Thesis for the Degree of Master of Engineering

Characterization of Dried Red Pepper (*Capsicum annuum*) Oil Extracted by Supercritical Carbon Dioxide

by

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(초임계 이산화탄소를 이용하여 추출한 건고추 오일의 특성에 관한 연구)

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A dissertation

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요 약

고추 (*Capsicum annuum*) 는 식생활에서 가장 많이 사용되는 조미 채소류로서 양념류, 김치류, 고추장 등의 가공뿐만 아니라 고춧가루로 만들어져 식품첨가용 향신료로 사용되고 있으며, 한국의 고춧가루 소비량은 국민 일인당 연간 2.0~2.5 kg에 이른다. 이는 주요 고추 소비국인 헝가리 200 g, 미국 50 g, 일본 20 g과 비교하여 평균 40~100배로 높아 한국이 세계에서 가장 많은 고추를 소비하고 있다. 일반적으로 고추는 수분함량이 많아 수확 후 건조, 분쇄하여 사용되며, 홍고추의 경우 수확 후 태양건조와 열풍건조 또는 이 두가지 방법을 병행한 방법으로 수분함량 15% 정도로 건조하여 저장 유통되고 있다. 고추의 주요 성분인 capsaicin, capsanthin, capaorubin, vitamin, 유기산 중에서 capsaicin이 매운맛을 나타내는 주성분인 것으로 알려져 있다. Capsaicin의 효능에 대한 연구로 체액성면역 증진과 암유전자 발현 조절작용 및 항성인병 작용, 에너지 대사율 증진, 살균작용, 3T3-L1 cells에서 세포자살 유도와 지방생성억제 등의 효과가 있음이 보고되었고, 이로 인하여 고추는 화장품, 의약품 및 기능성 식품 소재로서 높은 가치를 지닌다. 이산화탄소는 낮은 임계점과 무독성, 그리고 용매의 사용량이 적고 부식성이 없어 친환경적이기 때문에 가장 널리 이용되는 초임계 유체이며, 기존의 추출법이 가지는 어려움을 해결할 수 있는 새로운 혁신기술로서 주목 받고 있다.

본 연구에서는 친환경 공정인 초임계 이산화탄소 (SC-CO_2) 추출법과 hexane을 이용한 유기용매 추출법을 이용하여 상품성 있는 고추와 상품성 없는 고추로부터 오일을 추출 하였으며, 추출 조건이 capsaicin 함량

및 지방산 조성, 항산화 및 항균 효과에 어떠한 영향을 미치는지 알아보고자 하였다. SC-CO_2 추출은 SFE/SFC system (JASCO, Japan)을 이용하여 온도를 40℃로 고정하고, 압력조건은 150-300 bar에서 시행하였으며, 이산화탄소 유량은 2 mL/min로서 총 60분 동안 일정하게 흐르게 하였다. 추출 오일의 capsaicin 함량은 HPLC를 사용하여 측정하였으며, 측정 결과를 바탕으로 최적 추출 압력을 200 bar로 설정하였으며, 실험실 수준의 초임계 이산화탄소 추출 장치로 더 많은 양을 추출하여 항산화 및 항균 활성을 측정하였다. 오일의 지방산 분석에는 gas chromatography (GC)를 이용하였고, 특히 linoleic acid 함량이 상품성 있는 고추 오일에서 상품성 없는 고추 오일에 비하여 높게 나타났으며, SC-CO_2 추출 오일에서 hexane 추출 오일에 비하여 높게 나타났다. 산화 안정성 및 항산화 효과를 측정한 실험에서도 상품성 있는 고추 오일이 상품성 없는 고추 오일에 비하여 대체적으로 효과가 뛰어 났으며, 항균활성을 측정한 결과 또한 상품성 있는 고추 오일에서 효과가 가장 뛰어남을 확인할 수 있었다. 대부분의 실험에서 상품성 없는 고추 오일보다는 상품성 있는 고추 오일이, hexane 추출 오일보다는 SC-CO_2 추출 오일이 항산화 및 항균 활성 효과가 높게 나타났으며, 상품성 좋은 고추의 SC-CO_2 추출 오일이 가장 항산화 및 항균 활성 효과가 높은 것으로 나타났다. 이와 같은 결과를 통하여 고추로부터 기능성 오일을 추출함에 있어 hexane 추출 등과 같은 기존 재래식 유기용매 추출 공정에 비하여 SC-CO_2 추출법을 사용하는 것이 더 바람직하다는 것을 알 수 있었으며, 항산화 및 항균활성을 나타내는 고추 추출 오일의 화장품, 의약품 및 기능성 식품 소재로서 응용에 도움이 될 것으로 기대된다.

Introduction

Peppers are the fruits of plants from the genus *Capsicum* and belong to the family *Solanaceae*. There are several domesticated species of chili peppers, among them *Capsicum annuum, C. frutescens* and *C. chinense*, which include many common varieties (Zeid et al. 2011). *Capsicum annuum* L. has been used worldwide as chili peppers, vegetables, in folk medicines, and also as a source of food additives (Kyoko et al. 1999). *Capsicum annuum* L. is regarded as a healthy vegetable that possesses phytochemicals including carotenoids, ascorbic acid, tocopherol, flavonoids, and phenolic compounds, as well as natural food colorants (Jeong et al. 2006 and Al-Duais et al. 2009). Generally, their taste is representing a hot sense consisting of capsaicinoids as the major group of organic compounds closely related to the family of alkaloids (Pruthi J.S. 1976). Traditionally in Korea, pungent red pepper has been only used as a spice, fermented paste, and main ingredient of the fermented vegetable, (e.g., kimchi) (Ji-Sun Kim et al, 2011). Depending on the progress of studies about the efficacy of the pepper, the extracts of red pepper used as functional foods and pharmaceuticals is increasing.

Red pepper contains phenolic compounds such as ascorbic acid, casaicinoids, capsanthin, casorubin, cryptocapsin, quarcetin and luteolin (Manjeshwaret al.

2003), many of which have been reported as being antioxidants (Hasler et al. 2000). More than 20 capsaicinoids, differing only in the fatty acid structures, have been described (Thiele et al. 2008). Capsaicin (8-methyl-N-vanillyl-6-noneamid) is the most pungent of a group of compounds called capsaicinoids that can be isolated from hot peppers (*Capsicum annuum* L.) (Kwon et al. 2011). Capsaicin has been used in neurological research to stimulate sensory nerves and also to treat bladder inflammation. It is also found in topical ointments used for arthritis and neuralgia (Kaale et al. 2002), and exerts its effect on the sensory nerves by interacting with the vanilloid receptor, promoting the release of substance P as well as other cytokines (Surh et al. 2005).

The use of supercritical fluid extraction (SFE) offers numerous potential advantages over conventional extraction processes, such as reduced extraction time, reduced organic solvent volume, and more selective extractions (L.T. Taylor, 1996). Supercritical fluids have a relatively high liquid-like density as well as a relatively low viscosity and high diffusivity (Q. Lang and C.M. Wai, 2001). These properties provide a unique solvent that is both effective at dissolving materials as well as penetrating solid matrices.

Supercritical carbon dioxide (SC-CO_2), in particular, is an attractive supercritical solvent, low critical temperature (31.1℃), and the fact that it is both non-toxic and inert. Because of these properties, SC-CO_2 can be useful when

applied on food and pharmaceutical industries (Seung-Mi Lee, 2012). In recent years, the use of SFE for the removal of organic compounds from different liquid and solid matrices has attracted much attention. This technique has some advantages over more conventional separation techniques, largely due to the unique physical properties of supercritical fluids.

Red peppers have a high lipid content on account of seed, thus being a high source of nutritional energy. Also, high capsaicin contents in red pepper have functionalities. The functional matters including capsaicin are very sensitive, especially to heat. Using SC-CO_2 is better than hexane to extract oil containing functional matters from red pepper as SC-CO_2 extraction is performed at low temperature. In this study, the SC-CO_2 extraction was performed at ranges of pressure (150-300 bar) and constant temperature (40℃). The hexane extraction was performed for 12 h at 40°C for comparing.

The aim of this study was to extract oil from red pepper (*Capsicum annuum)* by SC-CO_2 with functional matters, and to analyze the capsaicin contents of extracts at different extraction conditions. And then, through the best condition for extraction using supercritical carbon dioxide and organic solvents, the effect for antioxidant activity and antimicrobial activity were investigated. In addition, the effect of merchantable quality was investigated.

Materials and Methods

1. Materials

The dried red pepper (*Capsicum annuum*) was collected from geochang-gun, Gyeongsangnam-do. Carbon dioxide (99.99 % pure) was supplied by KOSEM (Yangsan, Republic of Korea). Standards of capsaicin, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), FAME mix, ascorbic acid, gallic acid, catechin and Mueller-Hinton agar were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A). Microorganisms were supplied by Korean Culture Center of Microorganism (KCCM). All other reagents used in different analysis were analytical or HPLC grade.

2. Sample preparation

Red pepper samples were crushed in a mechanical blender (PHLIPS HR 1727 Mixer) and sieved by a mesh. Red pepper samples were used 500 μm and 700 μm mesh. The sieved samples were then stored at -20°C until using for supercritical carbon dioxide (SC-CO_2) and organic solvent extraction.

