A THESIS

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Effects of Far Infrared Radiation Drying on Antioxidant and Anticoagulant Activities of Extracts from *Ecklonia cava*

Seung-Hong Lee

Department of Aquatic Life Medicine

GRADUATE SCHOOL

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Seung-Hong Lee

(Supervised by Professor You-Jin Jeon)

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This thesis has been examined and approved by

Thesis director, Ki-Wan Lee, Professor of Marine Life Science

Soo-Hyun Kim, Professor of Food Bioengineering

You-Jin Jeon, Professor of Aquatic Life Medicine

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Date

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CONTENTS

국문초록

최근 노화와 성인병 질환의 원인으로 생체 내에서 발생하는 hydroxyl radical(·OH)과 superoxide anion($\cdot O_2^-$) 및 hydrogen peroxide(H_2O_2)와 같은 활성산소종(reactive oxygen species; ROS)의 산화대사 부산물이 중요 원인이 된다는 학설이 있으며, 이들 ROS는 세포막 지방질을 과산화시키고 세포막투과성의 변화를 초래하여 DNA 손상을 유발시킨다고 보고되었다. 이러한 활성산소는 정상적인 세포 대사과정에서도 일부 생성되어 체내에서 각종 항산화효소와 항산화제로 이를 제어하고 있다. 오늘날 널리 이용되고 있는 합성 항산화제는 butylated hydroxyanisole(BHA)와 butylated hydroxytoluene (BHT) 및 tertiary butylhydroquinone (TBHQ)이 알려져 있으며, 이들이 뛰어난 항산화력 페놀계 항산화제이다. 이러한 합성 항산화제는 약한 활성과 독성 및 발암성의 안전성 문제가 지적되어 활성산소종을 효과적으로 제어시켜 안전하고 경제적인 천연물 생리활성 물질에 관한 연구들이 진행되고 있다.

최근 우리나라도 식생활의 서구화와 환경의 오염 및 운동부족에 의한 성인병의 발병율이 높아지고 있다. 그 중 혈관 내 생성된 혈전이 혈관벽에 점착하거나 미세혈관을 막아 혈류를 방해함으로써 야기되는 혈액 순환계질환이 큰 비중을 차지하고 있다. 항응고 활성이란 혈액의 응고를 억제하는 활성으로 혈액의 응고는 혈전의 주성분인 불용성의 섬유소를 형성하는 과정으로 12단계 이상의 단백분해 과정이 cascade식으로 일어나는 복잡한 생화학적 반응이다. 비정상적인 혈전생성은 혈류부전과 혈관상해 및 고혈압 그리고 지질침착 등의 이유로 혈관내에서 유발되며 뇌출혈과 뇌혈전 및 심부전 그리고 심근경색과 동맥경화증 같은 중대한 성인병을 일으킨다.

제주도는 지형학적 특성으로 해산식물의 보고라고 할만큼 풍부한 해조류가 서

식하고 있다. 해조류는 다당류를 함유하고 다양한 미네랄과 비타민이 풍부하게 함유하여 어떤 특정 성분에서는 항균과 항산화 및 항바이러스 그리고 항암활성을 비롯하여 동맥경화와 심근경색 및 고혈압 그리고 협심증과 뇌졸증 같은 성인병 예방에 효과적이라는 연구결과에 의해 식품산업과 의약산업 및 화장품산업에서 다양한 용도로 산업적 측면에서 매우 큰 파급효과가 기대된다. 이들중 감태(*Ecklonia cava*)는 갈조류의 일종으로 polyphenol과 polysaccharide을 풍부하게 함유하고 항산화성과 항암성 및 항응고성 기능성들이 밝혀짐으로 새로운 기능성 소재로 충분히 개발할 가치가 있음이 많은 연구자들에 의해 보고되고 있다.

이러한 해조류는 가공할 때 건조과정을 거치는데 현재까지 해조류 건조는 천일건조와 고온건조법이 주로 이용되고 있다. 그러나 이러한 건조과정은 chemical composition과 biologically functional compounds의 변화를 초래하게 된다. 이러한 단점을 보완하기 위하여 식품산업에서 유용하게 쓰이는 동결건조법을 많이 이용하고 있지만 동결건조는 상대적으로 건조시간이 길고 비용이 비싸다는 단점을 가지고 있다. 이에 반해 원적외선 건조는 약 3.0~1,000 μm의 파장대로 에너지가 직접 열로 변환되어 전열 속도가 빠른 특징을 가진다. 이런 특성은 건조물을 균일하게 가열하여 시간을 단축시킴으로서 에너지 절감 효과를 얻을 수 있고 열분해에 의한 영양물질 또는 생리활성물질의 손실을 최소화 할 수 있다. 이로 인해 농산품 및 식품 분야에 최근 많이 적용되고 있다. 또한 의약 분야에서도 *in vivo* 상으로 실험한 결과 쥐의 생존력이 증가하며, 체력과 지구력이 증강되는 생리활성이 나타내기도 하였고, 천연 항산화 물질들은 중합체인 polyphenol과 tocophenol 및 flavonoid 등의 고분자를 가지고 있는데 원적외선 처리가 이들을 저분자로 유리시켜 항산화능이 증가되었다는 결과들을 보고하였다.

생리활성이 양호하다고 보고된바 있는 여러 식물들을 기존의 추출방법으로 추출할 때 낮은 추출효율과 이로 인한 에너지 소비가 많으며 열로 인한 많은 유용

성분의 파괴와 단백질의 변이 및 성분의 손실 그리고 가용성분 위주의 추출과열에 대하여 불안정한 것과 같은 단점을 드러내고 있다. 이에 반해 최근 대두되고 있는 초음파 추출 방법은 전통적인 용매 추출방법과는 달리 많은 경비가 소요되지 않으면서 추출공정의 효율을 높이고 식물성분의 용매 추출에 효과를 나타내는 등 최근 관심이 집중되고 있는 실정이다. 용매추출과 초음파 추출을 비교한 실험에서 초음파 에너지를 용매추출공정에 도입하여 추출한 결과 초음파 조사시간이 증가함에 따라 추출량은 증가하였고, 이는 용매추출에 비해 매우 짧은 시간에 추출이 완료된 것으로 초음파의 공동현상에 의한 높은 압력으로 세포 내부조직이 파괴되어 지방질의 이동거리가 짧아지고 확산이 용이하게 일어나기 때문이라는 결과를 보고한바 있다.

이에 이 연구는 항산화와 항응고에서 뛰어난 활성을 가지고 있다고 알려진 감태로부터 원적외선 건조에 의한 다양한 추출물을 제조하여 항산화활성 및 항응고활성을 조사하였으며 또한 초음파 추출법을 이용한 추출물의 항산화활성과 추출수율의 향상을 검토하였다. 원적외선 건조에 의한 감태 추출물의 항산화활성을 검토하기 위하여 라디칼 소거활성을 측정하였고, 정상세포인 Vero cell의 산화적 손상 억제활성을 측정하였다. 그 결과 원적외선 80℃의 건조조건를 제외한 모든 추출물에서 동결건조에 의한 추출물과 비교하여 높은 라디칼 소거활성과 우수한 세포 보호효과에 의한 Vero cell의 산화적 손상을 억제하였다. 특히, 원적외선 40℃의 건조조건에서의 모든 추출물이 가장 우수한 활성을 나타냈다. 원적외선 건조에 의한 감태 추출물의 항응고활성을 검토하기 위하여 동결건조에 의한 추출물과 비교해 APTT 활성을 측정하였다. 그 결과 crude polysaccharide fraction에서는 비슷한 결과를 나타냈지만 crude phenolic fraction에서는 원적외선 건조에 의한 추출물이 높게 나타났다. 초음파 추출법을 이용한 감태 추출물의 항산화활성과 추출수율의 향상을 검토한 결과는 초음파 추출시간이 증가함에 따라 항산화활성

과 추출수율이 증가하였고, alkyl radical, hydrogen peroxide 소거활성 및 DNA 손상 억제활성에서 12시간 초음파 추출법을 이용한 추출물이 24시간 전통적인 추출법에 비해 높은 활성을 나타내었다.

이러한 결과를 종합하면, 감태 추출물의 항산화활성과 항응고활성은 원적외선 건조가 효과적임을 입증하였으며 초음파 추출법 또한 효과적이라 판단된다. 이 연구에서 감태를 재료로 수행된 원적외선 건조와 초음파 추출법은 기존의 방법에 비하여 높은 활성과 시간 및 경비절감과 같은 장점이 있어 식품산업과 의약산업 및 화장품산업과 같은 분야에 경제적 파급효과를 기대할 수 있는 방법으로 판단된다.

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Fig. 3-5. The effect of supplementation in vitro with different concentrations of FIRD40-100MEC24h and FIRD40-100MEU12h on DNA damage of H_2O_2-induced L5178 cell. (A) FIRD40-100MEU12h, (B) FIRD40-100MEC24h. Mean±SE of determinations was made in duplicate experiments. (□ : % Fluorescence in tail, -◆- : Inhibitory effect of cell damage)

Fig. 3-6. Comet images of L5178 cells: (A) negative control; (B) 50 uM H_2O_2 (C) 25 ug/ml FIRD40-100MEU12h + 50 uM H_2O_2 (D) 50 ug/ml FIRD40-100MEU12h + 50 uM H_2O_2 (E) 100 ug/ml FIRD40-100MEU12h + 50 uM H_2O_2 (F) 25 ug/ml FIRD40-100MEC24h + 50 uM H_2O_2 (G) 50 ug/ml FIRD40-100MEC24h + 50 uM H_2O_2 (H) 100 ug/ml FIRD40-100MEC24h + 50 uM H_2O_2

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Part I

Effects of far infrared radiation drying on antioxidant and anticoagulant activities of extracts from *Ecklonia cava*

Abstract

This study investigated the effect of the antioxidant and anticoagulant activity of various water-soluble extracts (Water, Celluclast and Protamex extract) from *Ecklonia cava* dried by far infrared radiation drying at 40~80℃ (FIRD) as compared to those of extracts by freeze drying (FD). The total polyphenolic content were determined. In addition, the antioxidant activity was determined by measuring the free radical (DPPH, alkyl radical, hydroxyl radical and hydrogen peroxide) scavenging activity. The anticoagulant activity was determined by measuring activated partial thromboplastin time (APTT). In case of FIRD, total polyphenolic content decreased as the drying temperature was increased. But, FIRD extracts had a higher total polyphenolic content than FD extracts. Among them, Celluclast extracts from *E. cava* dried by FIRD at 40℃ showed the highest total polyphenolic content (5.50 g/100 g). All the extracts from the dried *E. cava* by FIRD at 40~80℃ and FD showed strong antioxidant activity. In case of FIRD, radical scavenging activity decreased as the drying temperature was increased. Most FIRD extracts except for a dry condition at 80℃ had a lower IC_{50} value than FD extracts. Among them, water extracts from *E. cava* dried by FIRD at 40℃ showed the lowest IC_{50} value (DPPH, hydroxyl radical, alkyl radical and hydrogen peroxide). All extracts were subjected to APTT assay to estimate their anticoagulant efficacy. In case of FIRD, APTT activity decreased as the drying temperature was increased. Comparatively, FD extracts showed higher APTT activity than FIRD extracts. In all the tested samples for anticoagulant activity of extracts, Celluclast extracts from *E. cava* dried by FD (FD-CE) and FIRD at 40℃ (FIRD40-CE) were selected and a crude polysaccharide fraction with molecular weight above 30 kDa was obtained. The crude polysaccharide fraction of FD-CE and FIRD40-CE showed the APTT activity (>500 s). The highly sulfated (0.87 and 0.84 sulfate/total sugar) active samples contain high amount

of fucose and less amount of galactose. This study provide information regarding its antioxidant and anticoagulant activity as affected by FIRD.

1. Introduction

Ecklonia cava, a brown seaweed, grows prolifically along the coast of Jeju Island in south Korea and is generally inedible due to its tannin-induced characteristic pucker taste. Previous studies revealed that bio-active compounds (polyphenol, polysaccharide) from *E. cava* were responsible for its antioxidant, anticoagulant, antitumor activities and/or cell damage protective effects (Heo et al., 2005 a, b; Kim et al., 2006 a, b; Athukorala et al., 2006a). Recently, it was proven that the cause of most adult diseases (such as aging) is combustion by free radical. So, taking into account that *E. cava* is rich in polyphenol and polysaccharide, compounds which were shown to have good potential in preventing serious chronic health problems including aging, *E. cava* would be very useful in new functional foods.

Several seaweeds are perishable in their fresh state and could deteriorate within a few days after harvest. The traditional way to preserve these plant products is by sun drying (Lim and Murtijaya, 2007). However, sun drying of fresh plant tissues may lead to significant changes in the composition and pigment of phytochemicals (Capecka et al., 2005). Thus, there is a need for drying techniques and methods that enhance product quality, reduce energy consumption and cost in drying processes and the use of artificial drying to preserve plant products has been more rapid and efficient (Afzal et al., 2005).

Among different drying processes, freeze drying is generally accepted as the best method to dry heat sensitive items and produce high quality products such as food items, but freeze drying results in low productivity due to long drying time and the relatively high operation cost (Livin et al., 1998; Ratti, 2001). On the other hand, far infrared radiation drying has significant advantages over conventional drying. These advantages are higher drying rate, energy saving, and uniform temperature distribution giving a better quality product (Wang and Sheng, 2006).

At present, many driers use far infrared radiation to improve drying efficiency, save space and provide clean working environment, among others (Ratti and Mujumdar, 1995; Yamazaki et al., 1992). Attempts have been reported on application of far infrared to the drying of agricultural materials and showed improved results (Ginzburg, 1969; Yagi and Kunii, 1951).

According to the previous reports, the aqueous extracts of powdered freeze dried *E. cava* (collected in Jeju Island in south Korea) showed high antioxidant and anticoagulant activities; however, there are a few information on the effect of far infrared radiation drying on the antioxidant and anticoagulant activity. Therefore, our objective of this paper is to evaluate the effect of far infrared radiation drying on the total phenol contents, antioxidant and anticoagulant activity of water-soluble extracts of *E. cava*.

2. Materials & Methods

2.1. Materials

The brown seaweed *Ecklonia cava* was collected along the coast of Jeju Island in Korea, during the period from March to May 2007. Salt, sand and epiphytes were removed using tap water. Finally, the samples were rinsed with fresh water and stored at -20℃ for further experiments. Carbohydrases such as Celluclast 1.5L FG (catalyzing the breakdown of cellulose into glucose, cellobiose and higher glucose polymers) and proteases such as Protamex (hydrolysis of food proteins) were obtained from Novo Co. (Novozyme Nordisk, Bagsvaerd, Danmark). 5,5-Dimethyl-1-pyrrolin N-oxide (DMPO), 2,2-azobis (2-amidinopropane) hydrochloride (AAPH), α-(4-pyridyl-1-oxide)-N-t-butylnitrone (4-POBN), DPPH (1,1-diphenyl-2-pricrylhydrazyl), peroxidase, ABTS (2,2-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid) were obtained from Sigma Chemical Co. (St. Louis, Mo, USA). All other reagents were of the highest grade available commercially.

2.2. The preparation of dried *E. cava*

The *E. cava* were cut into small pieces. For freeze drying, *E. cava* slices were dried with a freeze dryer (Bondiro, ilshin Lab Co., Ltd, Korea) for 3 days. For far infrared radiation drying, *E. cava* were dried in far infrared radiation dryer (JOURI-Q, KEC, Korea) at various conditions listed in Table 1-1. After drying, *E. cava* samples were ground and sieved through a 50 standard testing sieve.

Table 1-1. Drying conditions for FIRD and FD.

	FIRD[a]					FD[b]
Temperature (℃)	40	50	60	70	80	
Time (hr)	12	10	8	6	4	72

[a] FIRD: far-infrared radiation drying, [b] FD: freeze drying

2.3. Approximate chemical composition of dried *E. cava*

Approximate chemical composition of dried *E. cava* were determined according to AOAC method (1990). Crude carbohydrate was determined by phenol-sulfuric acid reaction (absorbance at 480 nm, using glucose as the calibration standard), crude lipid was performed by Soxhlet method and crude ash was prepared at 550℃ in the dry-type furnace. The moisture was determined keeping in a dry oven at 105℃ for 24 h and the crude protein was determined by Kjeldahl method.

2.4. Water extracts from *E. cava*

For water extracts from the dried *E. cava*, one gram of the ground *E. cava* powder was mixed with 100 ml of water and placed in shaking incubator for 24 h at room temperature. The mixtures were centrifuged at 3500 rpm for 20 min at 4°C and filtered with Whatman filter paper to remove the residue. All the samples were kept in −20°C for further experiments.

2.5. Enzymatic extracts from *E. cava*

The preparation of enzymatic extracts followed the method previously reported (Heo et al. 2003). One gram of the ground dried *E. cava* powder was homogenized with water (100 ml), and then 100 mg or 100 μl enzyme was added. The pHs of the homogenate were adjusted to its optimal pH value before the digestion. The enzymatic hydrolytic reactions were performed for 24 h to achieve optimum degree of the hydrolysis. As soon as the enzymatic reaction is complete, the digests were boiled for 10 min at 100°C to inactivate the enzyme. Each sample was clarified by centrifugation (3000 rpm, for 20 min at 4°C) to remove the residue. All the samples were kept in −20°C for further experiments.

2.6. Measurement of extraction yield

Yields of the extracts obtained by water extraction and enzymatic digest of *E. cava* were

calculated by dividing the dry weight of extracts filtrate over dry weight of the *E. cava* sample used.

2.7. Determination of total polyphenolic content

Phenolic contents were determined using a protocol similar to the method described by Chandler and Dodds (1983) as described by Shetty et al. (1995). Each 1 ml of *E. cava* extracts, 1 ml of 95% EtOH, 5 ml of distilled water, and 0.5 ml of 50% Folin-Ciocalteu reagent were mixed. The mixtures were allowed to react for 5 min, and then 1 ml of 5% Na_2CO_3 was added, and the mixture was thoroughly mixed and placed in the dark for 1 h. Absorbance was measured at 725 nm and gallic acid standard curve was obtained for the calibration of phenolic content.

2.8. DPPH radical scavenging assay using an ESR spectrometer

DPPH radical scavenging activity was measured using the method described by Nanjo et al. (1996). Methanol solution of 60 μl of each sample (or methanol itself as control) was added to 60 μl of DPPH (60 μmol/l) in methanol. After mixing vigorously for 10 seconds, the solutions were transferred into a 100 μl Teflon capillary tube and fitted into the cavity of the ESR (electron spin resonance) spectrometer (JES-FA machine, JEOL, Tokyo, Japan). The spin adduct was measured on an ESR spectrometer exactly 2 min later. Measurement conditions: central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 5 mW, gain 6.3×10^5, temperature 298 K.

2.9. Hydroxyl radical scavenging assay using an ESR spectrometer

Hydroxyl radicals were generated by Fenton reaction, and reacted rapidly with nitrone spin trap DMPO; the resultant DMPO-OH adducts was detectable with an ESR spectrometer (Rosen and Rauckman, 1984). The ESR spectrum was recorded 2.5 min after mixing in a phosphate buffer solution (PBS; pH 7.4) with 0.3 M DMPO 20 μl, 10mM $FeSO_4$ 20 μl and 10 mM 20 μl using an ESR spectrometer set at the following conditions: central field 3475 G, modulation

frequency 100 kHz, modulation amplitude 2 G, microwave power 1 mW, gain 6.3×10^5, temperature 298 K.

2.10. Alkyl radical scavenging assay using an ESR spectrometer

Alkyl radicals were generated by AAPH. The PBS (pH 7.4) reaction mixtures, containing 40 mmol/l AAPH, 40 mmol/l 4-POBN and indicated concentrations of tested samples, were incubated at 37℃ in a water bath for 30 min (Hiramoto et al., 1993), and then transferred to a 100 μl Teflon capillary tube. The spin adduct was recorded on JES-FA ESR spectrometer. Measurement conditions: central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 10 mW, gain 6.3×10^5, temperature 298 K.

2.11. Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging activity was determined according to the method of Muller (1985). A 100 μl of 0.1M phosphate buffer (pH 5.0) and the sample solution were mixed in a 96-well plate. A 20 μl of hydrogen peroxide was added to the mixture, and then incubated at 37℃ for 5 min. After incubation, 30 μl of 1.25 mM ABTS and 30 μl of peroxidase (1 unit/ml) were added to the mixture, and then incubated at 37℃ for 10 min. The absorbance was read with an ELISA reader at 405 nm.

