Appl. Phycol. 21:307-314.

Athukorala, Y., Jung, W. -K., Vasanthan, T. & Jeon, Y. -J. 2006. An anticoagulative polysaccharide from an enzymatic hydrolysate of *Ecklonia cava*. Carbohydr. Polym. 66:184-191.

Chang, H. -P., Huang, S. -Y. & Chen, Y. -H. 2005. Modulation of cytokine secretion by garlic oil derivatives is associated with suppressed nitric oxide production stimulated macrophages. J. Agric. Food Chem. 53:2530-2534.

Chen, I. -N., Ng, C. -C., Wang, C. -Y. & Chang, T. -L. 2008. Lactic fermentation and antioxidant activity of Zingiberaceae plants in Taiwan. Int. J. Food Sci. Nutr. 60:57-66.

Couto, S. R. & Sanromán, M. Á. 2006. Application of solid-state fermentation to food industry: a review. Food Engin. 76:291-302.

Cui, Y. -Q., Zhang, L. -J., Zhang, T., Luo, D. -Z., Jia, Y. -J., Guo, Z. -X., Zhang, Q. -B., Wang, X. & Wang, X. -M. 2010. Inhibitory effect of fucoidan on nitric oxide production in lipopolysaccharide-activated primary microglia. Clin. Exp. Pharmacol. Physiol. 37:422-428.

Đorđević, T. M., Šiler-Marinkovic, S. S. & Dimitrijević-Branković, S. I. 2010. Effect of fermentation on antioxidant properties of some cereals and pseudo cereals. Food Chem. 119:957-963.

Fan, J., Zhang, Y., Chang, X., Saito, M. & Li, Z. 2009. Changes in the radical scavenging activity of bacterial-type douchi, a traditional fermented soybean product, during the primary fermentation process. Biosci. Biotechnol. Biochem. 73:2749-2753.

Fernandez-Orozco, R., Frias, J., Muñoz, R., Zielinski, H., Piskula, M. K., Kozlowska, H. & Vidal-Valverde, C. 2008. Effect of fermentation conditions on the antioxidant compounds and antioxidant capacity of *Lupinus angustifolius cv. Zapaton*. Eur. Food Res. Technol. 227:979-988.

Frias, J., Miranda, M. L., Doblado, R. & Vidal-Valverde, C. 2005. Effect of germination and fermentation on the antioxidant vitamin content and antioxidant capacity of *Lupinus albus* L. var. Multolupa. Food Chem. 92:211-220.

Geisen, R. & Färber, P. 2002. New aspects of fungal starter cul-

tures for fermented foods. Appl Microbiol 2:13-29.

Guiry, M. D. & Blunden, G. 1991. *Seaweed resources in Europe: uses and potential*. John Wiley & Sons Ltd., Chichester, West Sussex, 432 pp.

Heo, S. -J., Lee, K. -W., Song, C. B. & Jeon, Y. -J. 2003. Antioxidant activity of enzymatic extracts from brown seaweeds. Algae 18:71-81.

Heo, S. -J., Park, E. -J., Lee, K. -W. & Jeon, Y. -J. 2005. Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresour. Technol. 96:1613-1623.

Iontcheva, I., Amar, S., Zawawi, K. H., Kantarci, A. & Van Dyke, T. E. 2004. Role for moesin in lipopolysaccharide-stimulated signal transduction. Infect. Immun. 72:2312-2320.

Joo, S. S., Won, T. J., Nam, S. Y., Kim, Y. -B., Lee, Y. C., Park, S. -Y., Park, H. Y., Hwang, K. W. & Lee, D. I. 2009. Therapeutic advantages of medicinal herbs fermented with *Lactobacillus plantarum*, in topical application and its activities on atopic dermatitis. Phytother. Res. 23:913-919.

Jung, J. -S., Shin, J. A., Park, E. -M., Lee, J. -E., Kang, Y. -S., Min, S. -W., Kim, D. -H., Hyun, J. -W., Shin, C. -Y. & Kim, H. -S. 2010. Anti-inflammatory mechanism of ginsenoside Rh1 in lipopolysaccharide-stimulated microglia: critical role of the protein kinase A pathway and hemeoxygenase-1 expression. J. Neurochem. 115:1668-1680.

Jung, W. -K., Heo, S. -J., Jeon, Y. -J., Lee, C. -M., Park, Y. -M., Byun, H. -G., Choi, Y. -H., Park, S. -G. & Choi, I. -W. 2009. Inhibitory effects and molecular mechanism of dieckol isolated from marine brown alga on COX-2 and iNOS in microglial cells. J. Agric. Food Chem. 57:4439-4446.

Kim, K. -N., Heo, S. -J., Song, C. B., Lee, J., Heo, M. -S., Yeo, I. -K., Kang, K. A., Hyun, J. W. & Jeon, Y. -J. 2006*a*. Protective effect of *Ecklonia cava* enzymatic extracts on hydrogen peroxide-induced cell damage. Process Biochem. 41:2393-2401.

Kim, M. -M., Ta, Q. V., Mendis, E., Rajapakse, N., Jung, W. -K., Byun, H. -G., Jeon, Y. -J. & Kim, S. -K. 2006*b*. Phlorotannins in *Ecklonia cava* extract inhibit matrix metalloproteinase activity. Life Sci. 79:1436-1443.

Kuo, C. -F., Chyau, C. -C., Wang, T. -S., Li, C. -R. & Hu, T. -J. 2009. Enhanced antioxidant and anti-inflammatory activities of *Monascus pilosus* fermented products by addition of turmeric to the medium. J. Agric. Food Chem. 57:11397-11405.

Lappas, M., Permezel, M., Georgiou, H. M. & Rice, G. E. 2002. Nuclear factor kappa B regulation of proinflammatory cytokines in human gestational tissues *in vitro*. Biol. Reprod. 67:668-673.

Link-Amster, H., Rochat, F., Saudan, K. Y., Mignot, O. & Aeschlimann, J. M. 1994. Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. FEMS Immunol. Med. Microbiol. 10:55-63.

Matsushita, H., Kobayashi, M., Tsukiyama, R., Fujimoto, M., Suzuki, M., Tsujii, K. & Yamamoto, K. 2008. Stimulatory effect of *Shoyu* polysaccharides from soy sauce on the intestinal immune system. Int. J. Mol. Med. 22:243-247.

Surh, Y. -J., Chun, K. -S., Cha, H. -H., Han, S. S., Keum, Y. -S., Park, K. -K. & Lee, S. S. 2001. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. Mutat. Res. 480-481:243-268.

Tzianabos, A. O. 2000. Polysaccharide immunomodulators as therapeutic agents: structural aspects and biologic function. Clin. Microbiol. Rev. 13:523-533.

Vane, J. R., Mitchell, J. A., Appleton, I., Tomlinson, A., Bishop-Bailey, D., Croxtall, J. & Willoughby, D. A. 1994. Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation. Proc. Natl. Acad. Sci. U. S. A. 91:2046-2050.

Xia, D., Lin, Z. B., Ma, L. & Li, Z. L. 1990. Effects of the polysaccharides isolated from mycelium and fermentation fluid of Schizophyllum commune on immune function in mice. Yao Xue Xue Bao 25:161-166.

Yun, H. Y., Dawson, V. L. & Dawson, T. M. 1996. Neurobiology of nitric oxide. Crit. Rev. Neurobiol. 10:291-316.

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