Table 1. Proximate compositions of dried red pepper

Composition (%)	Dried Red pepper
Moisture	25.52
Ash	8.75
Protein	35.41
Lipid	30.32

* Mean value of two replicates.

3. Method

3.1 Supercritical carbon dioxide extraction

The SFE/SFC system (JASCO, Japan) was used for extracting oil from red pepper. The flow diagram of the equipment is shown in Fig. 1. The sample, 5 g, was packed into the extraction vessel which was 10 mL in volume. A filter was placed at the top of the extraction vessel. Liquefied carbon dioxide was pumped to the extraction vessel up to the desired pressure which was regulated by a back-pressure regulator. The pressure of CO_2 was automatically maintained by the pump and computer program. The temperature of vessel was maintained heat by oven. Oil extraction by SC-CO_2 was performed at temperature of 40℃ and pressure of 150-300 bar. The total extraction time was 60 min and the flow rate of CO_2 was kept constant at 2 mL/min for all extraction conditions. The extracted oil was collected on the glass collect vessels. After SC-CO_2 extraction, the extracted oil was then stored at -20℃ until further analysis. The condition of extracts was shown in Table 2.

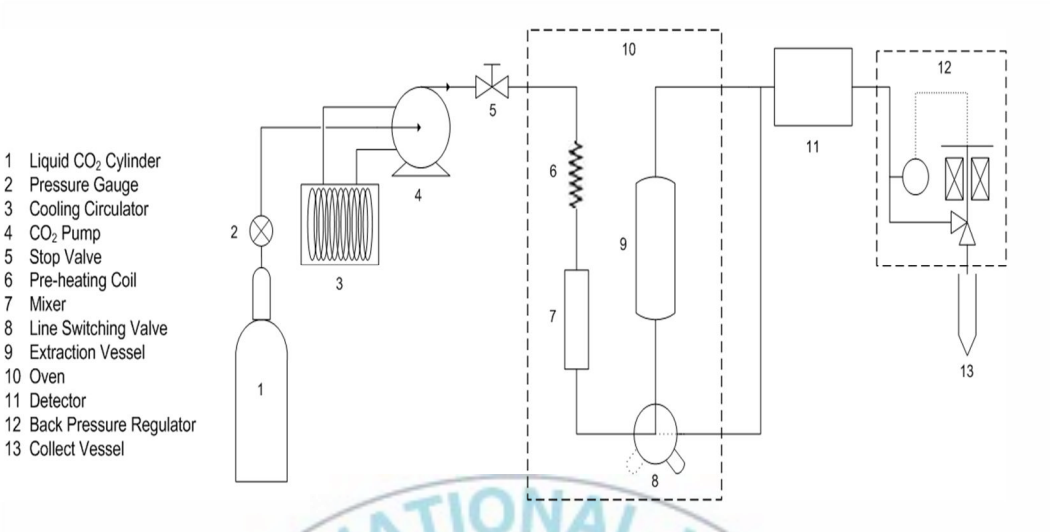

Fig. 1. Schematic diagram of supercritical carbon dioxide extraction.

Table 2. Operating conditions used in experiments of SC-CO_2 extraction

Parameter	Conditions
Sample	Red pepper (5 g)
Solvent	Carbon dioxide
Temperature	40℃
Pressure	150 bar, 200 bar, 250 bar, 300 bar
Operating time	60 min
Flow rate of carbon dioxide	2 mL/min

3.2 Organic solvent extraction

The extraction was carried out with Magnetic Stirring Extraction (MSE) using hexane according to Juangsamoot et al. (2012) with slight modification. 100 g of red pepper sample were macerated using magnetic stirring with 800 mL hexane into the beaker and stirred 12 h at 40°C and 400 rpm. The extracts were centrifuged at 3000 rpm for 10 min and then filtered through a CHMLAB No. F1093-125 filter paper. The filtered extract was evaporated in a rotary vacuum evaporator at 40°C. The extracted oil was collected and stored at -20°C until further analysis.

3.3 Analysis of capsaicin by HPLC

Capsaicin was analyzed by high-performance liquid chromatography (HPLC), according to Carla et al. (2004) and Juan et al. (2005) with slight modification. The HPLC analysis was carried out with a Waters HPLC equipped with a model 600E system controller, a model 484 UV/VIS detector and a XTerra® MS C18 column (5 μm, 4.6 x 250 mm, Waters, USA). The mobile phase was acetonitrile: water (70:30, v/v) and the flow rate was 1 mL/min. Capsaicin was detected at 280 nm. The amount of capsaicin in the extract was measured based on the peak area of standard capsaicin (purity > 99.0 %). Before injection, supercritical carbon dioxide extracts (20 μL) were dissolved in acetonitrile (20 mL) and the injection volume was 20 μL.

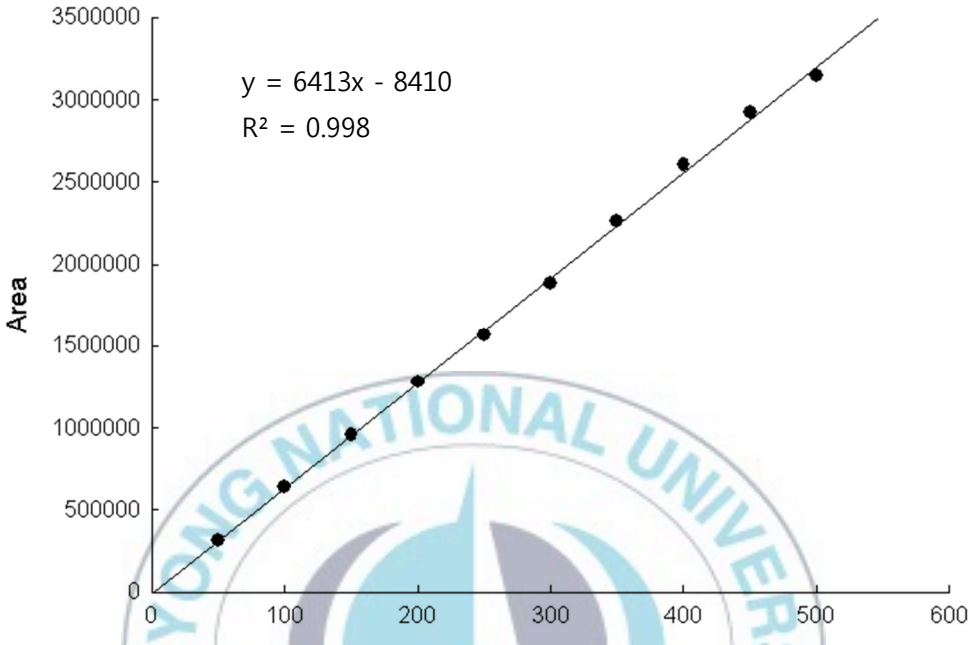

Fig. 2. Calibration curve of capsaicin.

3.4 Supercritical carbon dioxide extraction with laboratory scale

The set up of a SFE process of laboratory scale is shown in Fig. 3. The red pepper sample (200 g) was packed into the stainless steel extraction vessel which was 500 mL in volume. A thin layer of cotton was placed at the bottom of the extraction vessel. Before plugging with cap, another layer of cotton was used at the top of the sample. Liquefied carbon dioxide was pumped into the extraction vessel by high pressure pump (MILROYAL, MILTON ROY, USA) up to the desired pressure. A back pressure regulator was used to control the pressure of carbon dioxide. The extraction temperature was maintained by connecting the extraction vessel with water bath. Oil extraction by SC-CO_2 was performed at temperature of 40℃ and pressure of 200 bar. The flow rate of carbon dioxide was kept constant at 23 g/min for all conditions. The extracted oils were then stored at -20 °C until further analysis.

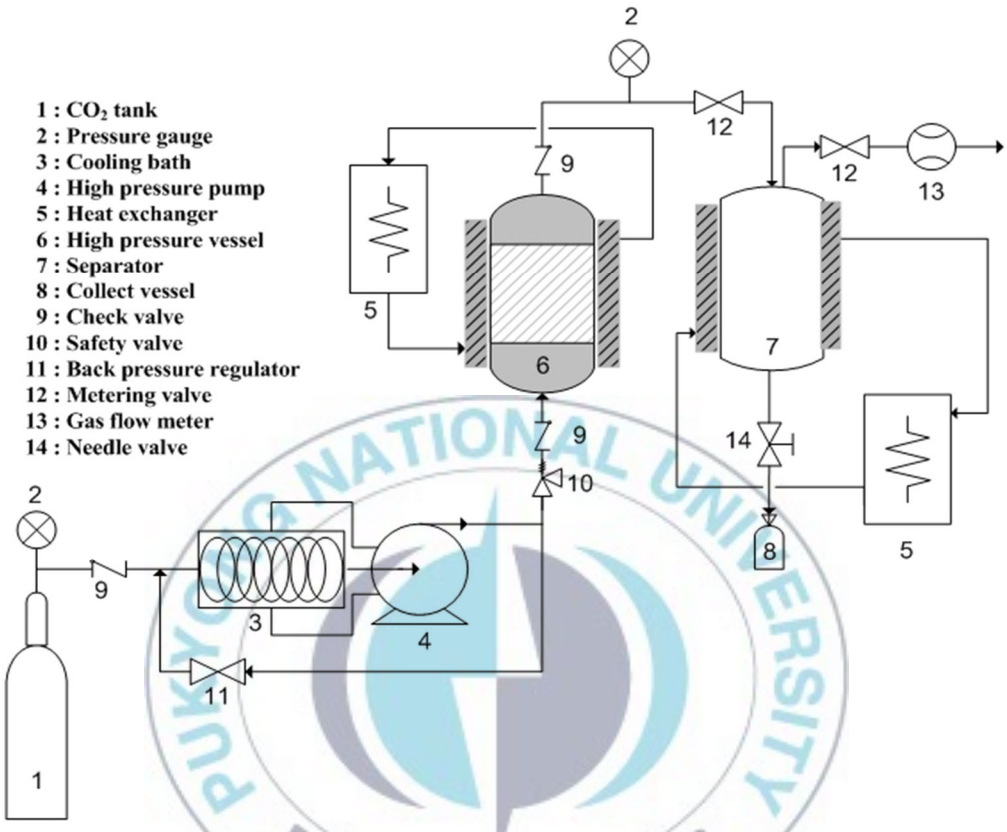

Fig. 3. Schematic diagram of SFE process of laboratory scale.