2.12. Separation of high molecular weight crude polysaccharide fraction (MW >30 kDa)

In all the tested samples for anticoagulant activity of extracts, two sample (Celluclast extracts from *E. cava* dried by FD and FIRD at 40℃ subjected to molecular weight fractionation to obtain the polysaccharide with molecular weight of above 30 kDa. Molecular weight fractionation of the Celluclast extracts from *E. cava* was conducted using previously reported method (Athukorala et al. 2006a, b). Celluclast extract was passed through micro-filtration membranes (30 kDa) using Millipore's Lab scale TFF system (Millipore Corporation, Bedford,

Massachusetts, USA) to obtain the desired molecular weight fraction (above 30 kDa). The collected fractions (100 ml) were mixed well with 300 ml of 99.5% ethanol and the mixture was allowed to stand for 30 min at room temperature. Then crude polysaccharides were collected by centrifugation at 10,000 g for 20 min at 4°C (Matsubara et al., 2000; Kuda et al., 2002). Hereafter, the collected precipitate was referred to as crude polysaccharide fraction (CpoF) and the resultant supernatant was referred to as crude phenolic fraction (CphF). CpoF and CphF were concentrated separately under vacuum at 40°C and removed all ethanol, then the samples were dissolved in water for the APTT assay and monosugar composition analysis.

2.13. Blood coagulation assay

Normal pooled plasma was obtained from ten individual healthy donors, without history of bleeding or thrombosis. Nine parts of blood collected by venipuncture was drawn into one part of 3.8% sodium citrate. Blood was centrifuged for 20 min at 2400 × g, and the plasma was stored at −60°C until use. All coagulation assays were performed with four individual replicates using dual-channel clot-2, (SEAC, Italy) and mean values were taken. For activated partial thromboplastin time (APTT) assay, citrated normal human plasma (90 μl) was mixed with a solution of algal extract (10 μl) and incubated for 1 min at 37°C, then APTT reagent (100 μl) was added to the mixture and incubated for 5 min at 37°C. Thereafter clotting was induced by adding 0.025 M $CaCl_2$ (100 μl) and clotting time was recorded.

2.14. Monosugar composition analysis

The active polysaccharide samples was hydrolyzed in a sealed glass tube 2 M of trifluoroacetic acid for 4 h at 100℃ to analyze neutral sugars. In order to analyze the mono-sugars the samples was digested using 6 N of HCl for 4 h. Then, 1 μg of sample were separately applied to CarboPac PA1 (4.5 × 250 mm, Dionex, Sunnyvale, CA, USA) with CarboPac PA1 cartridge (4.5 × 50 mm) column to analyze neutral and a mono sugar, respectively. The column was

eluted using 16 mM of NaOH at 1.0 ml/min flow rate. Each sugar of the sample was detected by using ED50 Dionex electrochemical detector and data were analyzed by peack Net on-line software.

2.15. Sulfate content analysis

After acid hydrolysis of the crude polysaccharides, the sulfate content was measured by the $BaCl_2$/gelation method (Satio, Yamagata and Suzuki, 1968).

3. Results

3.1. Approximate chemical composition

Approximate chemical compositions *E. cava* dried by FIRD and FD were shown in Table 1-2. The major chemical component of *E. cava* tested was found to be carbohydrate whose content occupied over 65% of the total dry weight. Protein contents determined from the *E.cava* were between 10.6% and 18.1%. Ash contents of most of the tested *E.cava* were around 1.5% but FIRD at 40 and 50℃ showed the value of 2.07 % and 2.15 %, respectively. Lipid contents of most of the tested *E. cava* were around 2.4% but FD showed the value of 3.78%.

3.2. Extraction yield

The extraction yield from *E. cava* dried by FD and FIRD were shown in Table 1-3. In case of FIRD, extraction yield increased as the drying temperature was increased. Expect for the water extract from FIR dried at 40 and 50℃, most of the extracts from FIRD showed higher yield than FD. All extracts from *E. cava* dried at 80℃ showed the highest extraction yield.

3.3. Total polyphenolic compounds

All extracts were subjected to total polyphenolic assay to determine their polyphenolic contents. The total polyphenolic amount of each of the extracts are shown in the Table 1-4. The highest polyphenolic content (5.50 g/100 g) was recorded in the Celluclast extract from *E. cava* dried by FIRD at 40℃, whereas the lowest content (3.23 g/100g) was shown the by water extract from *E. cava* dried by FD. In case of FIRD, total polyphenolic content decreased as the drying temperature was increased. But, FIRD extracts have higher total polyphenolic content than FD extracts.

Table 1-2. Chemical compositions of *E. cava* dried by FIRD and FD (%).

	FIRD[a]					FD[b]
	40℃	50℃	60℃	70℃	80℃	
Moisture	11.5±0.3[c]	11.7±0.3	10.8±0.4	10.7±0.3	10.1±0.3	11.6±0.3
Ash	1.47±0.2	2.07±0.1	2.15±0.2	1.84±0.4	1.55±0.3	1.39±0.4
Protein	12.4±0.1	14.7±0.2	13.0±0.4	16.3±0.3	10.6±0.1	18.1±0.1
Carbohydrate	72.2±0.2	68.7±0.3	71.6±0.3	68.9±0.3	75.4±0.4	65.1±0.3
Lipid	2.40±0.4	2.82±0.4	2.46±0.3	2.21±0.2	2.38±0.1	3.78±0.3

[a] FIRD: far-infrared radiation drying, [b] FD: freeze drying

[c] The values are averages of triplicate determinations.

Table 1-3. Yield of water and enzymatic extracts of *E. cava* dried by FIRD and FD.

Sample	Yield (%)					
	FIRD					FD
	40℃	50℃	60℃	70℃	80℃	
WE[a]	28.67±1.42[d]	30.67±1.99	32.33±1.29	38.33±1.66	41.67±1.69	31.67±1.30
CE[b]	52.67±1.45	54.00±1.47	54.67±1.62	57.33±1.79	63.67±1.69	52.00±1.68
PE[c]	51.33±1.45	52.00±1.68	52.33±1.47	55.67±1.45	60.00±1.62	50.33±1.39

[a] WE: water extract, [b] CE: Celluclast extract, [c] PE: Protamex extract

[d] The values are averages of triplicate determinations.

Table 1-4. Total phenolic contents of water and enzymatic extracts of *E. cava* dried by FIRD and FD.

Sample	Content (g/100 g)					
	FIRD					FD
	40℃	50℃	60℃	70℃	80℃	
WE[a]	4.77±0.3[d]	4.60±0.3	4.29±0.7	4.17±0.1	4.00±0.1	3.23±0.7
CE[b]	5.50±0.2	5.43±0.4	5.12±0.2	5.07±0.7	5.01±0.2	4.32±0.7
PE[c]	5.25±0.7	5.23±0.1	4.71±0.1	4.40±0.1	4.20±0.7	3.49±0.7

[a] WE: water extract, [b] CE: Celluclast extract, [c] PE: Protamex extract

[d] Mean±SE of determinations was made in duplicate experiments.

3.4. DPPH radical scavenging assay

DPPH is a stable free radical donor, which has been widely used to test free radical scavenging effect of natural antioxidants. In this study, the scavenging activities of all extracts from *E. cava* dried by FIRD and FD on DPPH radical are shown Fig. 1-1. All the tested extracts showed strong DPPH radical scavenging activity. Among them, water extract from *E. cava* dried by FIRD at 40℃ showed the highest DPPH radical scavenging activity (IC_{50} 11.6 μg/ml). And in case of same drying condition, water extracts exhibited DPPH radical scavenging activity higher than enzymatic extracts. In case of FIRD, DPPH radical scavenging activity decreased as the drying temperature was increased. But, except for the drying condition at 80℃, most FIRD extracts showed higher DPPH radical scavenging activity than FD extracts. In addition, the scavenging activity of those extracts increased with increasing concentrations from 5 μg/ml to 50 μg/ml.

3.5. Hydroxyl radical scavenging assay

Hydroxyl radicals generated in the Fenton system (Fe^{2+}/H_2O_2) were trapped by DMPO, forming a spin adduct as detected by the ESR spectrometer. As shown in Fig. 1-2, all the tested extracts showed good hydroxyl radical scavenging activity. Among them, water extract from *E. cava* dried by FIRD at 40℃ exhibited the highest scavenging activity (IC_{50} 223.5 μg/ml). Also, water extracts from the same drying condition showed higher hydroxyl radical scavenging activity than enzymatic extracts. In case of FIRD, hydroxyl radical scavenging activity decreased as the drying temperature was increased. But, except for a dry condition at 80℃, most FIRD extracts showed higher scavenging activity than FD extracts. In addition, the scavenging activity of those extracts increased with increasing concentrations from 125 μg/ml to 500 μg/ml.

3.6. Alkyl radical scavenging assay

The alkyl radical spin adduct was observed when AAPH was incubated with the spin trap 4-

POBN at 37℃ for 30 min. The scavenging activities of all extracts from *E. cava* dried by FIRD and FD on alkyl radical are shown Fig. 1-3. All the tested extracts showed strong alkyl radical scavenging activity. Among them, water extract from from *E. cava* dried by FIRD at 40℃ exhibited the highest scavenging activity (IC_{50} 9.1 μg/ml). Also at the same drying condition, water extracts showed higher alkyl radical scavenging activity than enzymatic extracts. In case of FIRD, alkyl radical scavenging activity decreased as the drying temperature was increased. Again, except for the drying condition at 80℃, most FIRD extracts showed higher alkyl radical scavenging activity than FD extracts. In addition, the scavenging activity of those extracts increased with increasing concentrations from 2.5 μg/ml to 25 μg/ml.

3.7. Hydrogen peroxide scavenging assay

The scavenging activities of the extracts from *E. cava* dried by FIRD and FD of on hydrogen peroxide are shown on Fig. 1-4. All the extracts showed good hydrogen peroxide scavenging activity. Among them, water extract from *E. cava* dried by FIRD at 40℃ exhibited the highest scavenging activity (IC_{50} 32.2 μg/ml); And at the same drying condition, water extracts showed higher hydrogen peroxide scavenging activity than enzymatic extracts. In case of FIRD, hydrogen peroxide scavenging activity decreased as the drying temperature was increased. But, except for the drying condition at 80℃, most FIRD extracts showed higher hydrogen peroxide scavenging activity than FD extracts. In addition, the scavenging activity of those extracts increased with increasing concentrations from 35 μg/ml to 180 μg/ml.

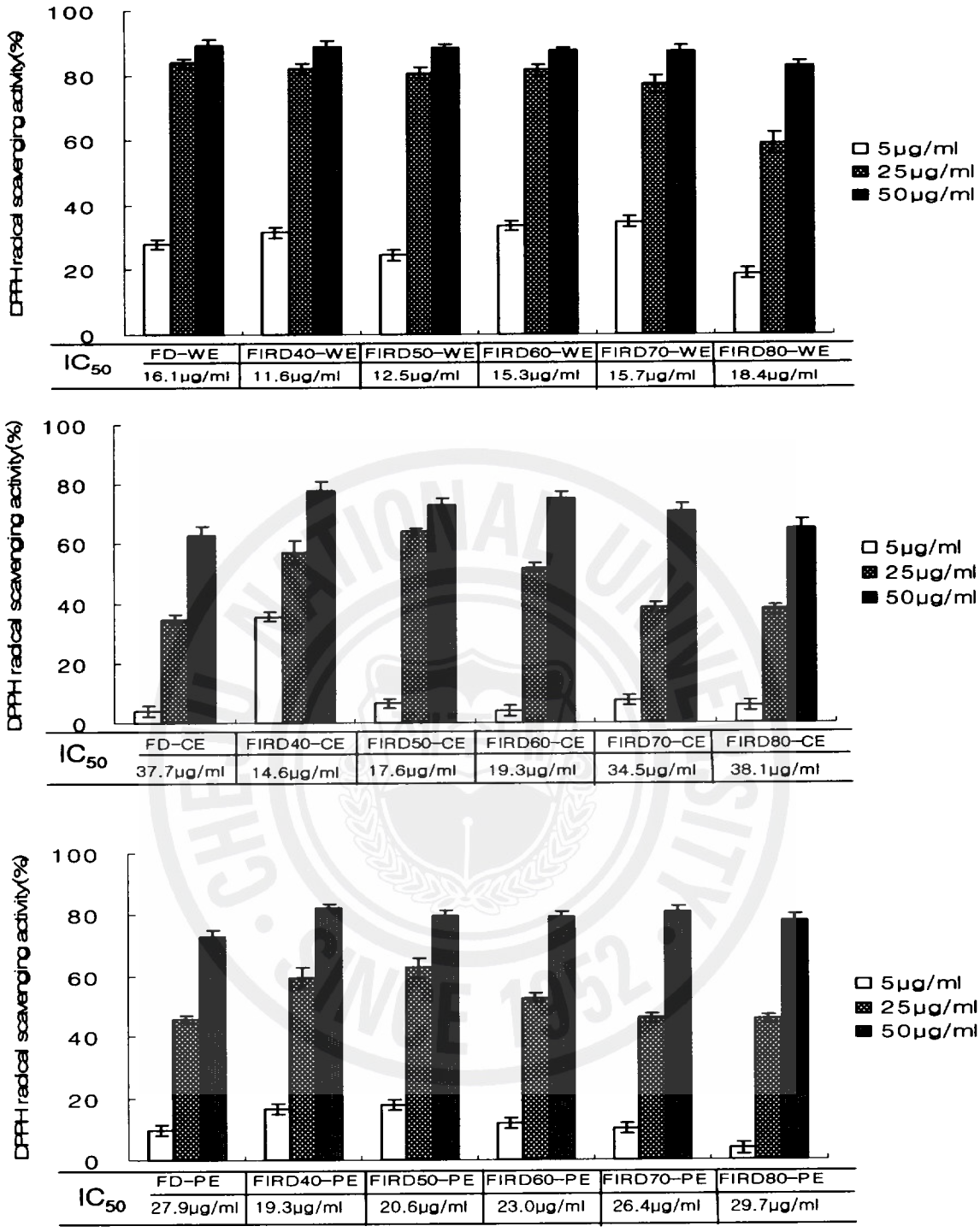

Fig. 1-1. DPPH radical scavenging activities of water and enzymatic extracts from *E. cava* dried by FIRD and FD. FD: freeze drying, FIRD: far-infrared radiation drying (40-80℃)
WE: water extract, CE: Celluclast extract, PE: Protamex extract
IC_{50} can be found from a plot of percent inhibition versus the concentration of extracts from *E. cava*. Mean±SE of determinations was made in duplicate experiments.

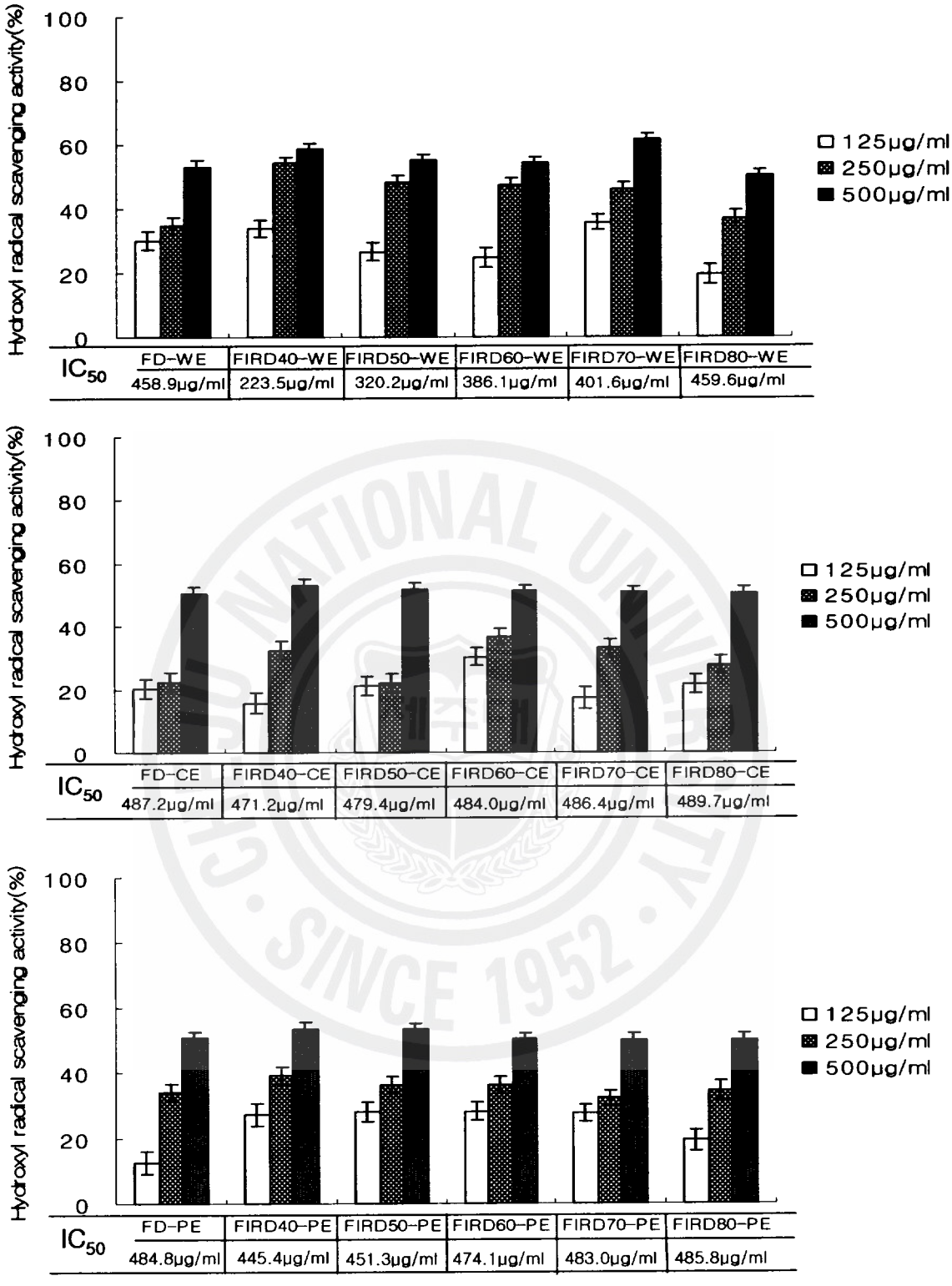

Fig. 1-2.Hydroxyl radical scavenging activities of water and enzymatic extracts from *E. cava* dried by FIRD and FD. Abbreviations are the same as in Fig. 1-1.

Mean±SE of determinations was made in duplicate experiments.

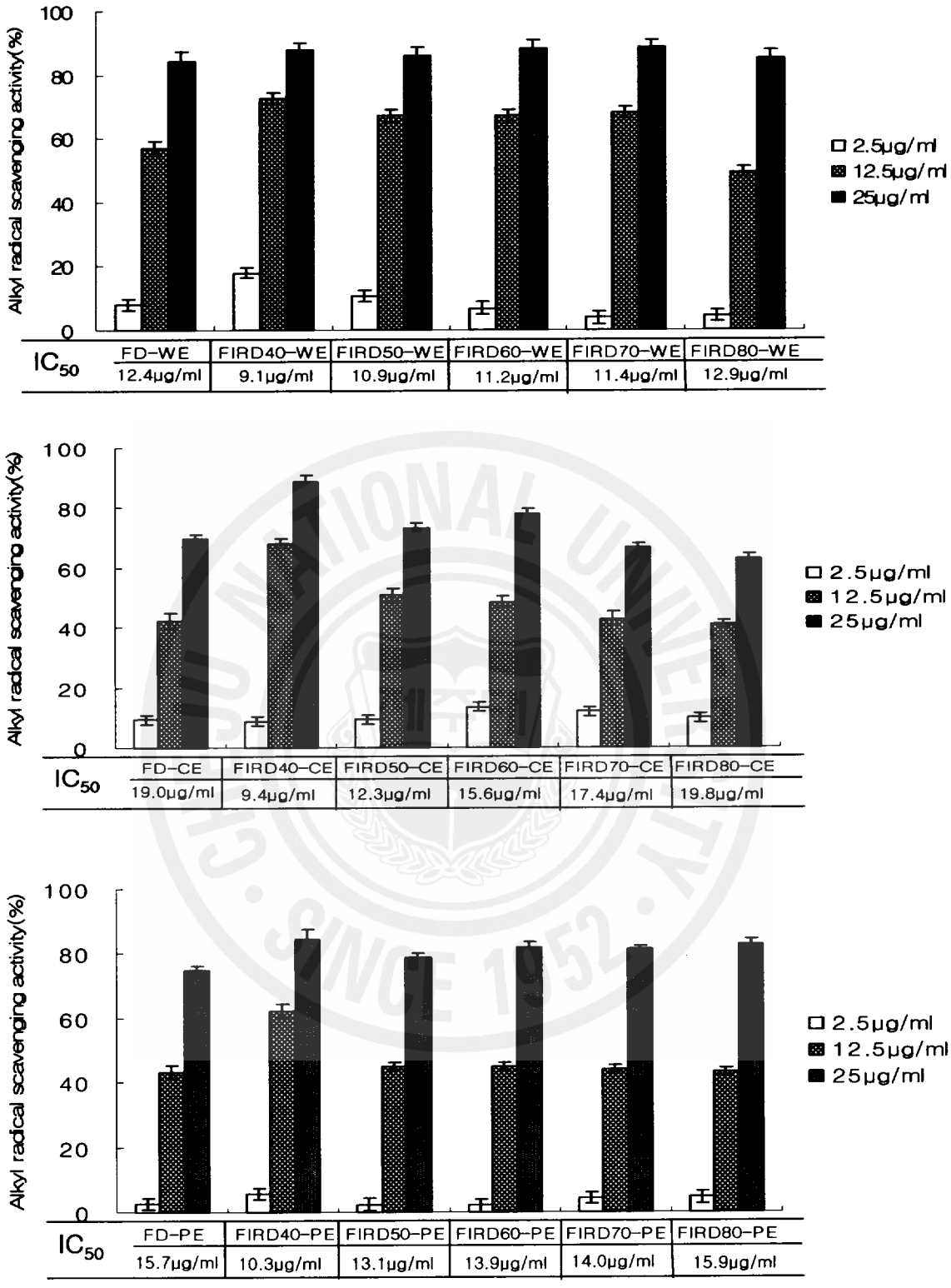

Fig. 1-3. Alkyl radical scavenging activities of water and enzymatic extracts from *E. cava* dried by FIRD and FD. Abbreviations are the same as in Fig. 1.