Table 3. Condition of extracts of red pepper samples

Sample form	Condition		
	Red pepper	Extraction solvent	Temperature
S-RP 500	Merchantable quality 500 μm	$SC\text{-}CO_2$ (200 bar)	40 ℃
S- RP 700	Merchantable quality 700 μm		
S-NRP 500	No merchantable quality 500 μm		
S-NRP 700	No merchantable quality 700 μm		
H- RP 500	Merchantable quality 500 μm	Hexane (MSE*)	
H- RP 700	Merchantable quality 700 μm		
H-NRP 500	No merchantable quality 500 μm		
H-NRP 700	No merchantable quality 700 μm		

* MSE : Magnetic Stirring Extraction.

3.5 Measurement of color

Color of the extracted oils was measured using a spectrophotometer (Lovi bond, U.K). Color of the powder before and after SC-CO_2 extraction also measured. For measurements, samples were placed in white cup and covered with optical glass and CIE L*a*b* color coordinates (considering standard illuminant D65 and observer 10) were calculated. L* value is lightness, a* value is redness and b* value is yellowness.

The range of L* value is 0 to 100 and it closer to 100, the sample is more bright. The color of the sample close to red, if the redness has positive value, and it close to green, if the redness has negative value. Also, the color of the sample close to yellow, if the yellowness has positive value, and it close to blue, if the yellowness has negative value.

3.6 Analysis of fatty acid composition by GC

The fatty acid compositions of extracts of red pepper obtained by SC-CO_2 extraction and organic solvent, hexane extraction were determined by gas chromatography using a Hewlett Packard gas chromatograph (6890 Series II GC system). The fatty acid methyl esters were prepared firstly according to AOCS official method Ce 2-66 (AOCS, 1998) and then separated using an Agilent Supleco fused silica capillary column (100.0 m × 0.25 μm × 0.20 μm). GC conditions for the detection of fatty acids were shown in Table 4. Fatty acid methyl esters were identified by comparison of retention time with standard fatty acid methyl esters mixture.

Table 4. GC conditions for the detection of fatty acids

Parameter	Conditions
Instrument	Agilent 6890N
Split	Splitless
Inject temperature	250 ℃
Detect temperature	260 ℃
Carrier gas flow	He, 1 mL/min
Oven time	140℃ (5 min) → 4℃/min → 250℃ (15 min)
Column	Agilent supleco fused silica capillary column

3.7 Free fatty acid contents (FFA)

FFA of extracted oil from each sample was analyzed as described by Benardez et al. (2005). Briefly, 50 mg of each extract was placed into pyrex tubes with the addition of 3 mL of cyclohexane and then 1 mL of cupric acetate-pyridine reagent was added. Tubes were vortexed for 30 sec. After centrifugation at 2000 g for 10 min, the upper layer was read at 710 nm with with UV-spectrophotometer (UVmini-1240, SHIMADZU CORPORATION, Japan). The FFA content of oil was measured on a calibration curve constructed from oleic acid standard. Copper reagent was prepared according to Lowry and Tinsley (1976). 5 % (w/v) aqueous solution of cupric acetate was prepared and filtered. Then the pH of cupric acetate solution was adjusted to 6.1 using pyridine.

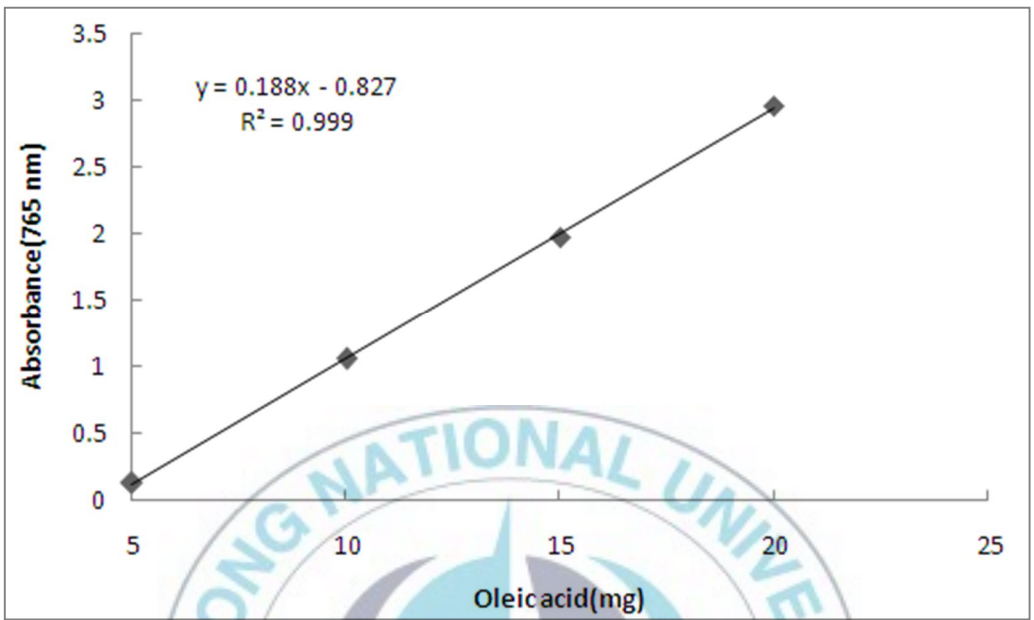

Fig. 4. Calibration curve of oleic acid for estimation of FFA contents.

3.8 Antioxidant activity of extracted oil

3.8.1 Analysis of total phenolic contents (TPC)

Total phenolic content of extracts was determined by using Folin-Ciocalteu assay according to Conforti et al. (2007) and Marinova et al. (2005) with slight modification. 50 mg of each extracts was weighed into the plastic tube and voltexed with 25 mL of extraction solution (40 mL of acetone : 40 mL of methanol : 20 mL of water : 0.1 mL of acetic acid). Then, the plastic tube was heated at 60℃ (water bath) for 1 h and after cooling to room temperature, homogenized for 30 s. 200 μL were introduced into test tube, 1.0 mL of Folin-Ciocalteu's reagent (FCR) were added. After 4 min, 1.0 mL of sodium carbonate solution (7.5% w/v) was added. Then, the mixture was allowed to stand at room temperature in dark environment for 2 h. The absorbance of mixture was read at 726 nm with UV-spectrophotometer (UVmini-1240, SHIMADZU CORPORATION, Japan). The measurements were carried out in duplicate and gallic acid was used for calibration of standard curve.

3.8.2 Analysis of total flavonoid contents (TFC)

Total flavonoid content of extracts was determined by using aluminum chloride colorimetric method according to Abu et al. (2011) and Marinova et al. (2005) with slight modification. 250 μL of each extract was mixed with 1 mL of distilled water and 75 μL of sodium nitrite solution (5% $NaNO_2$). After 6 min, 75 μL of aluminum trichloride solution (10% $AlCl_3$) was added and then added 1 mL of sodium hydroxide solution (4% NaOH) to the mixture. Immediately, water was added to bring the final volume to 2.5 mL, and then the mixture was thoroughly mixed and allowed to stand for 15 min at room temperature. Absorbance was measured at 510 nm with UV-spectrophotometer (UVmini-1240, SHIMADZU CORPORATION, Japan). The measurements were carried out in duplicate and catechin was used for calibration of standard curve.

3.8.3 DPPH radical scavenging activity

The DPPH radical scavenging capacity of extracts was determined based on the method described Williams et al. (1995) and Nihal et al. (2005) with some modifications. 20 mg of each sample was put into the plastic tube added 5 mL of methanol and heated in the water bath for 10 min. After 5 min, voltexed and cooling to room temperature. Then, the solution were centrifuged at 3000 rpm for 10 min and used supernatant as sample. 1.5 mL of 0.1 mM DPPH radical in methanol was added to a test tube with 0.5 mL of sample. Instead of sample, pure methanol was used as blank. The mixture was vortexed and let to stand at room temperature in the dark for 60 min. Absorbance was measured at 517 nm with UV-spectrophotometer (UVmini-1240, SHIMADZU CORPORATION, Japan). Ascorbic acid in methanol (10,000 ppm) was used for control and methanol was used as the blank. The percentage of DPPH radical scavenging capacity was calculated using this equation:

$$\text{Scavenging effect (\%)} = [1-(A_{517\ nm,\ sample}/A_{517\ nm,\ blank})] \times 100\ \%$$

All tests were carried out in triplicate.

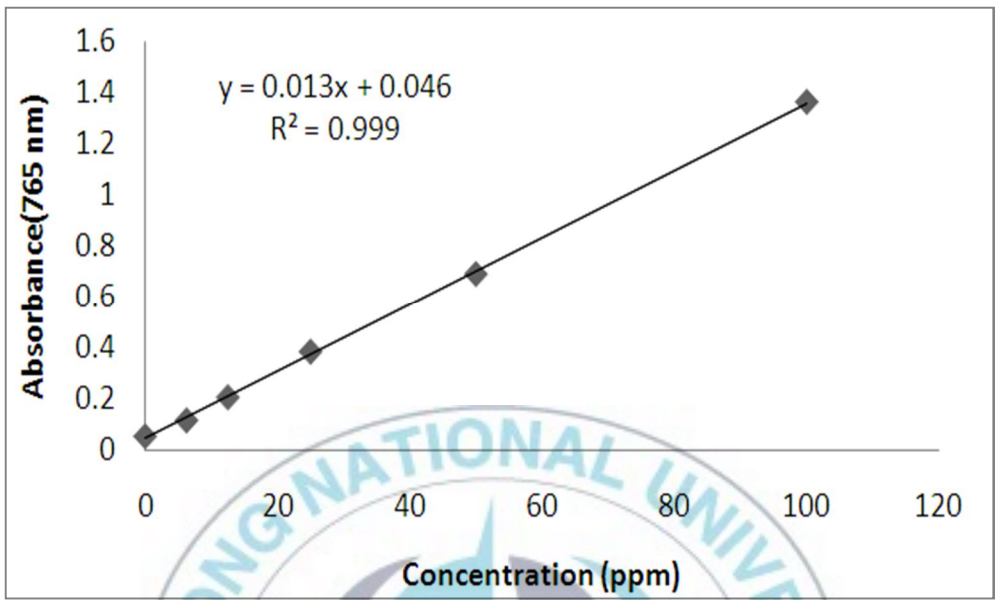

Fig. 5. Calibration curve of gallic acid for estimation of TPC.

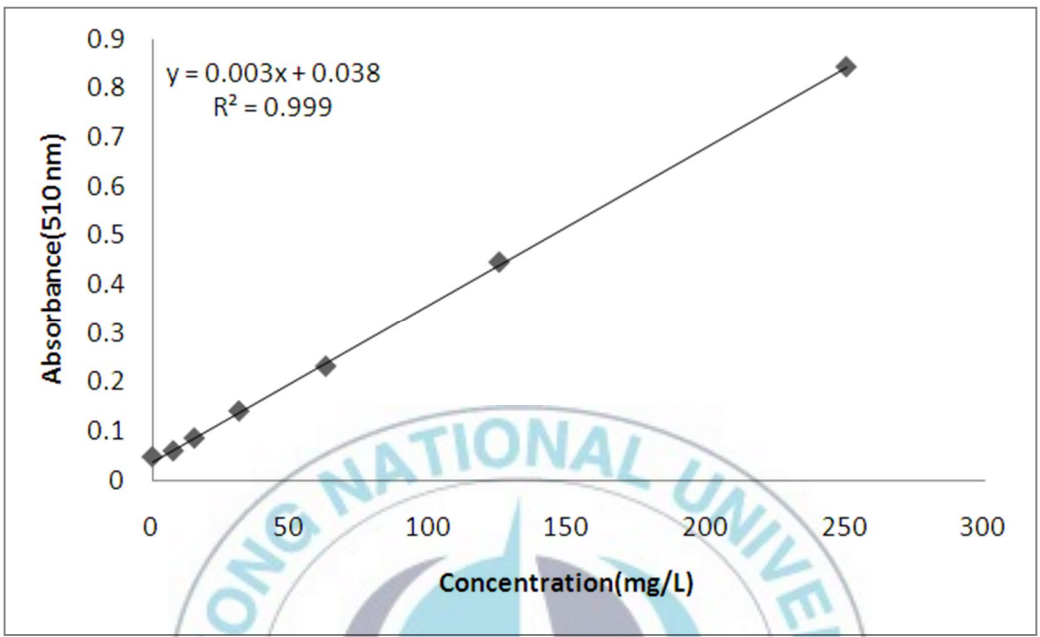

Fig. 6. Calibration curve of catechin for estimation of TFC.

3.9 Antimicrobial activity of extracted oil

3.9.1 Paper disc diffusion assay

The antimicrobial activities of the oils were determined by paper disc diffusion assay according Conner et al. (1984) and Hun et al. (1994) with slight modification. Shown in Table 5, six microorganisms were used in this study. All strains were obtained from the Korean Culture Center of Microorganisms (KCCM). McFarland standard No. 0.5 was used in the preparation of suspension of microorganism. The turbidity of bacterial suspension was adjusted according McFarland standard. The accurate turbidity of the bacterial suspension was confirmed by UV-spectrophotometer (UVmini-1240, SHIMADZU CORPORATION, Japan) on 625 nm with approximate cell density of each bacterial strain was 10^7 CFU/mL. Mueller-Hinton agar was used as growth medium for bacterial strains and sterilized at 121 ^{o}C for 15 min. The agar was poured into sterile petri dishes and after stiff agar forming, bacterial suspension was spread on the agar surface using sterile cotton. Then, Advantec paper disc (10 mm) contains extracts or control (methanol) were then placed in the middle of the plates and incubated for 24 h at 37℃ and the diameter of each inhibitory zone was measured (mm).

3.9.2 Minimum inhibition concentration (MIC) assay

The values of Minimum Inhibitory Concentration (MIC) were measured against bacteria tested according EUCAST (2000) and Baroty et al. (2010) with slight modification. It was performed with extracts on different concentration (10,000, 5,000 and 2,500 µg/mL). Sterile Mueller-Hinton agar was poured into petri dishes and allowed for 30 min to change solid state. The inoculums of bacteria spread on the surface of agar with cotton. After then, holes of 6.0 mm diameter were punched on the agar to create wells, wherein the certain concentration of extract was placed there. The MIC was the lowest concentration that inhibited the microorganisms, with an inhibition zone greater than 6.0 mm after 24 h incubation period at 37ºC.

Table 5. Microorganisms and culture medium used for antimicrobial activity tests

Microorganisms	Strain	Medium
Gram positive		
Bacillus cereus	KCCM 40022	Nutrient agar
Listeria monocytogenes	KCCM 40307	Nutrient agar
Staphylococcus aureus	KCCM 40050	Nutrient agar
Gram negative		
Escherichia coli	KCCM 11234	Nutrient agar
Pseudomonas aeruginosa	KCCM 11803	Nutrient agar
Salmonella typhimurium	KCCM 11862	Nutrient agar

Results and Discussion

1. Oil yield of red pepper oils by SC-CO_2

The total amount of oil obtained by SC-CO_2 shown in Fig. 7. The total amount of oil in red pepper of merchantable quality was higher than red pepper of no merchantable quality. The pressure and particle size of the sample also had effect on the amount of oils. In high pressure, the amount of oil was higher than that in low pressure. At low pressure, the amount of oils from 500 μm red pepper sample was higher than those from 700 μm red pepper sample. But in high pressure, comparing to the amount of oils from 700 μm red pepper samples, those from 500 μm red pepper samples was high. The total amount of oil in each sample showed almost constant amount over 200 bar.

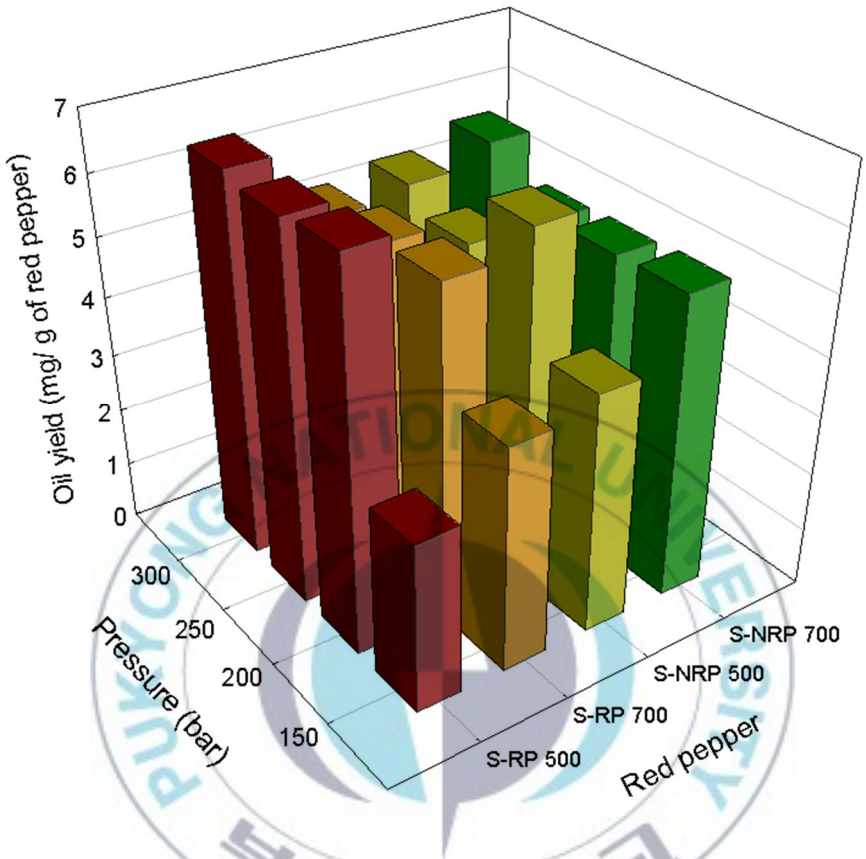

Fig. 7. Total amount of oil obtained by SC-CO_2.