Mean±SE of determinations was made in duplicate experiments.

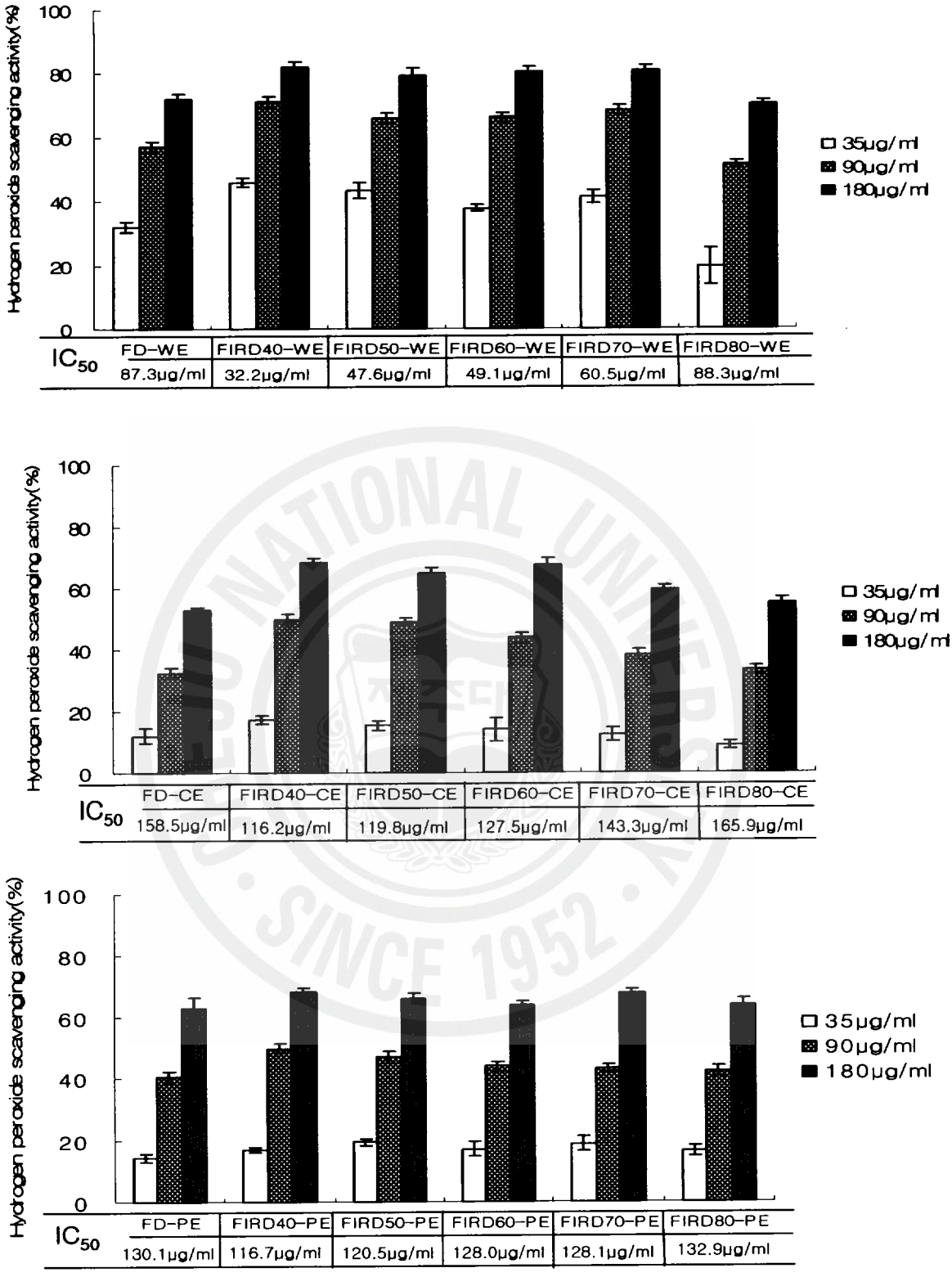

Fig. 1-4. Hydrogen peroxide scavenging activities of water and enzymatic extracts from *E. cava* dried by FIRD and FD. Abbreviations are the same as in Fig. 1.

Mean±SE of determinations was made in duplicate experiments.

3.8. Activated partial thromboplastin time (APTT) activity of crude polysaccharide with molecular weight fraction of above 30 kDa

All the extracts were subjected to APTT assay to estimate their anticoagulant efficacy. As shown Table 1-5, water and Celluclast extracts form *E. cava* dried by FD and FIRD showed good APTT activity. Among them, Celluclast extract from *E. cava* dried by FD exhibited the highest APTT activity (~303 s). Comparatively, all the FD extracts showed higher APTT activity than FIRD extracts. In case of FIRD, APTT activity was decreased with increasing drying temperature. In all the tested samples for anticoagulant activity of extracts, two sample Celluclast extracts from *E. cava* by FD (FD-CE) and FIRD at 40℃ (FIRD40-CE) were selected and applied to crude polysaccharide separate from molecular weight fraction (above 30 kDa). The resultant crude polysaccharide fraction (CpoF) and crude phenolic fraction (CphF) were further evaluated for their anticoagulant activities; the results are shown in Table 1-6. Among the tested samples, the CpoF of Celluclast extracts from *E. cava* by FD and FIRD at 40℃ showed the highest APTT activity (>500 s). Under the same conditions, the CphF of Celluclast extracts from *E. cava* by FIRD at 40℃ showed higher APTT activity (~205 s) than that of FD (~82 s).

3.9. Monosugar composition analysis

Approximate monosugar composition of polysaccharide isolated from *E. cava* dried by FIRD at 40℃ and FD were shown in Table 1-7 and Fig. 1-5. According to the results, both samples contain high amount of fucose ~57.44 and ~65.45% and less amount of galactose ~12.09, 15.46% respectively. Moreover both samples contain a small amount of other sugars. Polysaccharide isolated from *E. cava* dried by FIRD at 40℃ showed lower fucose content than FD. Whereas, the content of other sugars was exhibited a little higher in FIRD than in FD. The total sulfate content of the tested sample was 0.84 and 0.87 (sulfate/total sugar), respectively.

Table 1-5. Anticoagulant activities (APTT) of water and enzymatic extracts from *E. cava* dried by FIRD and FD.

Sample	APTT (s)					
	FIRD					FD
	40℃	50℃	60℃	70℃	80℃	
WE[a]	219	209	182	177	146	277
CE[b]	278	234	194	191	184	303
PE[c]	143	139	129	107	86	187
Control	37					

[a] WE: water extract, [b] CE: Celluclast extract, [c] PE: Protamex extract

Results are expressed as means of three determinations. Sample concentration is 70 μg/ml.

Table 1-6. Anticoagulant activity of high molecular weight crude polysaccharide fraction (>30 kDa) from celluclast extract of *E. cava* dried by FD and FIRD at 40℃.

Sample	APTT (s)	
	Supernatant (CphF)	Precipitate (CpoF)
FD-CE[a]	82	>500
FIRD40-CE	205	>500
Control	35	

[a] CE: Celluclast extract

Results are expressed as means of three determinations. Sample concentration is 70 μg/ml.

Table 1-7. Approximate monosugar composition of sulfated polysaccharide fraction (CpoF) isolated from the Celluclast extract of *E. cava* dried by FD and FIRD at 40℃.

Sugar	FIRD40-CE (%)	FD-CE (%)
fucose	57.44	65.45
galactose	15.46	12.09
glucose	6.39	2.84
mannose	8.19	7.35
xylose	12.52	12.27
Sulfate/total sugar[a]	0.84	0.87

[a] The mean degree of substitution of sulfate ester per total sugar.

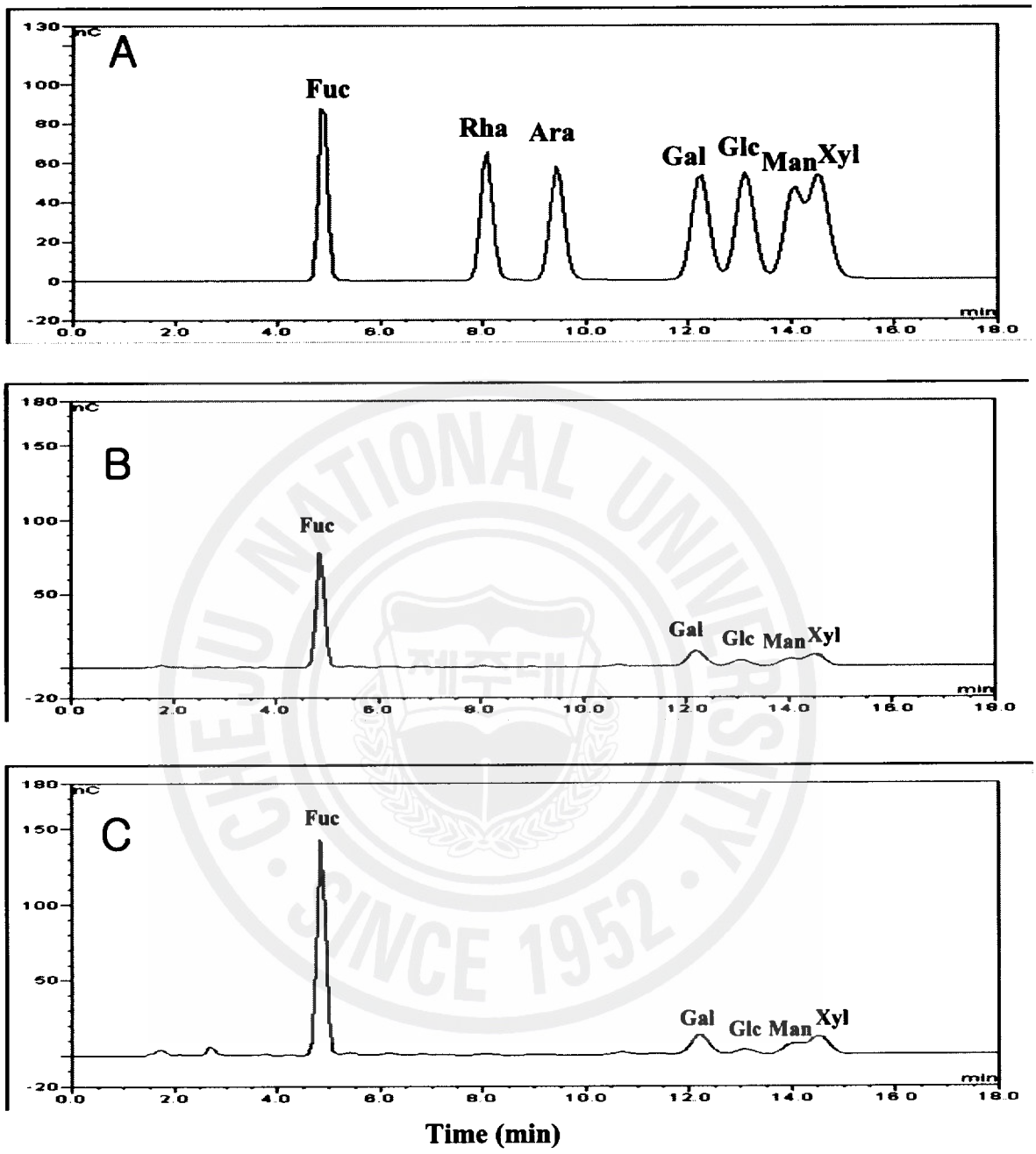

Fig. 1-5. The HPLC chromatograms for the monosugar composition sulfated polysaccharide fraction (CpoF) isolated from *E. cava*. The chromatogram for the sugar standards (A); The chromatogram for the FIRD40-CE polysaccharide sample (B); The chromatogram for the FD-CE polysaccharide sample (C).

4. Discussion

Ecklonia cava, a kind of brown seaweed is plentifully produced in Jeju Island in Korea (30,000 tons per year). It is popular in Korea and Japan where this valuable brown algae is utilized as food ingredients, animal feed, fertilizers and medicine. In addition, *E.cava* has pigment, fucoxathin, fucoidan, phycocolloid and especially is a good source of alginates (Ghiry and Bulunden, 1991). Many researchers have reported that aqueous extracts of freeze dried *E. cava* exhibits high antioxidant and anticoagulant activities (Heo et al., 2005 a, b; Kim et al., 2006 a; Athukorala et al., 2006); however, information regarding the effect of far infrared radiation drying on antioxidant and anticoagulant activity is relatively lacking.

FIR rays are defined as electromagnetic waves which have wavelengths longer than 4 μm but shorter than microwave (λ>0.1 cm). FIR rays are biologically active (Inoue and Kabaya, 1989) and transfer heat to the center of materials evenly without degrading the constituent molecules of surface (Niwa et al., 1988). FIR may have the capability to cleave covalent bonds and liberate antioxidants such as flavonoids, carotene, tannin, ascorbate, flavoprotein or polyphenols from polymers (Niwa and Miyachi, 1986).

In the present study, we focused on natural water soluble antioxidants from *E. cava*, which were prepared by water-soluble extraction from *E. cava* dried by far infrared radiation at 40~80℃ (FIRD), and their antioxidant and anticoagulant effects were evaluated in different reactive oxygen species assays and APTT activity.

Phenolic compounds are the most active antioxidant derivatives in plants (Bors, Michel and Stettmaier, 2001). They are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because they are stable radical intermediates (Cuvelier, Richard and Berset, 1992; Maillard et al., 1996). It has been reported, that total polyphenolic

compounds in *E. cava* are richer than other brown seaweeds (Heo et al., 2005a; Heo et al., 2003a, b). According to the total polyphenolic content results (Table 1-3), all extracts from *E. cava* dried by FD and FIRD were rich in polyphenolic compounds. In case of FIRD, total polyphenolic content was decreased with increasing drying temperature. but, FIRD extracts had a higher total polyphenolic content than FD extracts. Kim and Lee (2004) reported that the mineral content of *E. cava* was not affected by the drying methods. However, more vitamins were lost and total polyphenol content was reduced as a result of sun drying than by other drying methods used.

In this study, antioxidative effect were evaluated by ESR (DPPH radical, hydroxyl radical, alkyl radical) and hydrogen peroxide scavenging assay. ESR technique on spin trapping is the most direct method to detect highly reactive free radicals generated for short times (Janzen, Towner and Haire, 1987). DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule, and has often been used as a substrate to evaluate the antioxidative activity of natural compounds (Soares et al, 1997). Hydroxyl radicals are the major reactive oxygen species causing enormous biological damage and the initiation of lipid peroxidation. Hydroxyl radicals generated in the Fenton system ($Fe2+/H_2O_2$) was trapped by DMPO, forming a spin adduct as detected by the ESR spectrometer. The alkyl radical has been found to be a primary intermediate in many hydrocarbon reactions. These radicals were easily detected with ESR, a technique which has been found to be very useful in the characterization of solid surfaces and in the elucidation of active surfaces sites as well as surface reactions (Adebajo and Gesser, 2001). Hydrogen peroxide together with reactive oxygen species can damage several cellular components. Hydrogen peroxide is relatively an unstable metabolic product being responsible for the generation of hydroxyl radical and singlet oxygen, which is formed by fenton reaction and initiate lipid peroxidation or be toxic to cells.

The radical scavenging activities of all extracts from *E. cava* dried by FIRD and FD on DPPH, hydroxyl, alkyl, hydrogen peroxide are shown Fig. 1-1~4. All the extracts from *E. cava* by dried FD and FIRD at 40~80℃ showed strong radical scavenging activity. In case of FIRD, radical scavenging activity decreased with increasing drying temperature. But, except for the drying condition at 80℃, FIRD extracts had a lower IC_{50} value than FD extracts. Among them, water extracts from *E .cava* dried by FIRD at 40 ℃ showed the lowest IC_{50} value (DPPH: 11.6 μg/ml, hydroxyl radical: 223.5 μg/ml, alkyl radical: 9.1 μg/ml, and hydrogen peroxide: 32.2 μg/ml). Many researchers have reported positive correlation between free radical scavenging activity and total polyphenolic compound. Oki et al. (2002) observed that the radical scavenging activity increased with the increase of polyphenolic compound content. Two studies conducted by Lu and Foo (2000) and Siriwardhana et al. (2003) reported a high correlation between DPPH radical scavenging activities and total polyphenolic.

According to Lee et al. (2006), far-infrared radiation treatment on peanut hulls increased the antioxidant activity and total polyphenolic content of water extracts from peanut hulls. Also, this study showed that total polyphenolic content and radical scavenging activity in all extracts from dried *E. cava* increased significantly by FIRD. Therefore, FIRD of *E. cava* proved to be a more efficient drying procedure than FD, in terms of the effect on total polyphenolic content and radical scavenging activity of extracts from dried *E. cava*.

All extracts were subjected to APTT assay to estimate their anticoagulant efficacy. Among the extracts, water and Celluclast extracts from *E. cava* dried by FD and FIRD showed good APTT activity. Among them, Celluclast extract from *E. cava* dried by FD exhibited the highest APTT activity (~303 s). Athukorala et al. (2006a) reported that the high APTT activity of *E. cava* hydrolyzed with carbohydrases (such as Viscozyme, Celluclast, AMG, Termamyl, Ultraflo) are due to sulfated polysaccharides. Hence, two sample Celluclast extracts from *E. cava* dried by

FD (FD-CE) and FIRD at 40℃ (FIRD40-CE) were selected and applied to molecular weight fractionation (above 30 kDa). The resultant crude polysaccharide fraction (CpoF) and crude phenolic fraction (CphF) were evaluated for their anticoagulant activities and monosugar composition. In that results, the CpoF of FD-CE and FIRD40-CE showed high APTT activity (>500 s). Approximate monosugar composition showed both samples were rich in fucose ~57.44 and ~65.45% and have amount of galactose ~12.09, 15.46% respectively. The total sulfate content of the tested samples was 0.84 and 0.87 (sulfate/total sugar) respectively. Moreover, both samples contain a small amount of other sugars. The sulfated fucan are known for its high anticoagulant activity. In this study, fucose- rich sulfated polysaccharide showed strong anticoagulant activity. Several kinds of phenolic compounds, such as tannins (Dong et al., 1998), tea catechins (Kang et al., 1999) and triterpenes (O'Neill et al., 1998) have shown potent anticoagulant and/or antithrombotic activities. According to the results, under the same conditions, the CphF of FIRD40-CE showed higher APTT activity (~205 s) than that of FD-CE (~82 s). Which could be due to the high amount of total polyphenolic in the extracts.

In the present study, we used various water-soluble extracts from *E. cava* dried by FIRD, examined their total polyphenolic content, radical scavenging activity and anticoagulant effect. Far infrared radiation dried *E. cava* extract had greater antioxidant, anticoagulant activity and higher amounts of phenolics than freeze dried. Therefore, it was concluded that FIRD was more effective than FD in maintaining the antioxidant and anticoagulant phenolic compounds in *E. cava*. Further studies are required for identification and purification of antioxidant and anticoagulant activity of compounds from extracts of *E. cava* by FIRD.