2. Comparison of oil yield by SC-CO_2 and hexane extraction

The total amount of oil obtained by hexane with MSE from red pepper was 22.13% (w/w in dried sample). On the other hand, the amount of total oil in SC-CO_2 extraction was 23.11%. The total amount of oil obtained by soxhlet using hexane from red pepper was 25.80%. By considering that the extraction of oil using hexane was complete, the highest yield by SC-CO_2 extraction was almost 89 %. The maximum oil yield obtained by SC-CO_2 was 70% from peach seed (Sanchez-Vicente et al., 2009) and 75-80% from *Hippophae rhamnoides* L. seed (Jian-Zhong et al. 2005). The differences in maximum yield may be occurred due to variation of processing unit, operating conditions, sample size, percentage of lipid in sample etc (Md. Salim Uddin, 2011).

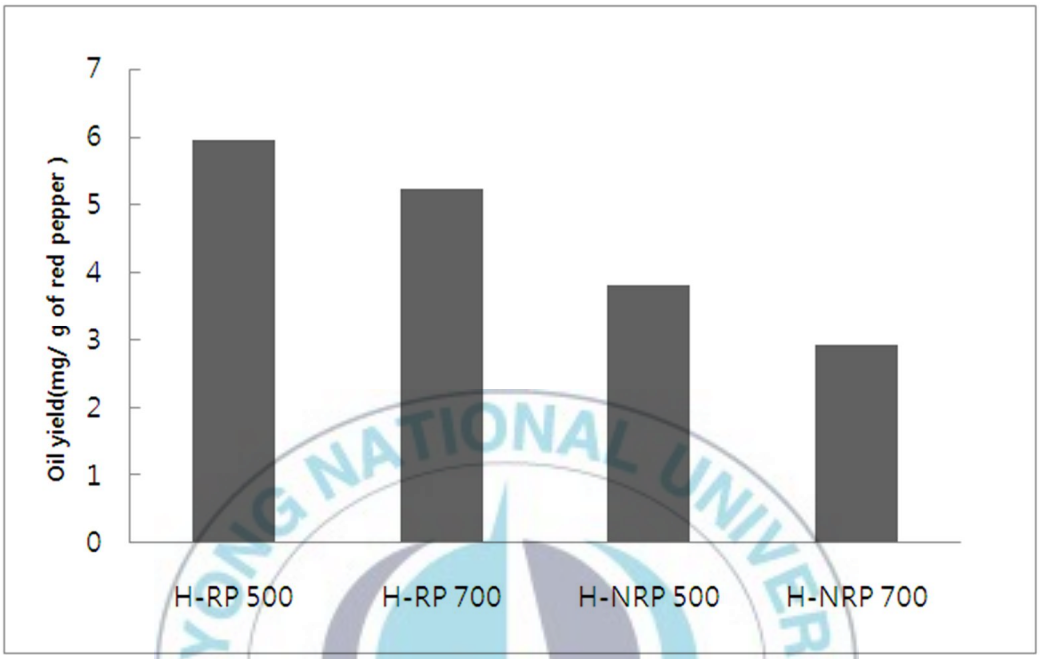

Fig. 8. Total amount of oil obtained by hexane with MSE.

3. Capsaicin contents

The concentration of capsaicin in the oil obtained from red pepper shown in Fig. 9 and Fig. 10. The highest concentration of capsaicin in the oil obtained by SC-CO_2 was 22.90 mg/g of extract at 250 bar from 500 μm red pepper of merchantable quality. The concentration of capsaicin in oil extracted at 250 bar was highest in 500 μm red pepper sample. But the concentration of capsaicin in oil extracted at 200 bar was highest in 700 μm red pepper sample. The increased pressure caused higher density of fluid resulting in higher yield of capsaicin (Gnayfeed et al. 2001). However, the highest yield was achieved at 200 and 250 bar in this study. This behavior of yield due to increasing pressure can be explained by a double effect of increased density of supercritical CO_2 and decreased diffusion coefficient. At 30 and 40℃, and 200 bar, the decrease in diffusion coefficient was more dominant than solvating power, resulting in high yield of capsaicin (Kwon et al. 2011). Careri et al. (2001) also reported similar effect for carotenoid extraction from algae.

The highest concentration of capsaicin in the oil obtained by hexane with MSE was 10.71 mg/g of extract from 500 μm red pepper of merchantable quality. The concentration of capsaicin in the oils obtained by from red pepper of

merchantable quality was higher than those of no merchantable quality. Comparing with the oil obtained by SC-CO_2, The concentration of capsaicin in the oils obtained by hexane with MSE was lower in all conditions.

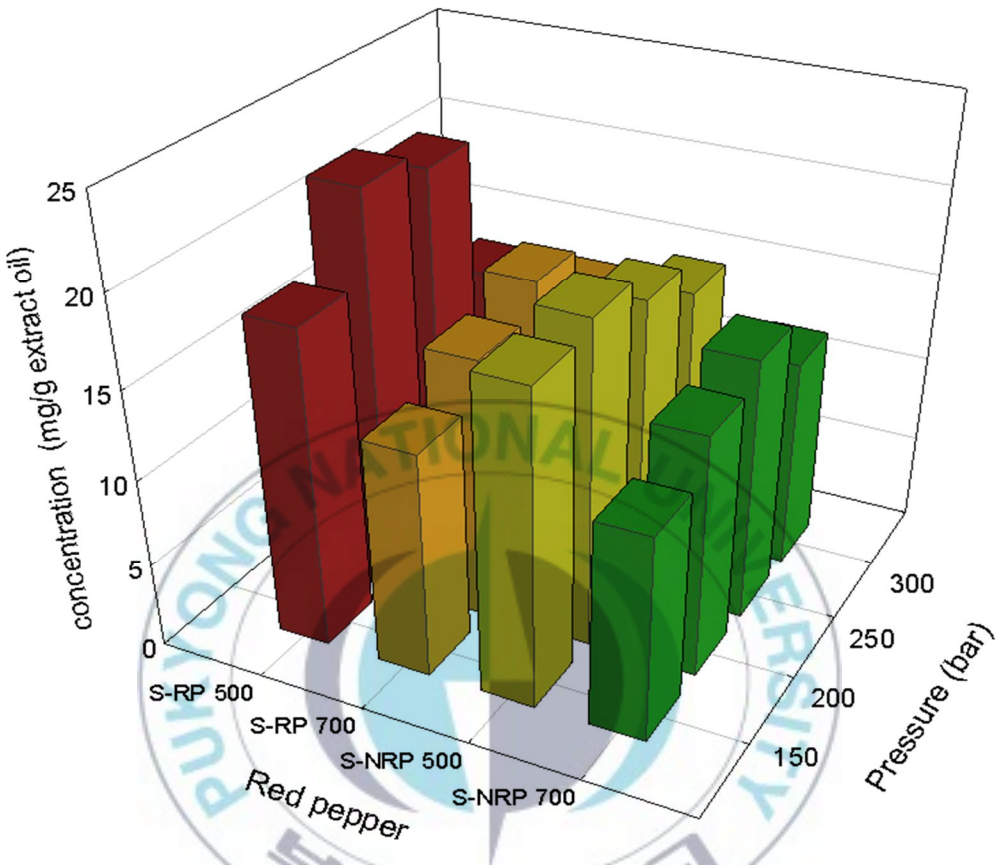

Fig. 9. Concentration of capsaicin in oil obtained by SC-CO_2.

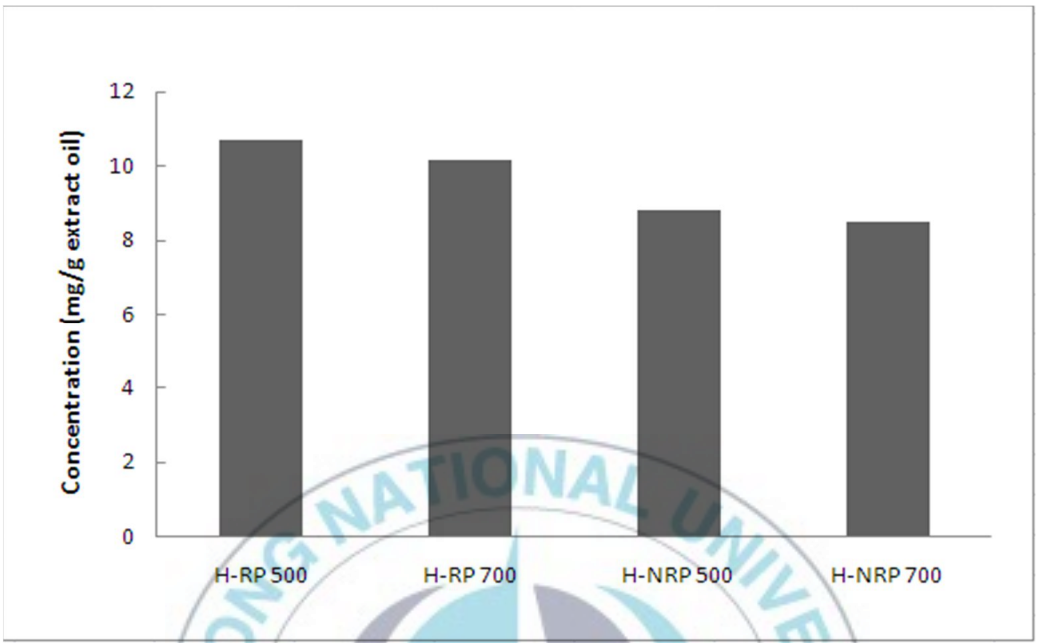

Fig. 10. Concentration of capsaicin in oil obtained by hexane.

4. Color

4.1 Color of the extracted oils

The difference in the lightness (L*), redness (a*) and yellowness (b*) over extracted oils are shown in Table 6. The lightness values in the oils obtained by SC-CO_2 were higher than the oils obtained by hexane with MSE and those of the oils obtained from 500 μm red pepper sample were higher than the oils obtained from 700 μm red pepper sample. Also, the lightness values of oils obtained from red pepper of merchantable quality were higher than those of no merchantable quality. The redness in the oils obtained by SC-CO_2 had also higher values than that of the oils obtained by hexane with MSE. The redness values of oils obtained from red pepper of merchantable quality were higher than those of no merchantable quality. It means the oils obtained by SC-CO_2 from red pepper of merchantable quality were redder than the oils obtained by hexane with MSE from red pepper of no merchantable quality. There were no significant differences with particle size of the red pepper samples. Yellowness also showed a similar pattern with redness and the oils obtained by SC-CO_2 were yellower than the oils obtained by hexane with MSE.

4.2 Color of the powder before and after SC-CO_2 extraction

The changes in the lightness (L*), redness (a*) and yellowness (b*) over powders of red pepper are shown in Table 7. The lightness values of the red pepper powders after SC-CO_2 extraction were higher than the lightness values of the red pepper powders before SC-CO_2 extraction. But the lightness values of the red pepper powder of merchantable quality were lower than those of no merchantable quality it because the color of red pepper of merchantable quality was darker than that of no merchantable quality. The lightness values were increased according to extraction pressure and the powder after extraction at 300 bar had highest lightness value. The redness and yellowness values were reduced after extraction and it also affected by extraction pressure. The redness and yellowness values were decreased according to extraction pressure. Therefore, the pigments in red pepper were extracted by SC-CO_2 and the extracted pigments were increased with increase in pressure.

Table 6. Color of extracted oil

Color	L*	a*	b*
S-RP 500	12.03	+16.95	+5.53
S-RP 700	6.16	+15.41	+3.58
S-NRP 500	8.00	+29.15	+9.99
S-NRP 700	4.12	+25.10	+6.41
H-RP 500	6.17	+1.40	+0.01
H-RP 700	1.82	+1.49	+0.27
H-NRP 500	2.45	+2.02	+0.34
H-NRP 700	1.65	+1.83	+0.31

Table 7. Color of powder before and after SC-CO_2 extraction

Color				L*	a*	b*
RP	500 μm	Before		13.48	+15.07	+18.52
		After	150 bar	17.47	+14.05	+18.11
			200 bar	17.25	+13.77	+17.24
			250 bar	23.54	+7.34	+15.36
			300 bar	23.27	+6.55	+14.53
	700 μm	Before		10.63	+15.04	+14.44
		After	150 bar	15.94	+13.73	+14.23
			200 bar	17.75	+10.07	+13.86
			250 bar	18.43	+9.55	+13.87
			300 bar	21.82	+7.85	+8.3
NRP	500 μm	Before		16.13	+6.38	+17.97
		After	150 bar	19.74	+5.75	+16.9
			200 bar	20.05	+4.64	+13.97
			250 bar	22.6	+3.25	+13.94
			300 bar	22.69	+2.81	+13.31
	700 μm	Before		17.19	+5.66	+17.13
		After	150 bar	17.59	+4.75	+13.56
			200 bar	21.89	+3.63	+13.09
			250 bar	21.63	+2.61	+12.25
			300 bar	22.01	+2.55	+11.95

5. Fatty acid compositions

In plant tissues, the most abundant saturated fatty acids are palmitic acid and stearic acid, and the most common unsaturated fatty acids are oleic acid, linoleic acid, and linolenic acid (D. Murphy, 1993). The comparison of the fatty acid composition of oil is shown in Table 8. The fatty acid composition of red pepper oils were palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2), and linolenic acid (C18:3). Among these fatty aicds, the major fatty acid was linoleic acid. It was same as result of Antonio ea al. (1999) and Sumi Mo (1975).

There were no differences in fatty acid composition of oils obtained by SC-CO_2 and hexane with MSE. But the contents of fatty acid were different and it depends on the samples and extraction conditions. The contents of linoleic acid of oils obtained by SC-CO_2 were higher than those of oils obtained by hexane with MSE and other fatty acids of oils obtained by SC-CO_2 were lower than those of oils obtained by hexane with MSE. Also, The contents of linoleic aicd and linolenic acid of oils obtained from red pepper of merchantable quality were higher than those of oils obtained from red pepper of no merchantable quality. The contents of palmitic acid and stearic acid from red pepper of merchantable quality were lower than those of oils obtained from red pepper of no

merchantable quality. Thus, it is observed that red pepper oil obtained from red pepper of merchantable quality by SC-CO_2 extraction contained high contents of linoleic acid and it is efficient to improve the nutritional functionality that oil from red pepper extract by SC-CO_2 .

Table 8. Fatty acid compositions of extracted oil

Fatty acid	C16 : 0 (Palmitic acid)	C18 : 0 (Stearic acid)	C18 : 2 (Linoleic acid)	C18 : 3 (Linolenic acid)
S-RP 500	4.45	7.91	67.83	19.81
S-RP 700	4.39	10.9	64.79	19.92
S-NRP 500	15.76	9.25	60.8	14.19
S-NRP 700	13.98	9.98	60.48	15.56
H-RP 500	5.29	12.33	61.06	21.32
H-RP 700	4.55	12.15	62.23	21.07
H-NRP 500	14.66	11.23	59.15	14.96
H-NRP 700	15.38	12.11	56.88	15.63

6. Free fatty acid contents

The quality of oil is deteriorated at production and storage conditions. The comparison of the oxidative rate of free fatty acids (FFA) and their esters have been published (Myers et al. 1941). Holman et al. (1947) reported that FFA's were oxidized more rapidly than their esters, and he suggested that this effect probably was due to participation of the carboxyl groups in the decomposition of peroxides. In fact, the prooxidant action of FFA have been published (Frega et al. 1999). It seems to be exerted by the carboxylic molecular group, which accelerates the rate of decomposition of hydroperoxides (Kazuo et al. 1986).

FFA contents of oil extracted by SC-CO_2 and hexane extraction is shown in Fig. 11. The highest content of FFA was found in the oil from red pepper of no merchantable quality by hexane with MSE. The FFA contents of oil extracted red pepper of no merchantable quality ranged from 16.52 to 19.16 g/100 g oil. The FFA contents of oils obtained from red pepper of merchantable quality by SC-CO_2 extraction was remarkably low compared with FFA contents of oils obtained in other conditions. Therefore, using SC-CO_2 for extraction was more effective to protect oil oxidation and it seems that the SC-CO_2 extraction was not exposed to air in process as hexane with MSE.

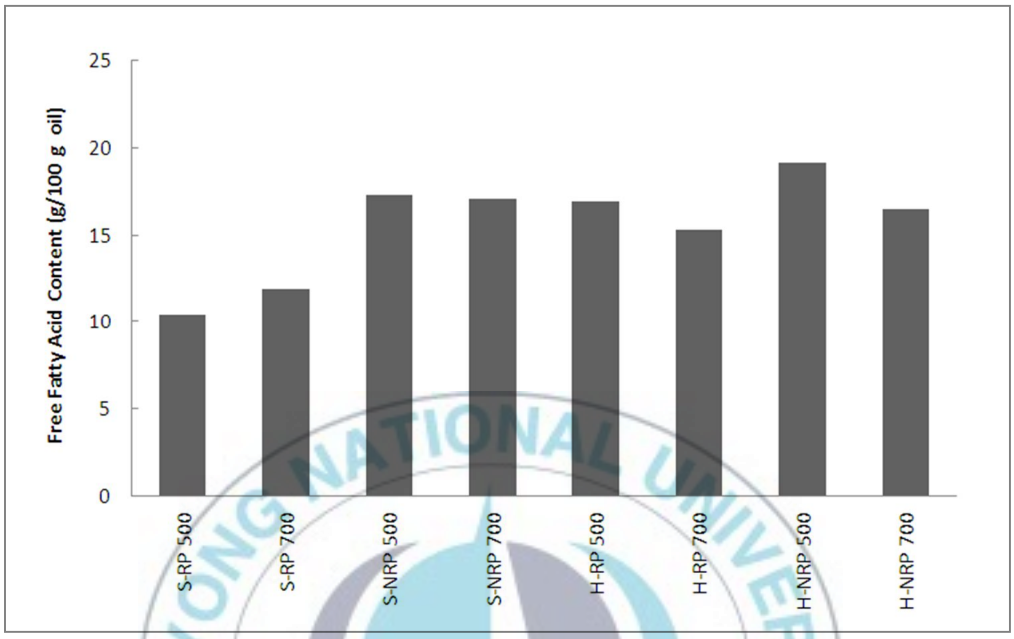

Fig. 11. Free fatty acid contents of extracted oil.