Part Ⅱ

Antioxidative effect of far infrared radiation drying on radical scavenging and H_2O_2-induced cell damage of methanolic extracts from *Ecklonia cava*

Abstract

In this study, methanolic extracts of *Ecklonia cava* dried by far infrared radiation drying at 40~80℃ (FIRD) were evaluated for their potential antioxidant activities, and then compared to the extracts from freeze drying (FD) sample. In case of FIRD, total polyphenolic content decreased as the drying temperature was increased. FIRD extracts had a higher total polyphenolic content than FD extracts. And, except for the drying condition at 80℃, dieckol and eckol content decreased as the drying temperature was increased. But, FIRD extracts had a higher dieckol and eckol content than FD extracts. All the methanolic extracts from *E. cava* dried by FD and FIRD at 40~80℃ showed strong antioxidant activity. In case of FIRD, radical scavenging activity decreased as the drying temperature was increased. But, except for the drying condition at 80℃, FIRD extracts had a lower IC_{50} value than FD extracts. Among them, 100% methanolic extract from *E. cava* dried by FIRD at 40 ℃ (FIRD40-100ME) showed the lowest IC_{50} value (DPPH, hydroxyl radical, alkyl radical and hydrogen peroxide). Therefore, 100% methanolic extract from *E. cava* dried by FIRD at 40 ℃ (FIRD40-100ME) and 100% methanolic extract from *E. cava* dried by FD (FD-100ME) was selected for use in the further experiments. Among the tested samples, FIRD40-100ME enhanced cell viability against H_2O_2 –induced oxidative damage, and relatively good hydrogen peroxide scavenging activity in a African green monkey kidney line (Vero) cell line as compared to FD-100ME. FIRD40-100ME also effected a reduction in the proportion of cells undergoing H_2O_2–induced apoptosis. These results illustrate that methanolic extracts from *E. cava* dried by FIRD exhibits a good antioxidant activity over H_2O_2- mediated cell damage *in vitro*.

1. Introduction

High levels of free radicals or ROS (reactive oxygen species) create oxidative stress, which leads to a variety of biochemical and physiological lesions and often results in metabolic impairment and cell death (Ames, 1998). Among a variety of ROS, H_2O_2 plays a pivotal role because it is generated from nearly all sources of oxidative stress and can diffuse freely in and out of cells and tissues (Halliwell and Aruoma, 1991). Cells are protected from ROS-induced damage by a variety of endogenous ROS scavenging enzymes, chemical compounds and natural products. Recently there has been increasing interest in the therapeutic potential of natural plants as antioxidants in reducing such free radical-induced tissue injury, suggesting that many plants have antioxidant activities that could be therapeutically useful.

Ecklonia cava has been widely used as a source of fucoidan, which has been well known as an antitumor, anticoagulant and antithrombin polysaccharide (Satoru et al., 2003; Takashi et al., 1999; Takashi et al., 2000). In our previous study (Heo et al., 2005; Ahn et al., 2007), we have screened several species of brown seaweeds from Jeju Island in Korea for their antioxidant activity. Among them, the *E. cava* methanolic extracts showed positive effect on scavenging DPPH radical, hydroxyl radical, hydrogen peroxide and protective effects against H_2O_2 – induced DNA damage.

Several seaweeds are perishable in their fresh state and could deteriorate within a few days after harvest. The traditional way to preserve these plant products is by sun drying (Lim and Murtijaya, 2007). However, sun drying of fresh plant tissues may lead to significant changes in the composition and pigment of phytochemicals (Capecka et al., 2005). Thus, there is a need for drying techniques and methods that enhance product quality, reduce energy consumption and cost in drying processes and the use of artificial drying to preserve plant products has been more

rapid and efficient (Afzal et al., 2005)

Among different drying processes, freeze drying is generally accepted as the best method for drying high quality and heat sensitive products such as quality foods but freeze drying results in low productivity due to long drying time and the relatively high operation cost is another concern (Livin et al., 1998; Ratti, 2001). On the other hand, far infrared radiation drying has significant advantages over conventional drying. These advantages are higher drying rate, energy saving, and uniform temperature distribution giving a better quality product (Wang and Sheng, 2006) At present, many driers use far infrared radiation to improve drying efficiency, save space and provide clean working environment, etc (Ratti and Mujumdar, 1995; Yamazaki et al., 1992). Attempts have been reported on application of far infrared to the drying of agricultural materials and showed improved results (Ginzburg, 1969; Yagi and Kunii, 1951).

Thus, the objective of the present study was to explore the antioxidative effects of far infrared radiation drying on the radical scavenging and H_2O_2-induced cell damage of methanolic extracts from *E. cava*.

2. Materials & Methods

2.1. Materials

The brown seaweed *Ecklonia cava* was collected along the coast of Jeju Island in Korea, during the period from March to May 2007. Salt, sand and epiphytes were removed using tap water. Finally, the samples were rinsed with fresh water and stored at -20℃ for further experiments. 5,5-Dimethyl-1-pyrrolin N-oxide (DMPO), 2,2-azobis (2-amidinopropane) hydrochloride (AAPH), α-(4-pyridyl-1-oxide)-N-t-butylnitrone (4-POBN), DPPH (1,1-diphenyl-2-pricrylhydrazyl), peroxidase, ABTS (2,2-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid), 2′,7′-Dichlorodi-hydrofluorescein diacetate (DCF-DA), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT), Propidium iodide (PI) and Hoechst 33342 were obtained from Sigma Chemical Co. (St. Louis, Mo, USA). All other reagents were of the highest grade available commercially.

2.2. Methanolic extracts from *E. cava*

For methanolic extracts from the dried *E. cava*, one gram of the ground *E. cava* powder was mixed with 100 ml of methanol (50%, 100%, respectively) and placed in shaking incubator for 24 h at room temperture. The mixtures were centrifuged at 3500 rpm for 20 min at 4°C and filtered with Whatman filter paper to remove the residue, there after evaporated under vacuum at 40°C to removed all methanol, and then dissolved in water. All samples were kept in −20°C for further experiments.

2.3. Measurement of extraction yield

Yields of the extracts obtained by methanolic extraction of *E. cava* were calculated by dividing the dry weight of extract filtrate over dry weight of the *E. cava* sample used.

2.4. Determination of total polyphenolic content

Phenolic contents were determined using a protocol similar to the method described by Chandler and Dodds (1983) as described by Shetty et al. (1995). Each 1 ml of *E. cava* extracts, 1 ml of 95% EtOH, 5 ml of distilled water, and 0.5 ml of 50% Folin-Ciocalteu reagent were mixed. The mixtures were allowed to react for 5 min, and then 1 ml of 5% Na_2CO_3 was added, and the mixture was thoroughly mixed and placed in the dark for 1h. Absorbance was measured at 725 nm and gallic acid standard curve was obtained for the calibration of phenolic content.

2.5. Determination of total eckol and dieckol content by HPLC system

The extracts were analyzed by HPLC according to the method of Ahn et al. (2007). The HPLC system (YoungLin Instrument, Korea) comprising the following components: Acme 9000 UV/Vis spectrometer, Gradient pump, Vaccum degasser & Mixer were used for the determination of total ekol and diekol content. The column used a RP-C18 column (250 × 4.6 mm, 5 μM, Waters, USA). For HPLC analysis, mobile phases used in the gradient elution consisted of primary eluant (A) consisting of 10% methanol and 0.04% trifluoroacetic acid (TFA), and a secondary eluant (B) consisting of 100% methanol and 0.04% trifluoroacetic acid (TFA). 10% solvent A changed, in the linear gradient, to 100% of B 40 minutes after injection. The flow rate was 1 ml/min, the column temperature was room temperature and sample volume injected was 20 μl. The absorbance was measured at a wavelength 230 nm for the detection of ekol and diekol. The total eckol and dieckol content were determined with the use of an external standard method (calibration curve obtained with identical standards).

2.6. DPPH radical scavenging assay using an ESR spectrometer

DPPH radical scavenging activity was measured using the method described by Nanjo et al (1996). Methanol solution of 60 μl of each sample (or methanol itself as control) was added to 60 μl of DPPH (60 μmol/l) in methanol. After mixing vigorously for 10 seconds, the solutions were transferred into a 100 μl Teflon capillary tube and fitted into the cavity of the ESR

(electron spin resonance) spectrometer (JES-FA machine, JEOL, Tokyo, Japan). The spin adduct was measured on an ESR spectrometer exactly 2 min later. Measurement conditions: central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 5 mW, gain 6.3×10^5, temperature 298 K.

2.7. Hydroxyl radical scavenging assay using an ESR spectrometer

Hydroxyl radicals were generated by Fenton reaction, and reacted rapidly with nitrone spin trap DMPO; the resultant DMPO-OH adducts was detectable with an ESR spectrometer (Rosen and Rauckman, 1984). The ESR spectrum was recorded 2.5 min after mixing in a phosphate buffer solution (PBS; pH 7.4) with 0.3 M DMPO 20 μl, 10mM $FeSO_4$ 20 μl and 10 mM 20 μl using an ESR spectrometer set at the following conditions: central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 5 mW, gain 6.3×10^5, temperature 298 K.

2.8. Alkyl radical scavenging assay using an ESR spectrometer

Alkyl radicals were generated by AAPH. The PBS (pH 7.4) reaction mixtures, containing 40 mmol/l AAPH, 40 mmol/l 4-POBN and indicated concentrations of tested samples, were incubated at 37℃ in a water bath for 30 min (Hiramoto et al., 1993), and then transferred to a 100 μl Teflon capillary tube. The spin adduct was recorded on JES-FA ESR spectrometer. Measurement conditions: central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 5 mW, gain 6.3×10^5, temperature 298 K.

2.9. Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging activity was determined according to the method of Muller (1985). A 100 μl of 0.1 M phosphate buffer (pH 5.0) and the sample solution were mixed in a 96-well plate. A 20 μl of hydrogen peroxide was added to the mixture, and then incubated at 37℃ for 5 min. After incubation, 30 μl of 1.25 mM ABTS and 30 μl of peroxidase (1 unit/ml)

were added to the mixture, and then incubated at 37℃ for 10 min. The absorbance was read using an ELISA reader at 405 nm.

2.10. Cell culture

Cells of an African green monkey kidney line (Vero) were maintained at 37℃ in an incubator, under a humidified atmosphere containing 5% CO_2. The cells were cultured in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum (FBS), streptomysin (100 μg/ml), penicillin (100 unit/ml) and sodium pyruvate (110 mg/l).

2.11. Hydrogen peroxide scavenging assay by DCF-DA

For the detection of intracellular H_2O_2, the Vero cells were seeded in 96-well plates at a concentration of 1.0×10^5 cells/ml. After 16 h, the cells were treated with *E. cava* samples (10 μl) and incubated at 37℃ under a humidified atmosphere. After 30 min, H_2O_2 was added at a concentration of 500 μM, and then the cells were incubated for an additional 30 min at 37℃. Finally, 2′,7′-Dichlorodi-hydrofluorescein diacetate (DCF-DA; 5 μg/ml) was introduced to the cells, and 2′,7′-Dichlorodi-hydrofluorescein fluorescence was detected at an excitation wavelength of 485 nm and an emission wavelength of 535 nm, using a Perkin-Elmer LS-5B spectrofluorometer.

2.12. Assessment of cell viability

Cell viability was then estimated via an MTT assay, which is a test of metabolic competence predicated upon the assessment of mitochondrial performance. It is a colorimetric assay, which is dependent on the conversion of yellow tetrazolium bromide to its purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells (Mosmann, 1983). The cells were seeded in 96-well plates at a concentration of 1.0×10^5 cells/ml. After 16 h, the cells were treated with *E. cava* samples (10 μl). Then, 10 μl of H_2O_2 (500 uM) was added to the cell culture medium, and incubated for 24 h at 37℃. MTT stock solution (50 μl; 2mg/ml) was then

applied to each of the wells, to a total reaction volume of 200 μl. After 4 h of incubation, the plates were centrifuged for 5 min at 800 × g, and the supernatants were aspirated. The formazan crystals in each well were dissolved in 150 μl of dimethylsulfoxide (DMSO), and the absorbance was measured via ELISA at a wavelength of 540 nm. Relative cell viability was evaluated in accordance with the quantity of MTT converted to the insoluble formazan salt. The optical density of the formazan generated in the control cells was considered to present 100% viability. The data are expressed as mean percentage of the viable cells versus the respective control.

2.13. Nuclear staining with Hoechst 33342

The nuclear morphology of the cells was evaluated using the cell-permeable DNA dye, Hoechst 33342. Cells with homogeneously stained nuclei were considered viable, whereas the presence of chromatin condensation and/or fragmentation was indicative of apoptosis (Lizard et al., 1995). The Vero cells were placed in 24-well plates at a concentration of 1.0×10^5 cells/ml. After 16 h plating, the cells were treated with various concentrations of the sample, and further incubated for 1 h prior to exposure to H_2O_2 (500 μM). After 24 h, 1.5 μl of Hoechst 33342 (stock 10 mg/ml), a DNA-specific fluorescent dye, were added to each well (1.5 ml), followed by 10 min of incubation at 37℃. The stained cells were then observed under a fluorescence microscope equipped with a CoolSNAP-Pro color digital camera, in order to examine the degree of nuclear of condensation.

3. Results

3.1. Extraction yield

The extraction yield from *E. cava* dried by FD and FIRD were shown in Table 2-1. In case of FIRD, extraction yield increased as the drying temperature was increased. All extracts showed FIRD higher yield than FD, and 50% methanolic extracts exhibited higher yield than 100% methanolic extracts. Among them, 50% methanolic extract from *E. cava* dried at 80℃ showed the highest extraction yield (45.33%).

3.2. Total polyphenolic compounds

All extracts were subjected to total polyphenolic assay to determine their polyphenolic contents. The total polyphenolic amount of each extract is shown in the Table 2-2. The highest polyphenolic content (6.35 g/100g) was recorded in the 50% methanolic extract from *E. cava* dried by FIRD at 40℃, whereas, the lowest content (3.89 g/100g) was shown the 100% methanolic extract from *E. cava* dried by FD. In case of FIRD, total polyphenolic content decreased as the drying temperature was increased. But, FIRD extracts have higher total polyphenolic content than FD extracts.

3.3. Total dieckol and eckol compounds

100% methanolic extract from *E. cava* dried by FIRD at 40 ℃ (FIRD40-100ME) and FD (FD-100ME) were subjected to HPLC analysis, to determine phlorotanin compounds. As shown in Fig. 2-1. two major compounds such as dieckol and eckol were observed. The dieckol and eckol amount of each extract are indicated in the Table 2-3. In case of FIRD, dieckol and eckol content decreased as the drying temperature was increased except for the drying condition at 80℃. But, in all the drying conditions, FIRD extracts had a higher dieckol and eckol content than FD extracts. Among the tested samples, the highest dieckol (1487 mg/100 g) and eckol (125 mg/100 g) content was recorded in the FIRD40-100ME.

Table 2-1. Yield of methanolic extracts of *E. cava* dried by FIRD and FD.

Sample	Yield (%)					
	FIRD[a]					FD[b]
	40℃	50℃	60℃	70℃	80℃	
50ME[c]	28.00±1.48[e]	30.00±1.47	32.00±1.54	34.33±1.12	45.33±1.32	27.00±2.08
100ME[d]	13.00±1.69	14.67±1.64	15.33±1.46	20.67±1.39	25.33±1.45	10.67±1.79

[a] FIRD: far-infrared radiation drying, [b] FD: freeze drying

[c] 50ME: 50% methanolic extract, [d] 100ME: 100% methanolic extract

[e] The values are averages of triplicate determinations.

Table 2-2. Total phenolic contents of methanolic extracts of *E. cava* dried by FIRD and FD.

Sample	Content (g/100 g)					
	FIRD					FD
	40℃	50℃	60℃	70℃	80℃	
50ME	6.35±0.2[a]	6.13±0.4	5.95±0.2	5.70±0.3	5.47±0.7	5.07±0.1
100ME	5.79±0.9	5.68±0.7	5.54±0.8	5.43±0.1	5.05±0.2	3.89±0.5

[a] Mean±SE of determinations was made in duplicate experiments.

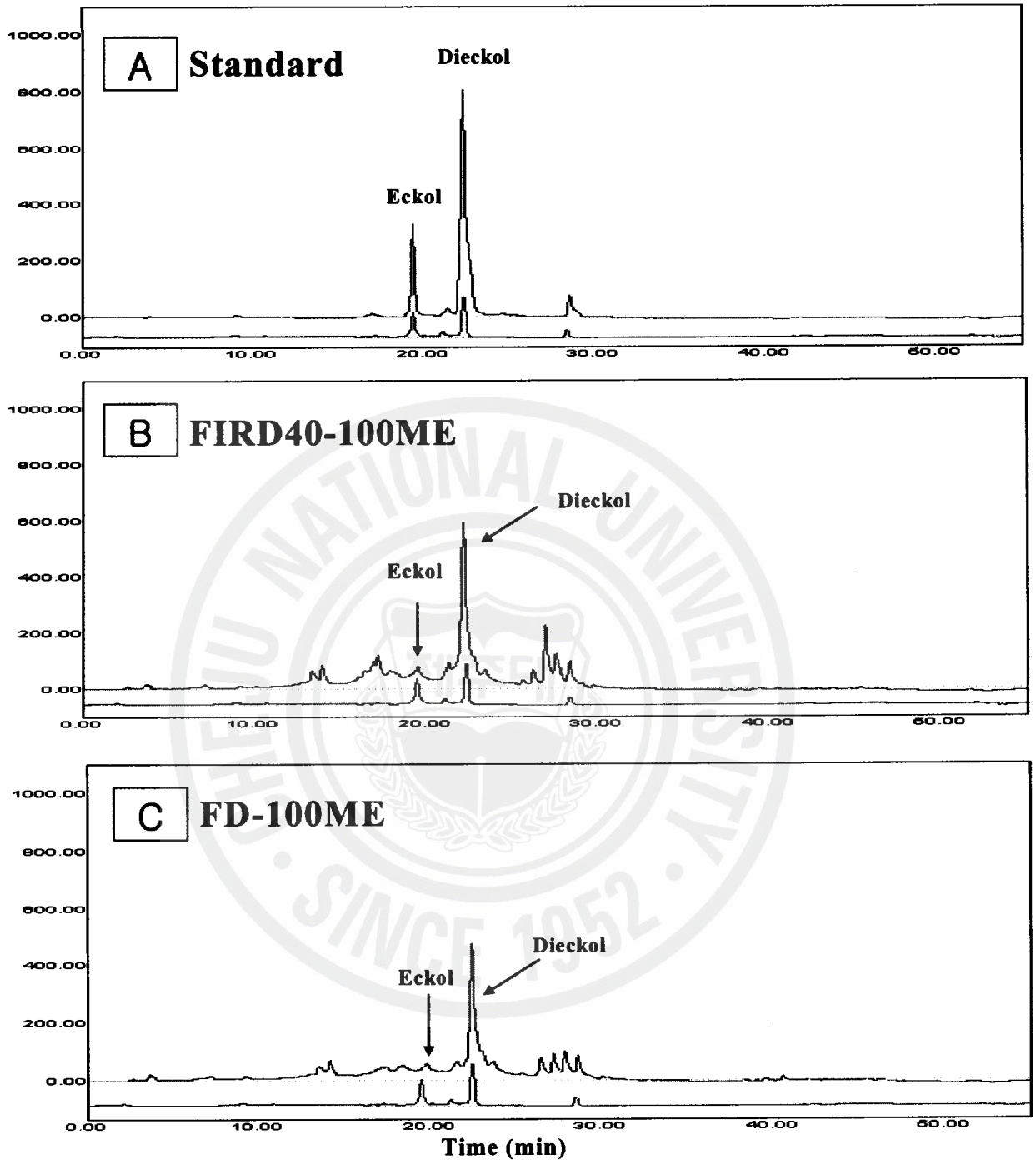

Fig. 2-1. The HPLC chromatograms for the eckol and dieckol of FIRD40-100ME and FD-100ME from *E. cava*. The chromatogram for the dieckol standards (A); The chromatogram for the FIRD40-100ME sample (B); The chromatogram for the FD-100ME sample (C).

Table 2-3. Dieckol and eckol contents of 100% methanolic extracts from *E. cava* dried by FIRD and FD.

100ME[a]	Content (mg/100 g)					
	FIRD					FD
	40℃	50℃	60℃	70℃	80℃	
Dieckol	1487±0.2[b]	1259±0.4	1163±0.2	1138±0.3	1328±0.7	1036±0.1
Eckol	125±0.9	112±0.7	111±0.8	109±0.1	123±0.2	70±0.5

[a] 100ME: 100% methanolic extract

[b] Mean±SE of determinations was made in duplicate experiments.