7. Antioxidant activity

7.1 Total phenolic contents

TPC analysis was based on measuring the color change caused by reduction of the Folin-Ciocalteu reagent. Phenolic compounds generated blue reducing substances with donate electron to phosphomolybdic/phosphotungstic acid complex in Folin-Ciocalteu reagent (Singleton et al. 1965 and Bray et al. 1954). Phenolic compounds have been associated with color, sensory qualities, and nutritional and antioxidant properties of food (Go-woon Jung, 2011). Phenolic compounds have been reported to have several biological activities including antioxidant activity (Chandimi et al., 2008). Total phenolic contents (TPC) of oils are shown in Fig. 12. Comparing with the oil obtained by hexane with MSE, the oil obtained by SC-CO_2 extraction had high phenolic content value. TPC value range from 7.63 to 11.48 mg GAE/g extracted oil obtained by SC-CO_2 extraction and it was maximum at 500 μm red pepper of merchantable quality. In terms of quality of red pepper samples, red pepper of merchantable quality was more effective than red pepper of no merchantable quality in total phenolic contents. It means that the antioxidant activity caused by phenolic compounds was best at the oil obtained from red pepper of merchantable quality by SC-CO_2 extraction.

Phenolic compounds in plants possess antioxidant activity, and may help protect cells against the oxidative damage caused by free radicals (Kirakosyan et al., 2003).

7.2 Total flavonoid contents

Flavonoids exist widely in the plant kingdom and are especially common in leaves, flowering tissues and pollens (Richard, 1988). Plant flavonoids are an important part of the diet because of their effects on human nutrition (Frankel, 1995). Some evidence suggests that the pharmacological effects of flavonoids are correlated with their antioxidant activities (Gryglewski et al. 1987). Total flavonoid contents (TFC) of oils are shown in Fig. 13. Comparing with the oil obtained by hexane extraction, the oil obtained by SC-CO_2 extraction had high flavonoid content value. TFC value range from 0.453 to 0.577 g CE/g extracted oil in SC-CO_2 extraction and it was maximum at 500 μm red pepper of merchantable quality. Comparing with the oil obtained from red pepper of no merchantable quality, the oil obtained from red pepper of merchantable quality had high flavonoid content value. There were slight differences in TFC value of the oil with particle size. TFC value also highest at the oil obtained from red pepper of merchantable quality by SC-CO_2 extraction. Thus, the best extracted condition for containing high flavonoid content value was SC-CO_2 extraction using red pepper of merchantable quality and the oil obtained from red pepper of merchantable quality by SC-CO_2 extraction had best antioxidant activity.

7.3 DPPH free radical scavenging effect

The DPPH radical scavenging effects of oils of are shown in Fig. 14. Generally, the red pepper showed DPPH scavenging activity (Sim et al. 2008). Comparing with the oils obtained by hexane extraction, the oils obtained by SC-CO_2 extraction had significantly stronger scavenging effect for DPPH radicals. Also, the oils from red pepper of merchantable quality had stronger DPPH scavenging activity than those obtained from red pepper of no merchantable quality. No significant difference of the DPPH radical scavenging activities in particle size of red pepper was found. Therefore, the DPPH radical scavenging effects were influenced by samples and extraction and the oil obtained from red pepper of merchantable quality by SC-CO_2 extraction had highest antioxidant activity than others.

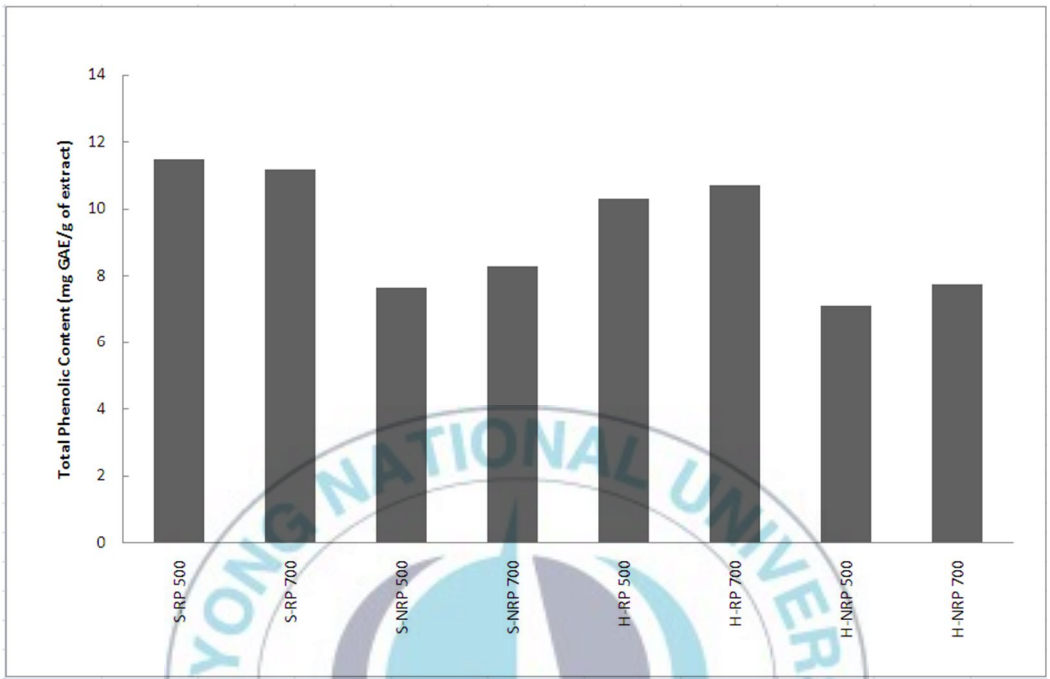

Fig. 12. Total phenolic contents of extracted oil.

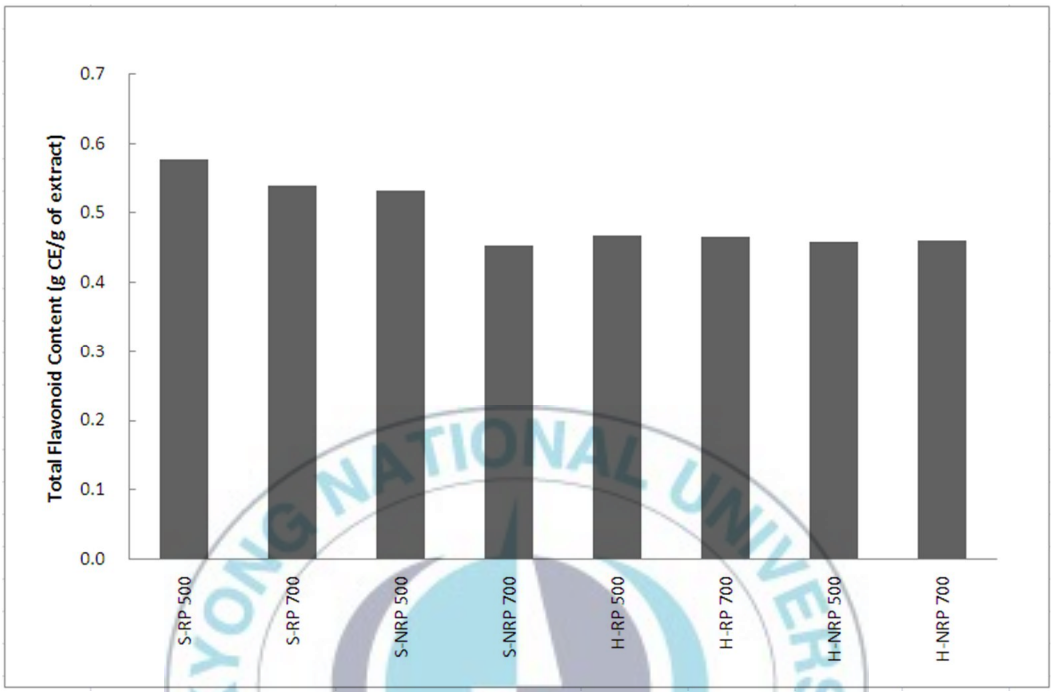

Fig. 13. Total flavonoid contents of extracted oil.

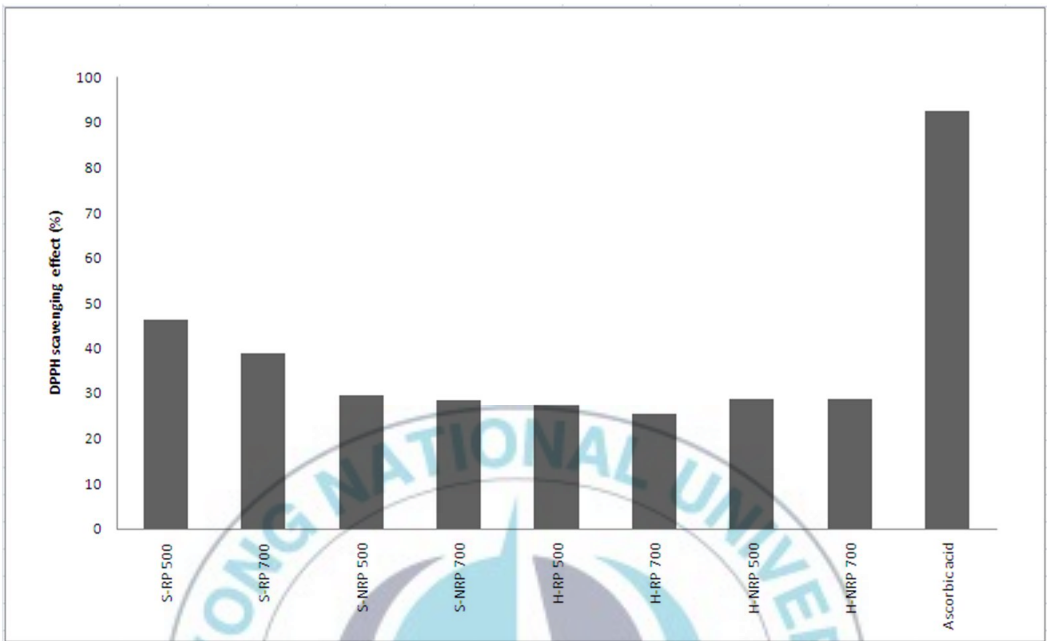

Fig. 14. DPPH free radical scavenging effect of extracted oil.

8. Antimicrobial activity

8.1 Paper disc diffusion

The antimicrobial activity in various oils was assessed by paper disc diffusion assay and the results were shown in Table 9. The antimicrobial activity of the oils obtained by hexane with MSE was not observed. The oils obtained by SC-CO_2 extraction were showed very slight antimicrobial activity against *B. cereus, L. monocytogenes, S. aureus, P. aeruginosa* and *S. typhimurium* and showed slight antimicrobial activity against *E. coli*. Thus, using SC-CO_2 extraction was the better way to gain the oil had high antimicrobial activity extracted from red pepper. A number of effects involving the micro-organisms were visually observed as circular zones surrounding the test extract-soaked disks and c*apsicum* species may contain an assortment of chemicals which were responsible for the inhibitory, stimulatory, and anti-hemolytic effects (Robert et al. 1996).

8.2 Minimum Inhibition Concentration

It was confirmed that the oil obtained by SC-CO_2 has antimicrobial activity, through the paper disc diffusion assay. The minimum inhibition concentration (MIC) results were shown in Table 10. While *E. coli* and *P. aeruginosa* did not appear the growth in 10,000 μg/mL in case of S-RP 500 and S-RP 700, the antimicrobial activity did not observe in others. The oils obtained by SC-CO_2 extraction were showed very slight antimicrobial activity in paper diffusion assay with undiluted sample, but antimicrobial activity of the oils was decreased with dilution. The oil obtained from red pepper of merchantable quality by SC-CO_2 extraction had highest antimicrobial activity and the oils were showed high antimicrobial activity without dilution.

Table 9. Clear zone of extracted oils in different condition

Microorganisms	Clear zone of the oil							
	S-RP 500	S-RP 700	S-NRP 500	S-NRP 700	H-RP 500	H-RP 700	H-NRP 500	H-NRP 700
B. cereus	±	±	±	±	-	-	-	-
L. monocytogenes	±	±	±	±	-	-	-	-
S. aureus	±	±	±	±	-	-	-	-
E. coli	+	+	+	+	-	-	-	-
P. aeruginosa	+	±	±	±	-	-	-	-
S. typhimurium	±	±	±	±	-	-	-	-

- No inhibition (10 mm)
± Very slight inhibition (10~11 mm)
+ Slight inhibition (11~12 mm)
++ Moderate inhibition (12~16 mm)

Table 10. Minimal inhibitory concentration of extracted oils in different condition

Microorganisms	MIC (μg/mL)							
	S-RP 500	S-RP 700	S-NRP 500	S-NRP 700	H-RP 500	H-RP 700	H-NRP 500	H-NRP 700
B. cereus	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <
L. monocytogenes	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <
S. aureus	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <
E. coli	10,000	10,000	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <
P. aeruginosa	10,000	10,000	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <
S. typhimurium	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <	10,000 <

Conclusion

In SC-CO_2 extraction, the total amount of oil in red pepper of merchantable quality was higher than red pepper of no merchantable quality and the total amount of oil in each sample showed almost constant amount over 200 bar. The oil amount of red pepper extracted by SC-CO_2 was higher than that of extract using hexane with MSE. The highest yield of capsaicin was achieved at 200 and 250 bar in SC-CO_2 extraction. The concentration of capsaicin in the oils obtained from red pepper of merchantable quality was higher than those of no merchantable quality. Comparing with the oil obtained by SC-CO_2, the concentration of capsaicin in the oils obtained by hexane with MSE was lower in all conditions. The values of lightness, redness and yellowness of the oils obtained from red pepper of merchantable quality were higher than those of no merchantable quality, and those of the oils obtained by SC-CO_2 had also higher values than that of the oils obtained by hexane with MSE. In fatty acid, the composition of fatty acid was followed with that red pepper seed. It was observed that red pepper oil obtained from red pepper of merchantable quality by SC-CO_2 extraction contained high contents of linoleic acid and it is efficient to improve the nutritional functionality that oil from red pepper extract by SC-CO_2.

In antioxidant activities for total phenolic contents (TPC), total flavonoid contents (TFC) and DPPH free radical scavenging activity, the oils obtained by SC-CO2 were better than that obtained by hexane with MSE and comparing with the oil obtained from red pepper of no merchantable quality, the oil obtained from red pepper of merchantable quality had high values. The antioxidant activity was influenced by samples and extraction and when the oil obtained from red pepper of merchantable quality by SC-CO_2 extraction, results was desirable. Otherwise, in the antimicrobial activities, the oils obtained by SC-CO_2 extraction were showed very slight antimicrobial activity against *B. cereus, L. monocytogenes, S. aureus, P. aeruginosa* and *S. typhimurium* and showed slight antimicrobial activity against *E. coli*. The oil obtained from red pepper of merchantable quality by SC-CO_2 extraction had highest antimicrobial activity and the oils were showed high antimicrobial activity without dilution. As a result, the oils obtained by SC-CO2 was excellent in antioxidant and antimicrobial activity and the oils obtained from red pepper of merchantable quality had higher antioxidant and antimicrobial activity than those of no merchantable quality. Thus, the extracts obtained from red pepper of merchantable quality by SC-CO_2 extraction will be more effective to human. Furthermore, the antioxidant activity and the antimicrobial activity were affected by not only concentration of capsaicin but also other parameters like pigments. Therefore, the study concerning effect of other parameters such as pigments will be required.

Acknowledgement

본 석사 논문이 완성되기 까지 많은 도움을 주신 많은 분들께 감사의 인사를 드립니다. 먼저 석사과정 동안 학문의 길로 인도해주시고 아낌없는 지도를 해주신 전병수 교수님께 깊은 감사의 인사를 드리며, 바쁘신 와중에도 논문을 심사해 주시고 소중한 조언과 충고를 해주신 김선봉 교수님, 안동현 교수님께도 깊은 감사를 드립니다. 또한 많은 가르침을 주신 조영제 교수님, 이양봉 교수님, 양지영 교수님, 김영목 교수님께도 진심으로 감사의 말씀 전합니다.

그리고 식품공학실험실 이름 하에 많은 도움을 주신 ASADUZZAMAN, TANBIRUL 박사님과 위로와 격려를 아끼지 않으셨던 심정은, 이승미 선배님 너무 감사합니다. 실험실 생활 동안 동고동락하며 많은 도움을 준 주희, 준호 오빠, EVI, MELLISA, 혜연언니, 정남오빠와 언제나 실험실 일들을 묵묵히 도와준 은주, 창주, 경진, 다혜, 태근, 동학, 지선, 효정, 미경에게도 고맙다는 말을 전합니다. 언제나 위로와 격려를 아끼지 않으며 힘이 되어준 찬이오빠, 홍희 오빠, 같이 졸업하는 다현이, 현지, 고생하는 민홍이오빠, 우영이, 연주언니, 은혜, 선영언니, 연중오빠에게도 감사하며, 제가 낯선 환경에서 잘 적응 할 수 있도록 많은 것을 가르쳐주고 도와주셨던 많은 선후배님들께 감사드립니다.

그리고 힘들 때마다 위로를 아끼지 않았으며 멀리서도 찾아와 격려해주었던 여진이, 현정이, 은아에게도 감사하며, 멀리 있어도 언제나 가까이 있는 것 같은 이과반 친구들과 이 논문이 나오기까지 가장 가까운 곳에서 지켜봐 주고 응원해준 경석이에게도 감사의 인사를 전합니다.

마지막으로 항상 저를 믿고 묵묵히 지켜봐 주시고 힘이 되어주신 부모님과 동생에게 이 논문을 바칩니다. 정말 사랑하고 감사합니다. 이 밖에 글로써 감사의 인사를 드리지 못한 도움주신 많은 분들께도 감사의 말을 전하고 싶습니다. 이 논문이 나오기까지 제가 얼마나 많은 분들의 사랑과 도움을 받으며 살고 있는지 알게 되었습니다. 다시 한번 모든 분들께 감사 드립니다.

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