3.4. DPPH radical scavenging assay

DPPH is a stable free radical donor, which has been widely used to test free radical scavenging effect of natural antioxidants. In this study, the scavenging activities of all the methanolic extracts from *E. cava* dried by FIRD and FD on DPPH radical are shown Fig. 2-2. All the tested extracts showed strong DPPH radical scavenging activity. Among them, 100% methanolic extract from *E. cava* dried by FIRD at 40℃ showed the highest DPPH radical scavenging activity (IC_{50} 4.6 μg/ml). In case of same drying condition, 100% methanolic extracts exhibited DPPH radical scavenging activity higher than 50% methanolic extracts. In case of FIRD, DPPH radical scavenging activity was decreased as the drying temperature was increased. But, except for the drying condition at 80℃, FIRD extracts showed higher DPPH radical scavenging activity than FD extracts. In addition, the scavenging activity of those extracts increased with increasing concentrations from 2.5 μg/ml to 25 μg/ml.

3.5. Hydroxyl radical scavenging assay

Hydroxyl radicals generated in the Fenton system (Fe^{2+}/H_2O_2) were trapped by DMPO, forming a spin adduct as detected by the ESR spectrometer. As shown in Fig. 2-3, all the tested extracts showed good hydroxyl radical scavenging activity. Among them, 50% methanolic extract from *E. cava* dried by FIRD at 40℃ exhibited the highest scavenging activity (IC_{50} 224.2 μg/ml). 50% methanolic extracts from the same drying condition showed higher hydroxyl radical scavenging activity than 100% methanolic extracts. In case of FIRD, hydroxyl radical scavenging activity decreased as the drying temperature was increased. But, except for the drying condition at 80℃, FIRD extracts showed higher scavenging activity than FD extracts. In addition, the scavenging activity of those extracts increased with increasing concentrations from 125 μg/ml to 500 μg/ml.

3.6. Alkyl radical scavenging assay

The alkyl radical spin adduct was observed when AAPH was incubated with the spin trap 4-POBN at 37℃ for 30 min. The scavenging activities of all the methanolic extracts from *E. cava* dried by FIRD and FD on alkyl radical are shown Fig. 2-4. All the tested extracts showed strong alkyl radical scavenging activity. Among them, 100% methanolic extract from *E. cava* dried by FIRD at 40℃ exhibited the highest scavenging activity (IC_{50} 2.0 μg/ml). Also at the same drying condition, 100% methanolic extracts showed higher alkyl radical scavenging activity than 50% methanolic extracts. In case of FIRD, alkyl radical scavenging activity decreased as the drying temperature was increased. Again, except for the drying condition at 80℃, FIRD extracts showed higher alkyl radical scavenging activity than FD extracts. In addition, the scavenging activity of those extracts increased with increasing concentrations from 1.25 μg/ml to 12.5 μg/ml.

3.7. Hydrogen peroxide scavenging assay

The scavenging activities of the methanolic extracts from *E. cava* dried by FIRD and FD on hydrogen peroxide are shown on Fig. 2-5. All the extracts showed good hydrogen peroxide scavenging activity. Among them, 100% methanolic extract from *E. cava* dried by FIRD at 40℃ exhibited the highest scavenging activity (IC_{50} 31.0 μg/ml). At the same drying condition, 100% methanolic extracts showed higher hydrogen peroxide scavenging activity than 50% methanolic extracts. In case of FIRD, hydrogen peroxide scavenging activity decreased as the drying temperature was increased. But, except for the drying condition at 80℃, FIRD extracts showed higher hydrogen peroxide scavenging activity than FD extracts. In addition, the scavenging activity of those extracts increased with increasing concentrations from 20 μg/ml to 90 μg/ml.

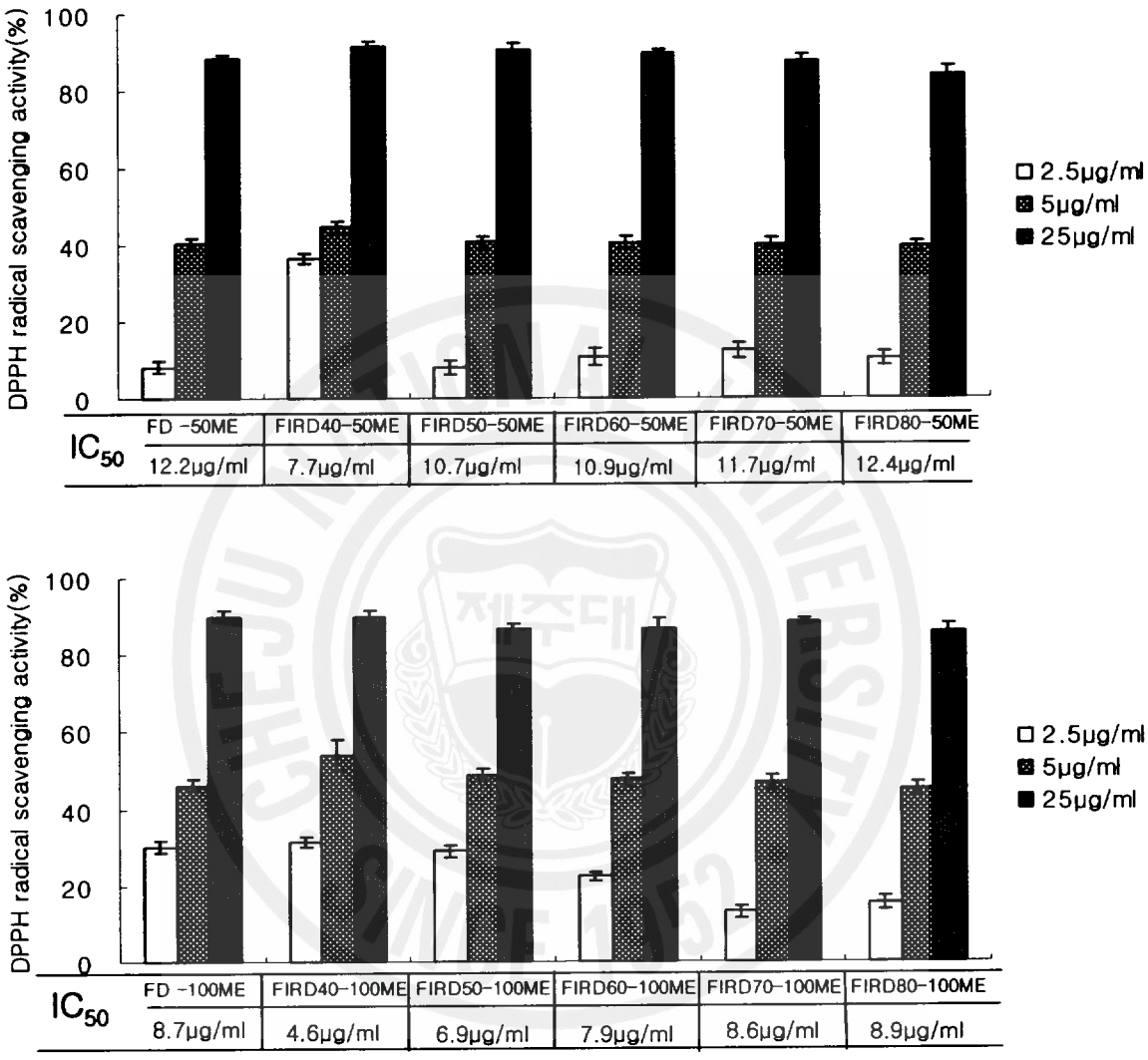

Fig. 2-2. DPPH radical scavenging activities of methanolic extracts from *E. cava* dried by FIRD and FD. FD: freeze drying, FIRD: far-infrared radiation drying (40-80℃). 50ME: 50% methanolic extract, 100ME: 100% methanolic extract

IC_{50} can be found from a plot of percent inhibition versus the concentration of extracts from *E. cava*. Mean±SE of determinations was made in duplicate experiments.

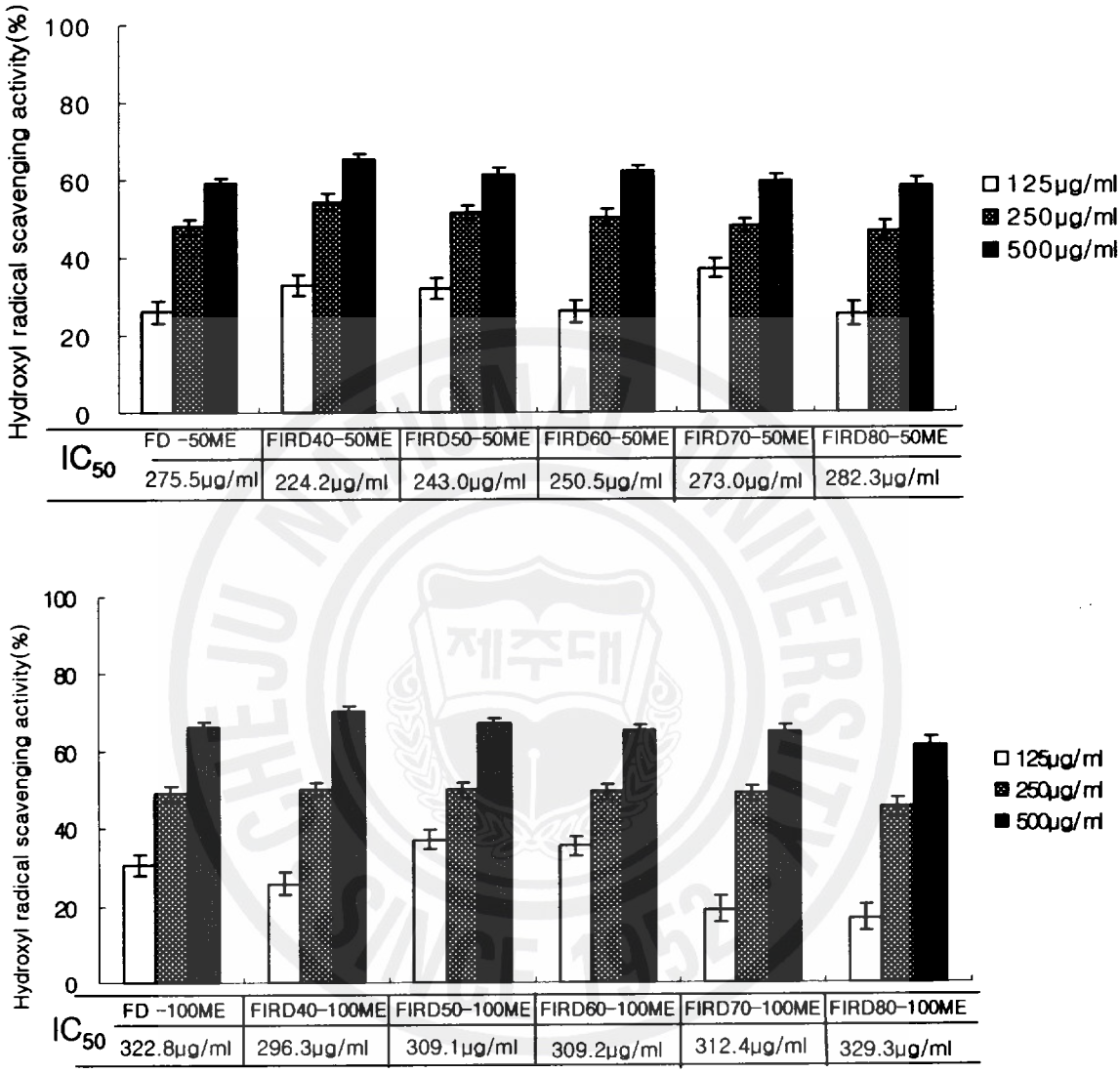

Fig. 2-3. Hydroxyl radical scavenging activities of methanolic extracts from *E. cava* dried by FIRD and FD. Abbreviations are the same as in Fig. 2-2.

Mean±SE of determinations was made in duplicate experiments.

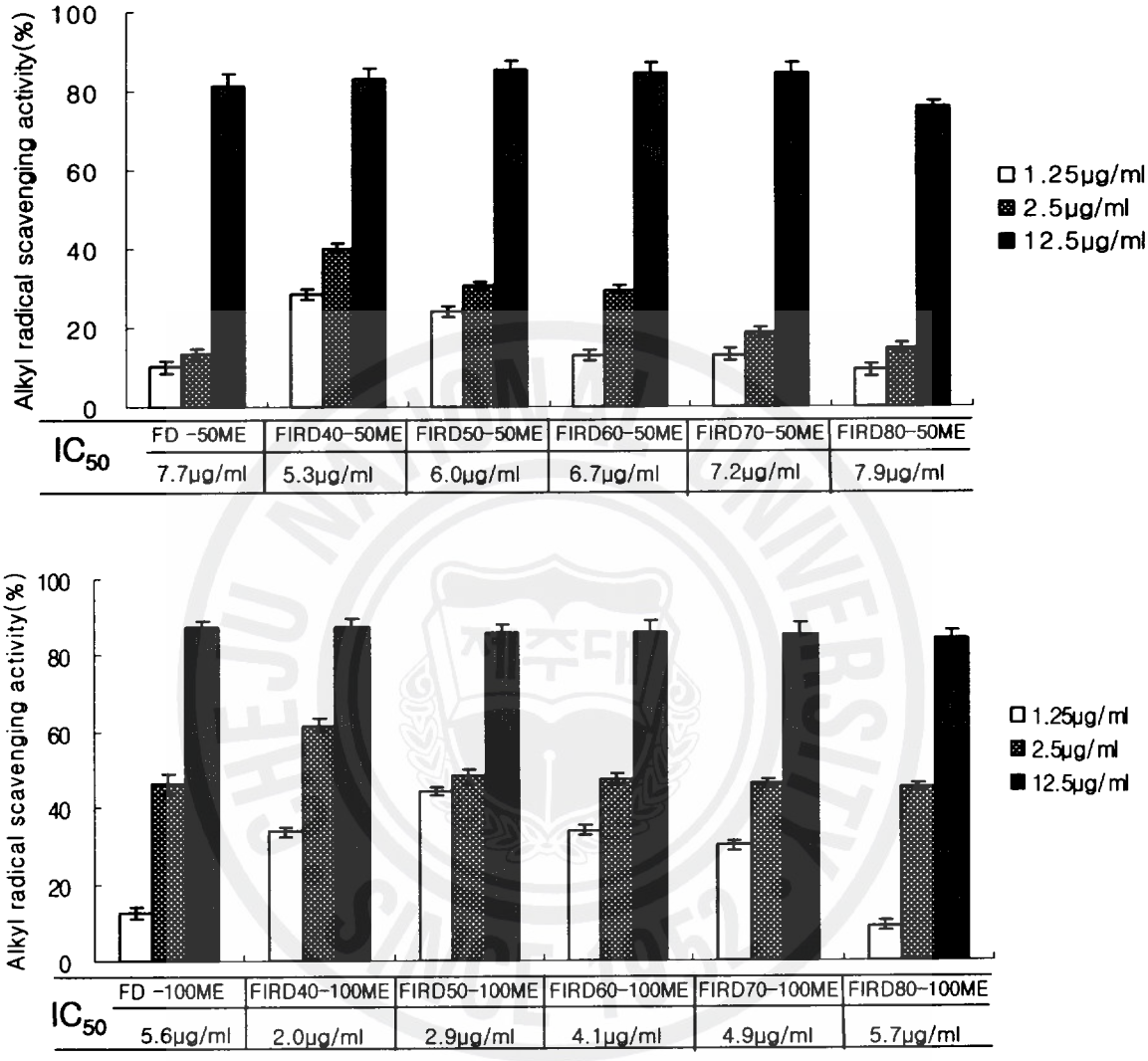

Fig. 2-4. Alkyl radical scavenging activities of water and enzymatic extracts from *E. cava* dried by FIRD and FD. Abbreviations are the same as in Fig. 2-2.

Mean±SE of determinations was made in duplicate experiments.

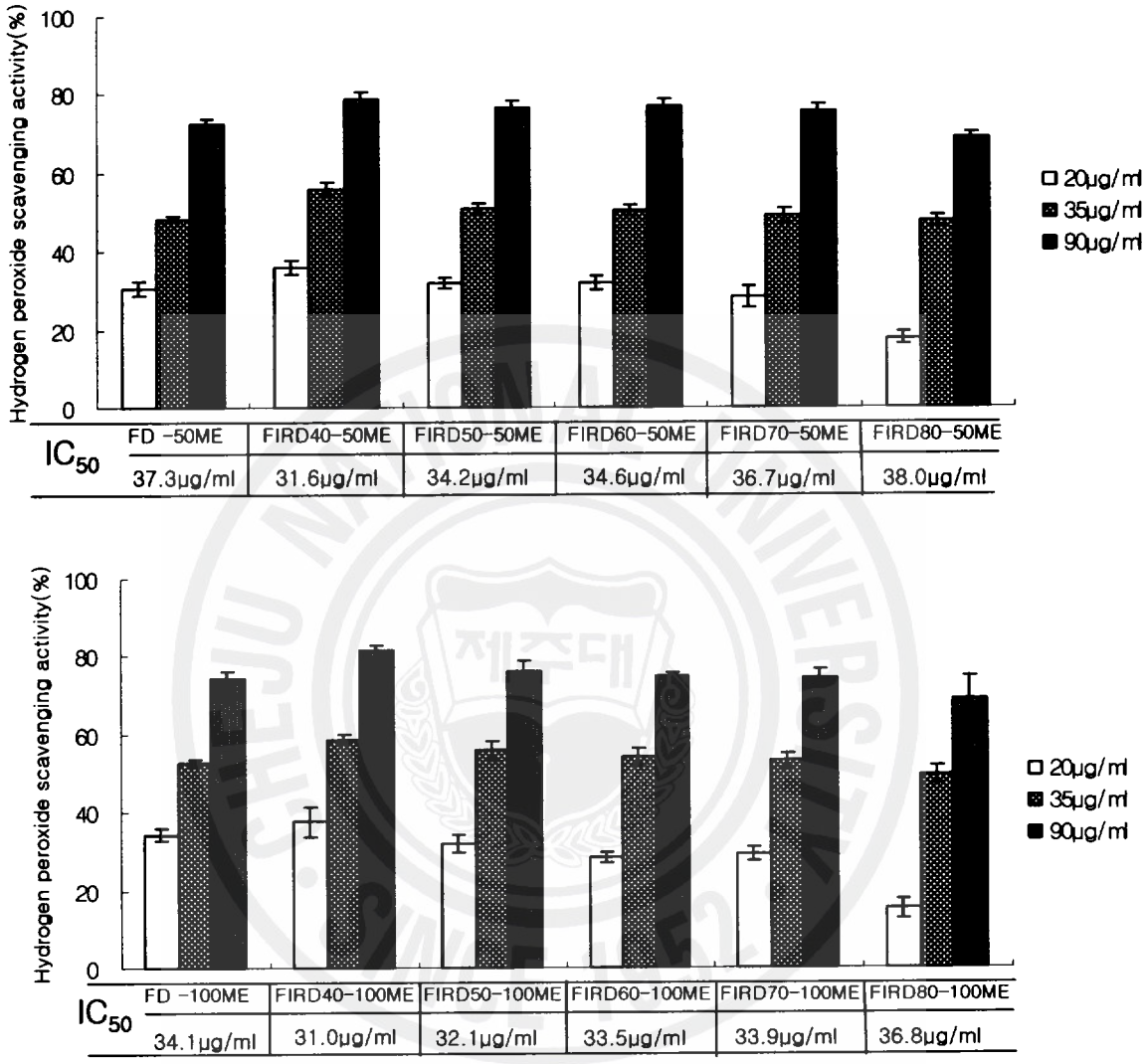

Fig. 2-5. Hydrogen peroxide scavenging activities of water and enzymatic extracts from *E. cava* dried by FIRD and FD. Abbreviations are the same as in Fig. 2-2.
Mean±SE of determinations was made in duplicate experiments.

3.8. Hydrogen peroxide scavenging assay by DCF-DA

As 100% methanolic extract from *E. cava* dried by FIRD at 40℃ possessed the highest hydrogen peroxide scavenging activity, this extract was further evaluated for its H_2O_2 scavenging activity by DCF-DA. The effect of the 100% methanolic extract from *E. cava* dried by FIRD at 40℃ (FIRD40-100ME) and FD (FD-100ME) on H_2O_2 scavenging activity by DCFH-DA is exhibited in Fig. 2-6. Both samples showed more than >40% H_2O_2 scavenging activity at 25 μg/ml. Comparatively, FIRD40-100ME showed better activity than FD-100ME. Both samples showed increased H_2O_2 scavenging activity in a dose-dependent manner. Especially, FIRD40-100ME had the highest H_2O_2 scavenging activity (70%) at 100 μg/ml.

3.9. Protective effect against H_2O_2-induced cell damage

As FIRD40-100ME possessed the highest hydrogen peroxide scavenging activity, this extract was further evaluated for its protecting ability against H_2O_2 -induced cell damages. The protective effect of the FIRD40-100ME and FD-100ME on H_2O_2-induced cell damage on Vero cells is exhibited in Fig. 2-7. In this study, both samples dose-dependently controlled H_2O_2-induced cellular damage. The addition of H_2O_2 to the cell medium without extracts rendered only 24.5% cell survival rate but addition of extracts along with H_2O_2 to the medium dose-dependently increased cell survival rate. However, the FIRD40-100ME and FD-100ME with concentrations of 25~100 μg/ml indicated almost similar activity enhancement. Especially, the highest cell viability with 65.2% was recorded on FIRD40-100ME at a concentration of 100 μg/ml.

3.10. Reduction of H_2O_2-induced apoptotic body

In order to analyze the protective effect of FIRD40-100ME and FD-100ME on H_2O_2-induced apoptosis, nuclei of Vero cells stained with Hoechst 33342 treated with H_2O_2 in the absence or presence of FIRD40-100ME and FD-100ME. Typical fluorescence photographs of

shrunken nuclei, chromatic condensation and appearance of apoptotic bodies were shown in Vero cell after the treatment with H_2O_2 (500 μM) for 24 h (Fig. 2-8 B). The negative control, treated without the sample and H_2O_2 showed clear image and exhibited no cell damage (Fig. 2-8 A). However, obvious cell damage was observed in the cell treated with H_2O_2. Cells treated with FIRD40-100ME and FD-100ME 1 h prior to H_2O_2 treatment exhibited a dramatic reduction in apoptotic bodies. Therefore, the photographs (Fig. 2-8 C~H) clearly suggest the ability of FIRD40-100ME and FD-100ME to protect cell damage against H_2O_2 attack. Especially, the highest reduction of H_2O_2 –induced apoptotic body was observed on FIRD40-100ME at a concentration of 100 μg/ml (Fig. 2-8 E).

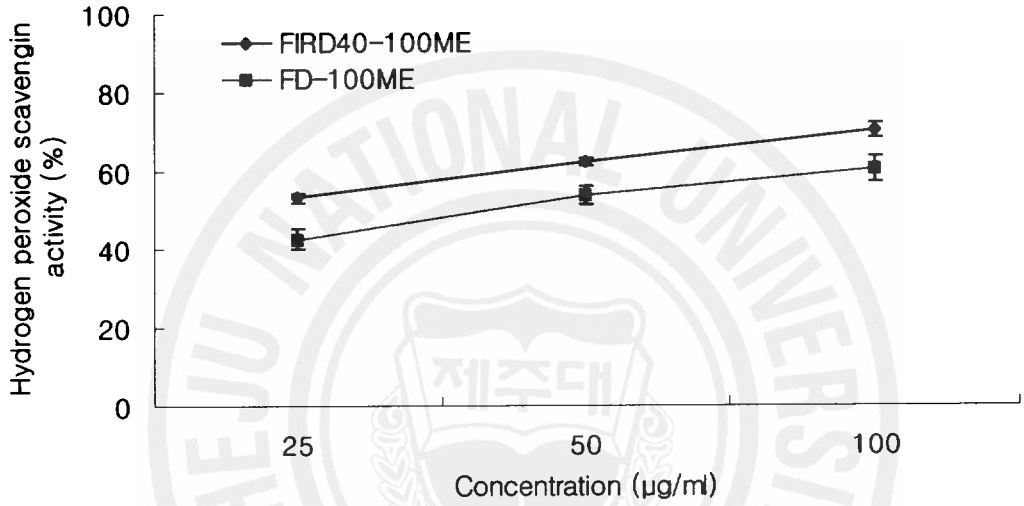

Fig. 2-6. Hydrogen peroxide scavenging activities of FIRD40-100ME and FD-100ME from *E. cava* on H_2O_2-induced oxidative damage in Vero cells.
Mean±SE of determinations was made in duplicate experiments.

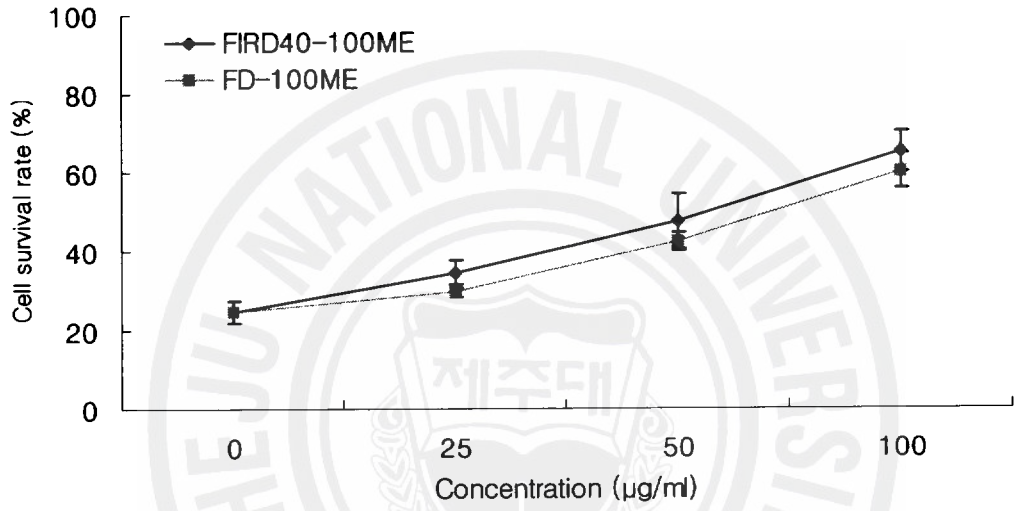

Fig. 2-7. Protective effect of FIRD40-100ME and FD-100ME from *E. cava* against H_2O_2-induced oxidative damage in Vero cells.
Mean±SE of determinations was made in duplicate experiments.

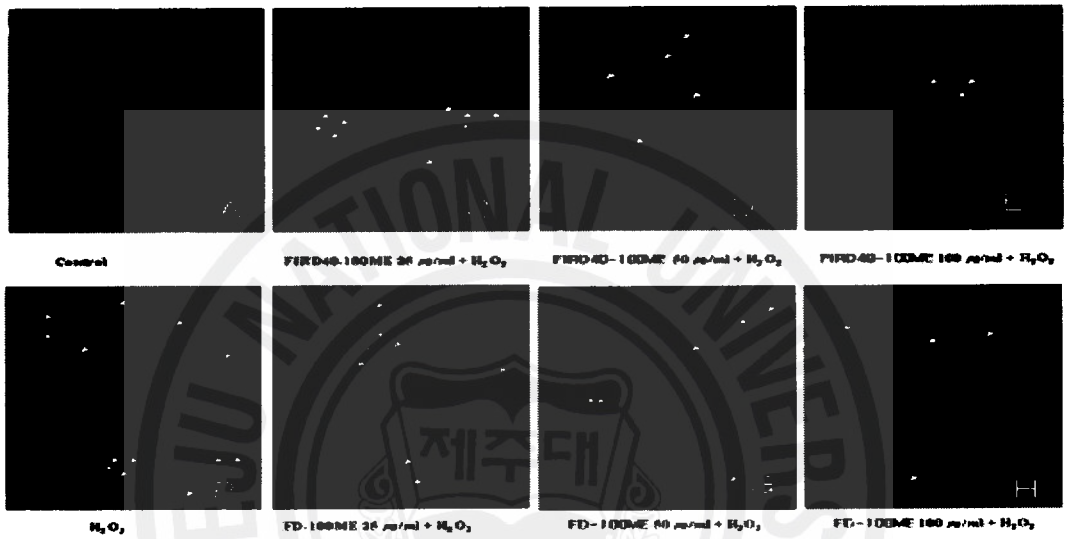

Fig. 2-8. Protective effect of FIRD40-100ME and FD-100ME from *E. cava* against H_2O_2-induced apoptosis in Vero cells. Cellular morphological changes were observed using fluorescence microscope. Photomicrographs of Vero cells treated with (A) vehicle only; (B) 500 μM H_2O_2 (C) 25 μg/ml FIRD40-100ME + 500 μM H_2O_2 (D) 50 μg/ml FIRD40-100ME + 500 μM H_2O_2 (E) 100 μg/ml FIRD40-100ME + 500 μM H_2O_2 (F) 25 μg/ml FD-100ME + 500 μM H_2O_2 (G) 50 μg/ml FD-100ME + 500 μM H_2O_2 (H) 100 μg/ml FD-100ME + 500 μM H_2O_2. Apoptotic bodies are indicated by arrows.

4. Discussion

Reactive oxygen species (ROS) such as DPPH, hydroxyl radical, hydrogen peroxide are physiological metabolites formed during aerobic life as a result of the metabolism of oxygen. DNA, cell membranes, proteins and other cellular constituents are target site of the degradation processes, and consequently induce different kinds of serious human diseases including atherosclerosis, rheumatoid arthritis, muscular dystrophy, cataracts, some neurological disorders and some types of cancer as well as aging (Kovatcheva et al., 2001; Ruberto et al., 2001).

E.cava has pigment, fucoxathin, fucoidan, phycocolloid and especially is a good source of alginates (Ghiry and Bulunden, 1991). According to Heo et al. (2005), methanolic extracts of freeze dried *E. cava* showed positive effect on scavenging DPPH radical, hydroxyl radical, hydrogen peroxide. Also, methanolic extract have higher total phenolic content than water extracts.

Many natural plant antioxidants, however, exist either as bound forms to high molecular weight compounds or as part of the repeating subunits of high molecular weight polymers (Niwa and Miyachi, 1986). Several methods including far-infrared radiation are known to liberate and activate low molecular weight natural antioxidants (Niwa et al., 1988). In the present study, methanolic extracts from *E. cava* dried by far infrared radiation drying at 40~80°C were investigated on the DPPH, hydroxyl radical, alkyl radical, hydrogen peroxide scavenging activity and protective effect against H_2O_2–induced cell damage.

Many researchers have reported positive correlation between free radical scavenging activity and total polyphenolic compound. Oki et al. (2002) observed that the radical scavenging activity increased with the increase of polyphenolic compound content. According to the polyphenolic content results (Table 2-2), all the extracts from *E. cava* dried by FD and FIRD were rich in

polyphenolic compounds. FIRD extracts have higher total polyphenolic content than FD extracts. The radical scavenging activities of all the extracts from *E. cava* dried by FIRD and FD on DPPH, hydroxyl, alkyl, hydrogen peroxide are shown Fig. 2-2~5. All the extracts from *E. cava* dried by FD and FIRD at 40~80℃ showed strong radical scavenging activity. Except for the drying condition at 80℃, FIRD extracts had lower IC_{50} values than that of the FD extract. Among them, 100% methanolic extracts from *E. cava* dried by FIRD at 40 ℃ (FIRD40-100ME) showed the lowest IC_{50} value (DPPH: 4.6 μg/ml, Alkyl radical: 2.0 μg/ml, and Hydrogen peroxide: 31.0 μg/ml). Lee et al. (2003) reported far-infrared radiation treatment on rice hulls increased the radical scavenging activity and total polyphenolic content of methanolic extracts from rice hulls compared to oven drying at 100℃. Also, this study showed that total polyphenolic content and radical scavenging activity in all extracts from dried *E. cava* increased significantly by FIRD (except for the drying condition at 80℃).

As the FIRD40-100ME generated in this study evidenced such high H_2O_2 radical scavenging activity, this sample was evaluated on the H_2O_2 radical scavenging activity by DCF-DA and protective effect against H_2O_2-induced cell damage. The DCF-DA method was used to detect the intracellular hydrogen peroxide level (Rosenkranz et al., 1992). DCF-DA diffuses into cells, where it is hydrolyzed by intracellular esterase to polar 2′,7′-Dichlorodihydrofluorescein. This non-fluorescent fluorescein analog gets trapped inside the cells and is oxidized by intracellular oxidants to a highly fluorescent, 2′,7′-Dichlorodi-hydrofluorescein. FIRD40-100ME from *E. cava*, The H_2O_2 radical scavenging activity showed better activity than FD-100ME and then enhanced the viability of Vero cells exposed to H_2O_2. The protective effects of FIRD40-100ME on H_2O_2 -induced apoptosis was observed under microscopy. The cells exposed to H_2O_2 exhibited distinct morphological features of apoptosis, such as nuclear fragmentation and apoptotic body. However, the cells pretreated with FIRD40-100ME had significantly reduced

H_2O_2 –mediated apoptosis in Vero cells. According to the previous reports, polyphenolic compounds like eckol and dieckol which are rich in *E. cava* effectively control V79-4 cells against H_2O_2 damage by enhancing the cellular antioxidant activity and free radical scavenging activities (Kang et al., 2005; Ahn et al., 2007). This study showed FIRD40-100ME extract had a higher eckol and dieckol content than FD-100ME extract (Table 2-3).

Far-infrared radiation drying of *E. cava* liberated phenolic compounds and thus increased the amounts of active compounds in extracts. Far-infrared radiation drying released more phenolic compounds in *E. cava* and thus increased the radical scavenging activity and enhanced the viability of Vero cells exposed to H_2O_2 of *E. cava* extract. Therefore, far-infrared radiation drying with its simple process would be more effective in releasing antioxidant compounds from marine seaweeds by products such as *E. cava* at an industrial scale.

Part Ⅲ

Antioxidative effect on radical scavenging and H_2O_2-induced DNA damage of *Ecklonia cava* extracts prepared by far infrared radiation drying and ultrasonic extraction

Abstract

Water and methanolic extracts of *Ecklonia cava* dried in far infrared radiation drying at 40℃ were prepared under ultrasonic extraction (UE) and conventional extraction (CE) investigated for radical scavenging activity, as well as inhibitory effect on H_2O_2-induced DNA damage. In addition, the total polyphenolic content and extraction yield were measured. Extraction yield and total polyphenolic content increased as the ultrasonic extraction time was increased. Water extract showed higher extraction yield than methanolic extract. Also, all the extracts by UE exhibited higher than the yield of CE. On the other hand, in case of total polyphenolic content, 50% methanolic extract showed higher total polyphenolic content than water extract, and all the extracts by CE exhibited higher total polyphenolic contents than UE. Among the tested extracts, 100% methanolic extracts by UE and CE showed the lowest IC_{50} value (DPPH, hydroxyl radical, alkyl radical and hydrogen peroxide). In case of all the extracts by UE, radical scavenging activities increased as the ultrasonic extraction time was increased. All the extracts by CE showed higher DPPH and hydroxyl radical scavenging activity than UE. But, in case of alkyl radical and hydrogen peroxide, extracts by UE showed higher scavenging activity than CE. In all the tested samples for hydrogen peroxide scavenging activity of extracts, 100% methanolic extract with UE 12 h (100MEU12h) and 100% methanolic extract with CE 24 h (100MEC24h) were selected and evaluated by comet assay for inhibitory effect against H_2O_2-induced DNA damage. The protective effect of 100MEU12h and 100MEC24h increased as their concentratios increased from 25 to 100 μg/ml. 100MEU12h showed slightly higher protective effect against H_2O_2-induced DNA damage than 100MEC24h. These results indicate that the extracts obtained by UE from *E. cava* have the high extraction yield, potential inhibitory effect on H_2O_2-induced DNA damage and harmful free radicals. Thus, ultrasonic extraction can be effectively used as seaweeds extraction technique and a potential for scale up of the extraction process.

1. Introduction

Ecklonia cava is a brown alga that is abundant in the subtidal regions of Jeju Island in Korea. *E. cava* has pigment, fucoxanthin, fucoidan, phycocolloid and especially is a good source of alginates, which can be used as viscosifiers of thickeners in a wide variety of products (Ghiry and Bulunden, 1991). Recently, it has been reported that *Ecklonia* species exhibits radical scavenging activity (Heo et al., 2005 a, b; Kang et al., 2003), anticoagulant activity (Athukorala et al., 2006a), antitumor activities and/or cell damage protective effects (Kim et al., 2006 a, b), anti-plasmin inhibiting activity (Fukuyama et al., 1990), bactericidal activity (Nagayama et al., 2002) and HIV-1 reverse transcriptase and protease inhibiting activity (Ahn et al., 2004). The wide range of biological activities associated with natural compounds of *E. cava* may expand its value in food industry and pharmaceutical industry in whole over the world.

The benefit using ultrasonication in plant extraction has already been demonstrated for a number of compounds interested to both the pharmacology and food industries (Vinatoru, Toma and Mason, 1999). Specific examples of the benefits include the extraction of tea solids from dried leaves with water using ultrasound giving an improvement of almost 20% in yield at 60℃, approaching the efficiency of thermal extraction at 100℃ (Mason and Zhao, 1994). The main improvement of sonication for the extractions are related to the yield and shortening of the extraction time (Salisova and Toma, 1997; Valachovic, Pechova and Mason, 2001). Ultrasounds produce cell disruption, particle size reduction and ultrasonic jet towards solid's surfaces leading to a greater contact area between solid and liquid phase, better access of solvent to valuable components (Mason and Cordemans 1996; Sun, Peng and Yang, 1999).

Nowadays, sonication has been employed to extract active compounds such as rutin and quercetin (Yang and Zhang, 2007), antioxidants (Albu et al., 2004), polysaccharides (Yang et al.,

2007) and bioactive principles (Vinatoru et al., 1999) from plant materials. So far, for the extraction of dried *E. cava* using ultrasound, no such work has been reported in the literature. Hence, the objective of this study was to investigate the effect of ultrasonic technique on extraction yield, total polyphenol content and radical scavenging activity, and inhibitory effect of H_2O_2-induced DNA damage in the preparation of *E. cava* extracts.

2. Materials & Methods

2.1. Materials

The brown seaweed *Ecklonia cava* was collected along the coast of Jeju Island in Korea, during the period from March to May 2007. Salt, sand and epiphytes were removed using tap water. Finally, the samples were rinsed with fresh water and stored at -20℃ for further experiments. 5,5-Dimethyl-1-pyrrolin N-oxide (DMPO), 2,2-azobis (2-amidinopropane) hydrochloride (AAPH), α-(4-pyridyl-1-oxide)-N-t-butylnitrone (4-POBN), DPPH (1,1-diphenyl-2-pricrylhydrazyl), peroxidase, ABTS (2,2-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid) obtained from Sigma Chemical Co. (St. Louis, Mo, USA). All other reagents were of the highest grade available commercially.

2.2. The preparation of dried *E. cava*

The *E. cava* were cut into small pieces. *E. cava* were dried in far infrared radiation dryer (JOURI-Q, KEC, Korea) at 40℃ drying temperature. After drying, *E. cava* samples was ground and sieved through a 50 standard testing sieve.

2.3. The preparation of extracts from *E. cava* by ultrasonic extraction

For the ultrasonic extraction experiments, an ultrasonic bath was used as an ultrasound source. The bath, JAC 2010 (Kodo Technical Research Co. Ltd., Korea) was a rectangular container (30 cm × 24 cm × 15 cm), to which 40 kHz transducers were annealed at the bottom, and the bath power rating was 200 W. The extraction of *E. cava* was performed by adding 1 g of the ground *E. cava* powder into 100 ml of water and methanol (50%, 100%, respectively) in a 150 ml flask. The flask was then partially immersed into the ultrasonic bath for 6 h and 12 h at 30℃, which contains 2.0 l of water. The extracts were centrifuged at 3500 rpm for 20 min at 4°C and filtered with Whatman filter paper to remove the residue, there after evaporated under vacuum at 40°C

to removed all methanol, and then dissolved in water. All samples were kept in −20°C for further experiments.

2.4. The preparation of extracts from *E. cava* by conventional extraction

For extracts from *E. cava* by conventional extraction, one gram of the ground *E. cava* powder was mixed with 100 ml of water, methanol (50%, 100%, respectively) and placed in shaking incubator for 24 h at room temperature. The mixtures were centrifuged at 3500 rpm for 20 min at 4°C and filtered with Whatman filter paper to remove the residue, there after evaporated under vacuum at 40°C to removed all methanol, and then dissolved in water. All samples were kept in −20°C for further experiments.

2.5. Measurement of extraction yield

Yields of the extracts obtained by ultrasonic extraction (UE) and conventional extraction (CE) of *E. cava* were calculated by dividing the dry weight of extract filtrate over dry weight of the *E. cava* sample used.

2.6. Determination of total polyphenolic content

Phenolic contents were determined using a protocol similar to the mothod described by Chandler and Dodds (1983) as described by Shetty et al. (1995). Each 1 ml of *E. cava* extracts, 1 ml of 95% EtOH, 5 ml of distilled water, and 0.5 ml of 50% Folin-Ciocalteu reagent were mixed. The mixtures were allowed to react for 5 min, and then 1 ml of 5% Na_2CO_3 was added, and the mixture was thoroughly mixed and placed in the dark for 1h. Absorbance was measured at 725 nm and gallic acid standard curve was obtained for the calibration of phenolic content.

2.7. DPPH radical scavenging assay using an ESR spectrometer

DPPH radical scavenging activity was measured using the method described by Nanjo et al. (1996). Methanol solution of 60 μl of each sample (or methanol itself as control) was added to

60 μl of DPPH (60 μmol/l) in methanol. After mixing vigorously for 10 seconds, the solutions were transferred into a 100 μl Teflon capillary tube and fitted into the cavity of the ESR (electron spin resonance) spectrometer (JES-FA machine, JEOL, Tokyo, Japan). The spin adduct was measured on an ESR spectrometer exactly 2 min later. Measurement conditions: central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 5 mW, gain 6.3×10^5, temperature 298 K.

2.8. Hydroxyl radical scavenging assay using an ESR spectrometer

Hydroxyl radicals were generated by Fenton reaction, and reacted rapidly with nitrone spin trap DMPO; the resultant DMPO-OH adducts was detectable with an ESR spectrometer (Rosen and Rauckman, 1984). The ESR spectrum was recorded 2.5 min after mixing in a phosphate buffer solution (PBS; pH 7.4) with 0.3 M DMPO 20 μl, 10 mM $FeSO_4$ 20 μl and 10 mM 20 μl using an ESR spectrometer set at the following conditions: central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 5 mW, gain 6.3×10^5, temperature 298 K.

2.9. Alkyl radical scavenging assay using an ESR spectrometer

Alkyl radicals were generated by AAPH. The PBS (pH 7.4) reaction mixtures, containing 40 mmol/l AAPH, 40 mmol/l 4-POBN and indicated concentrations of tested samples, were incubated at 37℃ in a water bath for 30 min (Hiramoto et al., 1993), and then transferred to a 100 μl Teflon capillary tube. The spin adduct was recorded on JES-FA ESR spectrometer. Measurement conditions: central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 5 mW, gain 6.3×10^5, temperature 298 K.

2.10. Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging activity was determined according to the method of Muller (1985). A 100 μl of 0.1M phosphate buffer (pH 5.0) and the sample solution were mixed in a

96-well plate. A 20 μl of hydrogen peroxide was added to the mixture, and then incubated at 37℃ for 5 min. After the incubation, 30 μl of 1.25 mM ABTS and 30 μl of peroxidase (1 unit/ml) were added to the mixture, and then incubated at 37℃ for 10 min. The absorbance was read with an ELISA reader at 405 nm.

2.11. Cell culture

To study the inhibition effect of extracts on H_2O_2-mediated DNA damage, we used the L5178 mouse T-cell lymphoma cell line (L5178Y-R). The L5178 mouse T-cell lymphoma cell line (L5178Y-R) were maintained at 37℃ in an incubator with a humidified atmosphere of 5 % CO_2 and cultures in RPMI 1640 medium supplemented with 10 % (v/v) heat inactivated fetal bovine serum (FBS), streptomysin (100 μg/ml), penicillin (100 unit/ml).

2.12. Comet assay

The alkaline comet assay was conducted according to Ahn et al. (2007). The number of cultured cells was adjusted as 4×10^4 cells/ml and the cells were incubated with each samples that have the concentrations of from 25 to 100 μg/ml determined according to the hydrogen peroxide scavenging activity for 30 min at 37℃ in a dark incubator. After preincubation, the cells were centrifuged at a minimum rpm for 5 min and washed using phosphate buffer saline (PBS). Then, the cells were resuspended in PBS with 50 μM H_2O_2 for 5 min on ice. The untreated control cells were resuspended only in PBS without H_2O_2. The cells were washed with 1 ml PBS and centrifuged. The cell suspension was mixed with 100 μl of 0.7% low melting point agarose (LMPA), and added to 1.0% normal melting point agarose (NMPA)-coated slides. After keeping them for 10 min at 4℃, the slides were covered with another 100 μl of 0.7% LMPA and kept for 40 min at 4℃ for solidification of the agarose. And the slides were immersed in lysis solution (2.5 M NaCl, 100 μM EDTA, 10 mM Tris, 1%sodium laurylsarcosine and 1% Triton X-100) for 1 h at 4℃. The slides were unwinded and applied for

electrophoresis with the electric current of 25 V/300 mA for 20 min. Then, the slides were neutralized in 0.4 M Tris buffer (pH 7.5) for 10 min two times and dehydrated with 70% ethanol. The percentage of fluorescence in the DNA tail of each cell (tail intensity, TI; 50 cells from each of two replicate slides) on the ethidium bromide stained slides were measured by image analysis (Kinetic Imaging, Komet 5.0, UK) and fiuorescence microscope (LEICA DMLB, Germany).

2.13. Statistical analysis

Data were analyzed using the SPSS package for windows (Version 10). Values were expressed as mean ± standard error (SE). The mean values of the tail intensity from each treatment were compared using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. P-value of less than 0.05 was considered significant.

3. Results

3.1. Extraction yield

The extraction yield of extracts obtained by UE and CE from *E. cava* were shown in Table 3-1. Extraction yield increased as the ultrasonic extraction time was increased. Among all the extracts, the water extracts showed higher extraction yield than the methanolic extracts, and the 50% methanolic extracts exhibited higher extraction yield than the 100% methanolic extracts. Among them, the water extract with UE 12 h from *E. cava* showed the highest extraction yield (34.33%). Also, all the extracts by UE 6 h and 12 h exhibited higher extraction yield than CE 24 h.

3.2 Total polyphenolic compounds

All the extracts were subjected to total polyphenolic assay to determine their polyphenolic contents. The total polyphenolic amount of each extract is shown in Table 3-2. The highest polyphenolic content (6.35 g/100 g) was recorded in the 50% methanolic extract by CE 24 h from *E. cava*, whereas, the lowest content (3.42 g/100 g) was shown by the 100% methanolic extract by UE 6 h from *E. cava*. In case of UE, total polyphenolic content increased as the ultrasonic extraction time was increased. The 50% methanolic extracts showed higher total polyphenolic content than the water extracts, and, all the extracts treated by CE for 24 h exhibited higher total polyphenolic content than UE treated for 6 and 12 h.

3.3. DPPH radical scavenging assay

DPPH is a stable free radical donor, which has been widely used to test free radical scavenging effect of natural antioxidants. In this study, the scavenging activities of all the extracts by UE and CE from *E. cava* on DPPH radical are shown Fig. 3-1. All the tested extracts showed strong DPPH radical scavenging activity. Among them, the 100% methanolic extract by CE 24 h from

E. cava showed the highest DPPH radical scavenging activity (IC_{50} 4.6 μg/ml). And in case of the same condition, the 100% methanolic extracts exhibited DPPH radical scavenging activity higher than 50% methanolic extracts. In case of UE, DPPH radical scavenging activity increased as the ultrasonic extraction time was increased. But, extracts obtained by UE 6 h and 12 h showed lower DPPH radical scavenging activity than the extracts by CE 24 h. In addition, the scavenging activity of those extracts increased with increasing concentrations from 2.5 μg/ml to 25 μg/ml.

3.4. Hydroxyl radical scavenging assay

Hydroxyl radicals generated in the Fenton system (Fe2+/H2O2) were trapped by DMPO, forming a spin adduct as detected by the ESR spectrometer. As shown in Fig. 3-2, all the tested extracts showed good hydroxyl radical scavenging activity. Among them, the 50% methanolic extract by CE 24 h from *E. cava* exhibited the highest scavenging activity (IC_{50} 224.2 μg/ml). The 50% methanolic extracts from the same condition showed higher hydroxyl radical scavenging activity than 100% methanolic extracts. In case of UE, hydroxyl radical scavenging activity increased as the ultrasonic extraction time was increased. But, extracts by UE 6 and 12 h showed lower hydroxyl radical scavenging activity than extracts by CE 24 h. In addition, the scavenging activity of those extracts increased with increasing concentrations from 125 μg/ml to 500 μg/ml.

Table 3-1. Yield of extracts using UE and CE from *E. cava* dried by FIRD[a] at 40℃.

	UE[b]-6h (%)	UE-12h (%)	CE[c]-24h (%)
FIRD40-Water	31.33±1.64[d]	34.33±1.45	28.67±1.42
FIRD40-50MeOH	28.33±1.68	30.67±1.75	28.00±1.48
FIRD40-100MeOH	16.00±1.47	16.33±1.87	13.00±1.69

[a] FIRD40: far-infrared radiation drying at 40℃

50MeOH: 50% Methanol, 100MeOH: 100% Methanol

[b] UE: ultrasonic extraction, [c] CE: conventional extraction

[d] The values are averages of triplicate determinations.

Table 3-2. Total phenolic contents of extracts using UE and CE from *E. cava* dried by FIRD[a] at 40℃.

	UE[b] -6h (g/100 g)	UE-12h (g/ 100 g)	CE[c] -24h (g/100 g)
FIRD40-Water	4.14±0.1[d]	4.76±0.1	4.77±0.2
FIRD40-50MeOH	5.58±0.1	6.15±0.1	6.35±0.2
FIRD40-100MeOH	3.42±0.3	3.85±0.2	5.79±0.9

[a] FIRD40: far-infrared radiation drying at 40℃

50MeOH: 50% Methanol, 100MeOH: 100% Methanol

[b] UE: ultrasonic extraction, [c] CE: conventional extraction

[d] Mean±SE of determinations was made in duplicate experiments.

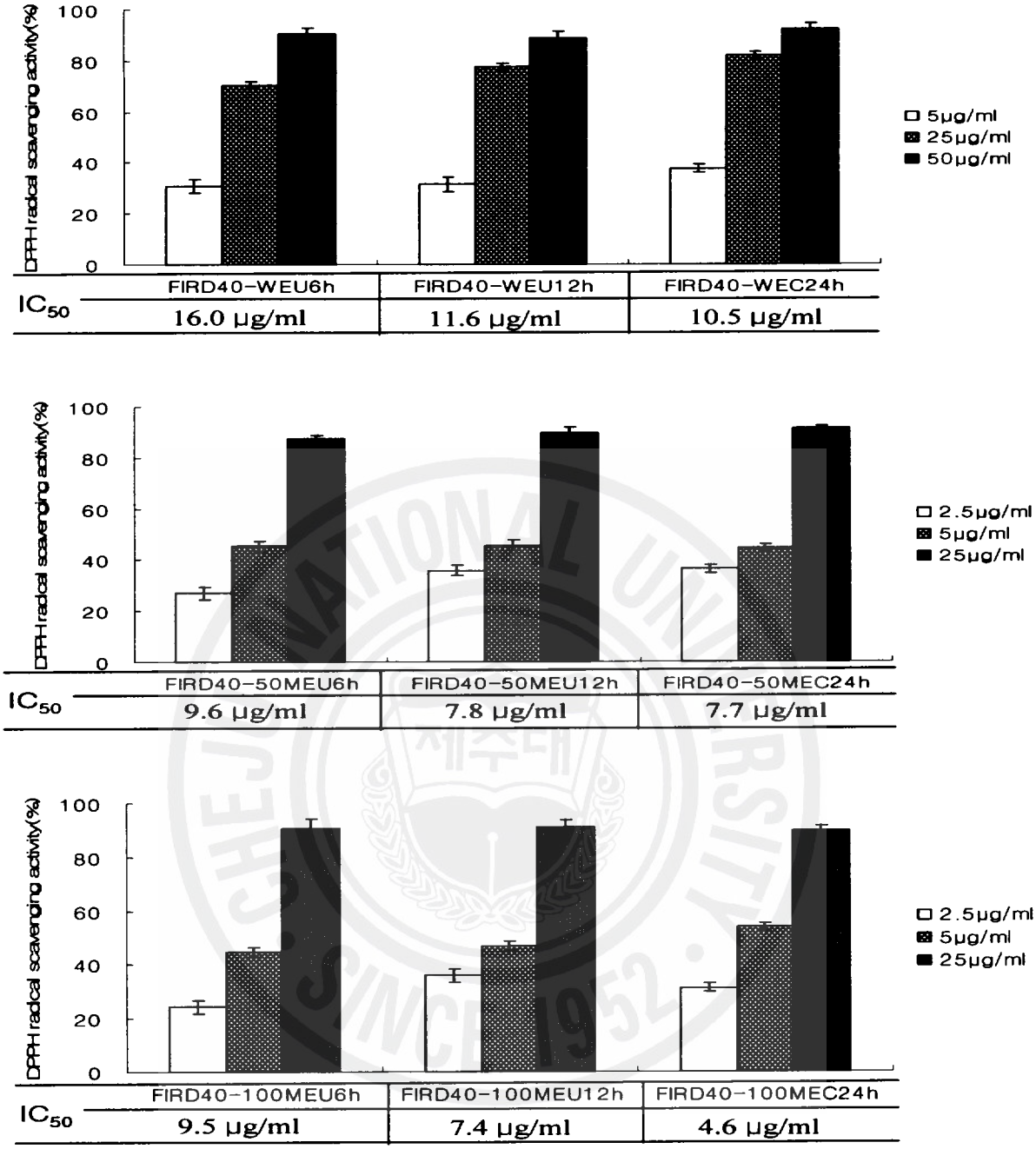

Fig. 3-1. DPPH radical scavenging activities of extracts using UE and CE from *E. cava* dried by FIRD at 40℃. WEU6h (12h): water extract by ultrasonic extraction 6h (12h), WEC24h: water extract by conventional extraction 24h, 50 (100) MEU6h (12h): 50% (100%) methanolic extract by ultrasonic extraction 6h (12h), 50 (100) MEC24h: 50% (100%) methanolic extract by conventional extraction 24h.

IC_{50} can be found from a plot of percent inhibition versus the concentration of extracts from *E. cava*. Mean±SE of determinations was made in duplicate experiments.

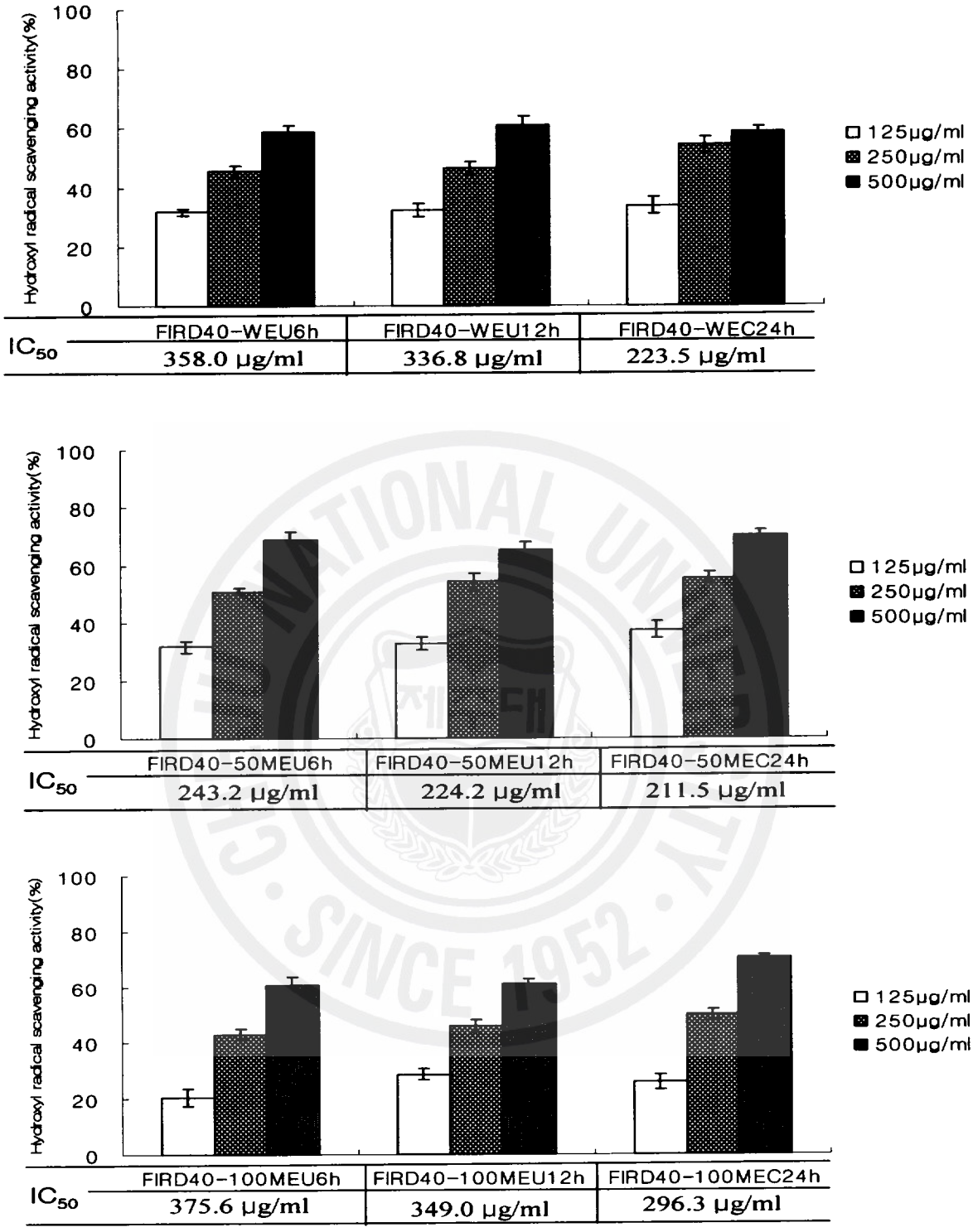

Fig. 3-2. Hydroxyl radical scavenging activities of extracts using UE and CE from *E. cava* dried by FIRD at 40℃. Abbreviations are the same as in Fig. 3-1.

Mean±SE of determinations was made in duplicate experiments.

3.5. Alkyl radical scavenging assay

The alkyl radical spin adduct was observed when AAPH was incubated with the spin trap 4-POBN at 37℃ for 30 min. The scavenging activities of all the extracts by UE and CE from *E. cava* on alkyl radical are shown in Fig. 3-3. All the tested extracts showed strong alkyl radical scavenging activity. Among them, the 100% methanolic extract by UE 12 h from *E. cava* exhibited the highest scavenging activity (IC_{50} 1.8 μg/ml). Also at the same condition, the 100% methanolic extracts showed higher alkyl radical scavenging activity than 50% methanolic extracts. In case of UE, hydroxyl radical scavenging activity was increased as the ultrasonic extraction time was increased. Moreover, all the extracts by UE 6 and 12 h showed higher alkyl radical scavenging activity than the extracts by CE 24 h. In addition, the scavenging activity of those extracts increased with increasing concentrations from 1.25 μg/ml to 12.5 μg/ml.

3.6. Hydrogen peroxide scavenging assay

The scavenging activities of the all extracts by UE and CE from *E. cava* on hydrogen peroxide are shown on Fig. 3-4. All the extracts showed good hydrogen peroxide scavenging activity. Among them, the 100% methanolic extract by UE 12 h from *E. cava* exhibited the highest scavenging activity (IC_{50} 25.4 μg/ml). And at the same condition, the 100% methanolic extracts showed higher hydrogen peroxide scavenging activity than the 50% methanolic extracts. In case of UE, hydrogen peroxide scavenging activity was increased as the ultrasonic extraction time was increased. Moreover, all the extracts by UE 12h showed higher alkyl radical scavenging activity than extracts by CE 24h. On the other hand, the extracts by UE 6h showed lower alkyl radical scavenging activity than extracts by CE 24h. In addition, the scavenging activity of those extracts increased with increasing concentrations from 20 μg/ml to 90 μg/ml.

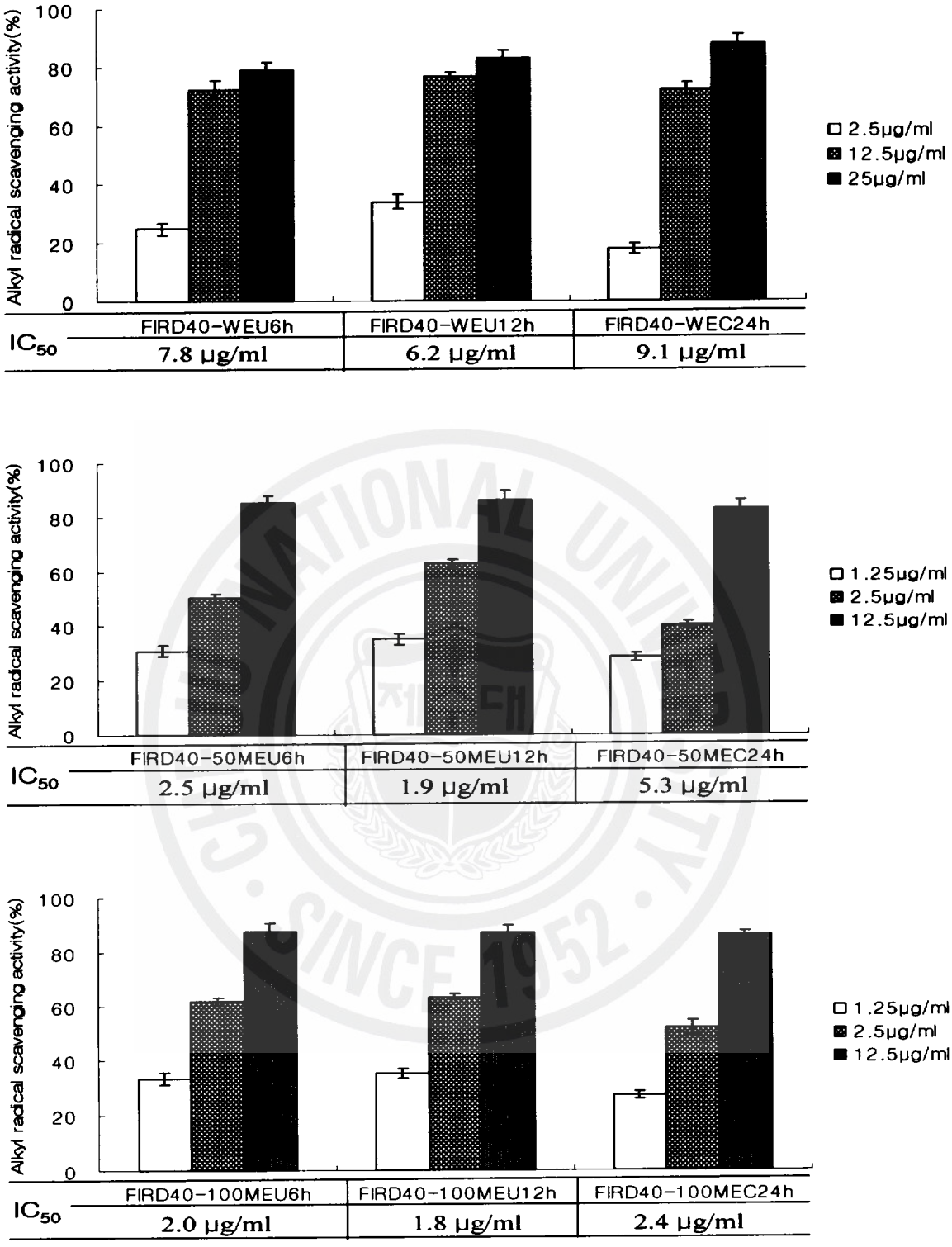

Fig. 3-3. Alkyl radical scavenging activities of extracts using UE and CE from *E. cava* dried by FIRD at 40℃. Abbreviations are the same as in Fig. 3-1.

Mean±SE of determinations was made in duplicate experiments.

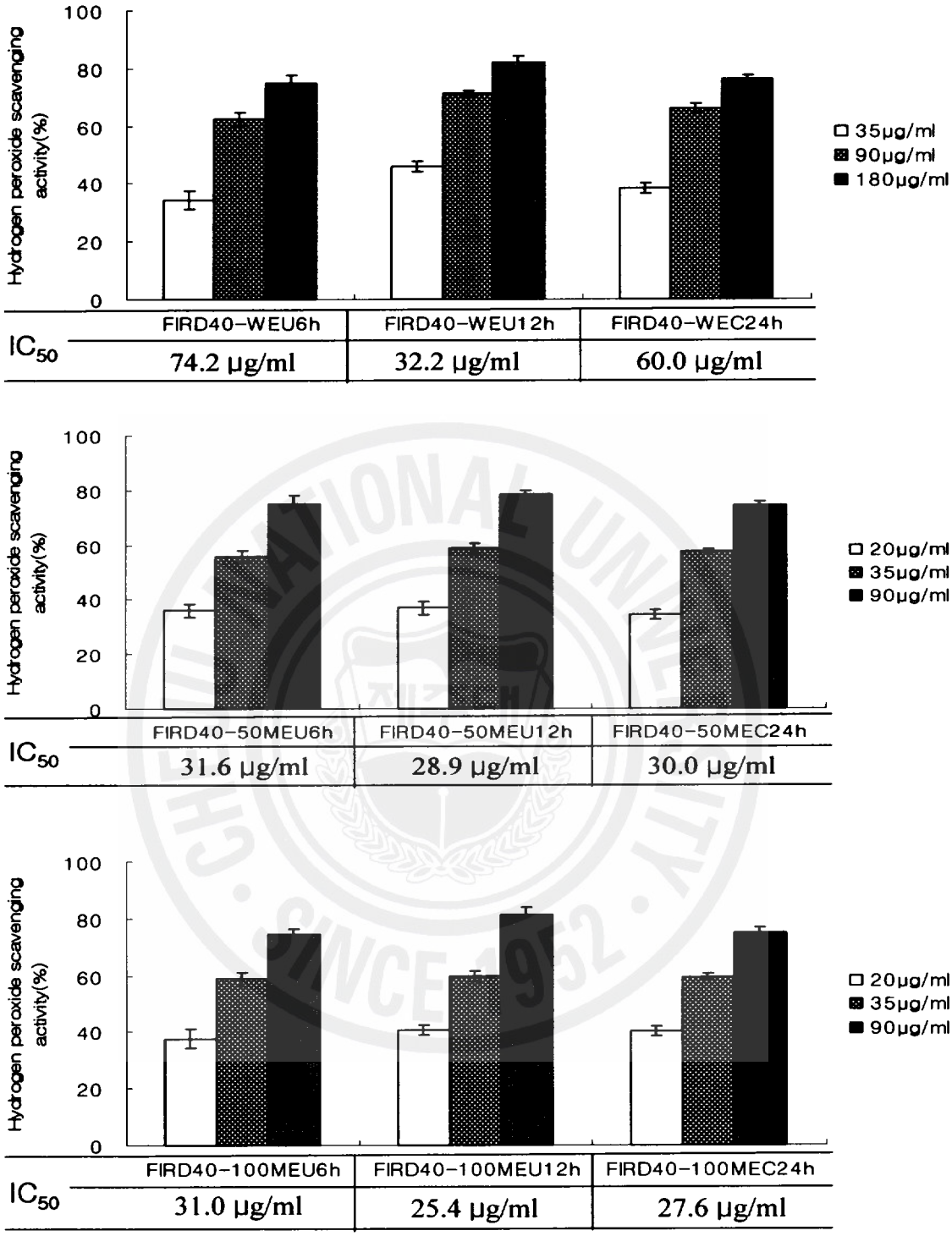

Fig. 3-4. Hydrogen peroxide scavenging activities of extracts using UE and CE from *E. cava* dried by FIRD at 40℃. Abbreviations are the same as in Fig. 3-1.

Mean±SE of determinations was made in duplicate experiments.

3.7. Inhibitory effect of DNA damage of H_2O_2-induced cells

Inhibitory effect of the 100% methanolic extracts by UE 12h (100MEU-12h) and CE 24h (100MEC-24h) against H_2O_2-induced DNA damage was investigated using comet assay (Fig. 3-5 and 6). The percent fluorescence in DNA tail intensity of L5178 cells was significantly increased in cells treated with only H_2O_2. This level of DNA damage induced by H_2O_2 was significantly controlled dose-dependently by preincubating H_2O_2 together with 100MEU-12h and 100MEC-24h at the concentrations of 25, 50 and 100 μg/ml in PBS (Fig. 3-5). At the same concentrations, 100MEU-12h exhibited slightly higher protective effect H_2O_2-induced DNA damage than 100MEC24h. Fig. 3-6 showed photomicrographs of different DNA migration profiles obtained from L5178 cells, with the presence of different concentrations of 100MEU-12h (Fig. 3-6 C~E) and 100MEC-24h (Fig. 3-6 F~H). In the cells exposed to only H_2O_2, the DNA was completely damaged but the addition of 100MEU-12h and 100MEC-24h with H_2O_2 effectively suppressed DNA damage. Especially, 100MEU-12h at 100 μg/ml showed highest inhibitory effect of DNA damage.

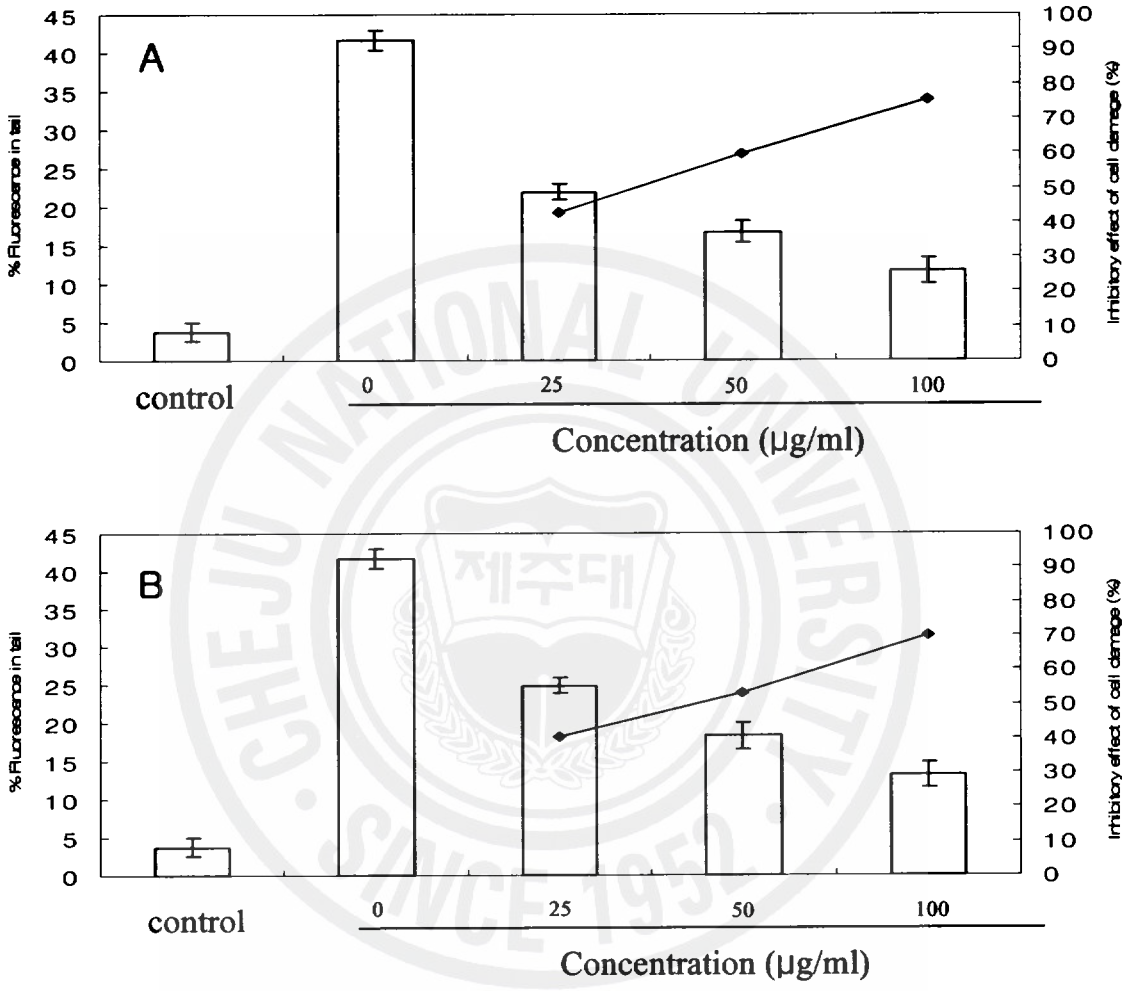

Fig. 2-5. The effect of supplementation in vitro with different concentrations of FIRD40-100MEC24h and FIRD40-100MEU12h on DNA damage of H_2O_2-induced L5178 cell. (A) FIRD40-100MEU12h, (B) FIRD40-100MEC24h. Mean±SE of determinations was made in duplicate experiments. (□ : % Fluorescence in tail, -◆- : Inhibitory effect of cell damage)

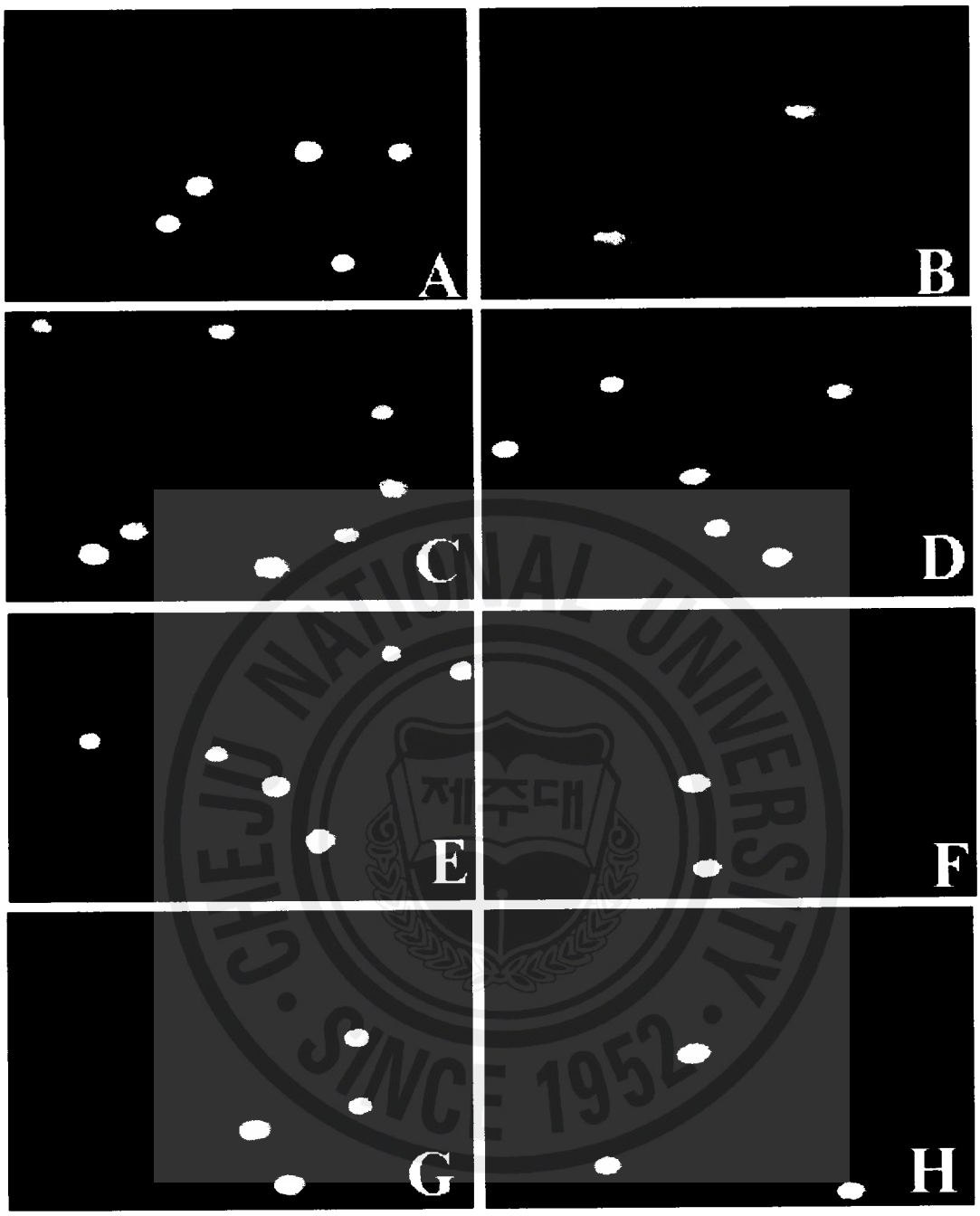

Fig. 2-6. Comet images of L5178 cells: (A) negative control; (B) 50 uM H_2O_2 (C) 25 ug/ml FIRD40-100MEU12h + 50 uM H_2O_2 (D) 50 ug/ml FIRD40-100MEU12h + 50 uM H_2O_2 (E) 100 ug/ml FIRD40-100MEU12h + 50 uM H_2O_2 (F) 25 ug/ml FIRD40-100MEC24h + 50 uM H_2O_2 (G) 50 ug/ml FIRD40-100MEC24h + 50 uM H_2O_2 (H) 100 ug/ml FIRD40-100MEC24h + 50 uM H_2O_2

4. Discussion

Conventional extraction methods brought the consumption of large amount of solvent and long extraction time (Yan et al., 1999; Yu, Vasanthan and Temelli, 2001). Nevertheless, extraction of bio-compounds from plant is still more widely conventionally performed. Other techniques which include supercritical carbondioxide extraction, subcritical water extraction, ultrasonic extraction, microwave extraction have also become of interest as alternatives for the conventional methods. Among these, ultrasonic extraction (UE) is the extraction of organic compounds contained within the body of plants and seeds by a solvent is significantly improved. The mechanical effects of ultrasound provide a greater penetration of solvent into cellular materials and improves mass transfer. There is an additional benefit for the use of power ultrasound in extractive processes which results from the disruption of biological cell walls to facilitate the release of contents (Mason, Paniwnyk and Lorimer, 1996).

Seaweeds that can be divided into three basic types: brown (Phaeophyta), red (Rhodophyta), and green (Chlorophyta) seaweeds are known to be especially rich in components such as fucoxanthin, catalase and polyphenol-containing phlorotannins that have antioxidant effects. Among them, *Ecklonia cava* has become good candidate for the source of natural antioxidants revealed by a number of recent studies (Heo et al., 2005b; Kim et al., 2006a; Ahn et al., 2007). Thus, in the present study, the water and the methanolic extracts obtained by UE from far infrared radiation dried *E. cava* were investigated for extraction yield, total polyphenol content and free radical scavenging activity, as well as inhibitory effect of H_2O_2-induced DNA damage.

According to the extraction yield and total polyphenolic content results (Table 3-1 and 2), extraction yield and total polyphenolic content increased as the ultrasonic extraction time was increased. All the extracts by UE exhibited higher extraction yield than CE. The water extract

by ultrasonic extraction treated for 12h showed improvement of 6% yield as compared with conventional extraction treated for 24h. On the other hand, in case of total polyphenolic content, all the extracts by CE exhibited slightly higher total polyphenolic content than UE. The results indicate that in both cases, the extraction yield and total polyphenol content is highly time-dependent. It has been reported that in almost all of the ultrasonic cases the amount of extract is similar or greater compared with the conventional technique. The ultrasonic procedure thus seems to be a significant improvement when extraction time is taken into account (Vinatoru et al., 1997). The antioxidative effect of all the extracts by UE and CE from *E. cava* on free radical scavenging and H_2O_2-induced DNA damage are shown in Fig. 3-1~6. In case of extracts by UE, free radical scavenging activities increased as the ultrasonic extraction time was increased. All the extracts by CE showed higher DPPH and hydroxyl radical scavenging activity than UE. But, in case of alkyl radical and hydrogen peroxide, extracts by UE showed higher scavenging activity than CE. In addition, 100MEU12h showed good inhibitory effect of H_2O_2-induced DNA damage than 100MEC24h. Many researchers have reported positive correlation between free radical scavenging activity and total polyphenolic compound. Oki et al. (2002) observed that the radical scavenging activity increased with the increase of polyphenolic compound content. In this study, all the extracts by UE from *E. cava* have low amount of total polyphenol content as compared with CE. Nevertheless, all the extracts by UE from *E. cava* showed higher alkyl radical, hydrogen peroxide scavenging activity and inhibitory effect of H_2O_2-induced DNA damage than CE. It is thought that another materials in the extracts by UE from *E. cava*, such as small molecular weight polysaccharide, protein or pigments, probably influence the activity. According to the previous reports, ultrasonically assisted extraction can be applied to the production of medicinal compounds from Chinese plants, protein from soya bean and polysaccharides from longan fruit pericarp (Zhao, Bao and Mason, 1991; Wang, 1981; Moulton

and Wang, 1982; Yang et al., 2007).

Two extraction techniques for antioxidative effect on radical scavenging and H_2O_2-induced DNA damage in the far infrared radiation dried *E. cava* were investigated and compared. UE showed higher yield and alkyl radical, hydrogen peroxide scavenging activity and inhibitory effect of H_2O_2-induced DNA than CE. The UE technique was shown to be very efficient in the extraction of *E. cava*. Therefore, the application of sonication method can be useful in the extraction industry of seaweeds.

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