Thesis for the Degree of Master of Engineering

Characterization of Extracts Recovered from Mealworm (*Tenebrio molitor*) using Sub- and Supercritical Fluids

by

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아임계 및 초임계 유체를 이용한 갈색 거저리(*Tenebrio molitor*)로부터 회수된 추출물의 특성에 관한 연구

Advisor: Prof. Byung-Soo Chun

by

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Contents

List of Table

List of Figure

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Abstract

Tenebrio molitor is known as a yellow mealworm or mealworm. It is mass-cultured worldwide as a pet insect and is used for food or feed purposes. The mealworm has a high content of fat and protein, and the fat is composed of high amounts of unsaturated fatty acids, e.g., oleic acid (C18:1) and linoleic acid (C18:2) which make it potential to use widely in the food industry. The lipids and proteins are usually extracted by organic solvents or other physical methods which are hazardous for the consumers and the environment. So, at present there is attention for the safe and environmentally friendly process. In this regard, supercritical carbon dioxide (SC-CO2) and subcritical water hydrolysis processes are considered to be a preferable method to extract the lipid and hydrolysis of protein of deoiled mealworm residue. This study was aimed to optimize the SC-CO2 extraction parameters of lipids from meal worm ranging temperature 40~50°C and pressure 15~30 MPa. The extraction yield and a number of physicochemical and stability parameters of extracted oil at different SC-CO2 conditions were compared with organic solvent extracted oil. In SC-CO2 extraction, the extraction yield was found

maximum at 45°C and 25 MPa pressure. The oxidation stability of oil was evaluated by acid value, peroxide value, p-anisidine value and free fatty acid value. The stability of lipid extracted by SC-CO2 was higher than that of organic solvent extracted lipid. Among the various lipid extraction conditions, lipid stability was found to be the highest at 45°C and 250 bar. The SC-CO2 extraction is capable to extract only nonpolar lipids and co-solvents like ethanol can be added to extract amphiphilic compounds like phospholipids. The phospholipids contained in the extracted oil were analyzed by Stewart (1980). The de-oiled mealworm powder was hydrolysed by using sub-critical water and the properties of the hydrolysate was evaluated. The hydrolysis was performed at conditions of temperature 150 to 225°C, pressure 3 MPa, stirring speed of 200 rpm. The protein content, sugar content, antioxidant ability, water soluble protein content and reducing sugar content were showed to increase in hydrolysate with increasing temperature. The radical scavenging activity of produced hydrolysates such as DPPH, ABTS and FRAP were measured. The phenolic compounds and flavonoids related to antioxidant activity were also high in the high temperature produced mealworm proteins extracted by subcritical water hydrolysis.

Introduction

Yellow mealworm, *Tenebrio molitor* is commonly called mealworm and is cultivated in large quantities and used as a human food material. The mealworm contains 45% protein, 35% lipid and 10% carbohydrate. Mealworm oil can be used as food due to its high content of unsaturated fatty acid. Mealworm oil contains more than 70% of unsaturated fatty acids such as linoleic acid and oleic acid. Many countries of the world including European are producing and developing products using mealworm oil. Low level of trans-fat and good flavor of mealworm oil make it use as food. In particular, foods and feeds with high protein and lipid content using insect proteins and lipids have been developed [1]. Recently, in Korea, mealworms have been using as a food ingredient and are emerging as future food resource. Currently, the insects that can be used as food in Korea are *Tenebrio molitor* larva, *Protaetia brevitarsis seulensis* larvae, *Gryllus bimaculatus*, and *Allomyrina dichotoma* larvae. The insect industry is expected to expand globally, the number of farmers are increasing, and the growth potential of edible insects is highly appreciated. The domestic edible insect industry is expected to grow to 159 billion won in 2020 from 2011 and 2015, and the global market size is estimated at 38 thousand billion won. As of 2014, the insect industry occupied 696 people and the number of production farms were 464 [2].

In particular, foods and feeds by insect proteins and lipids are being developed.

Insect protein is healthy, nutritious alternatives to chicken, pork, beef and even fish. Mealworm protein is rich in various amino acids and supplies nutritional supplements to the diet. The absorption of amino acid is rapid and it is necessary unit for human body metabolism to grow and maintain the body. Efficient separation of protein and lipids of mealworm can make their use easier. Oil can be extracted by organic solvents and physicochemical methods, but these methods are expensive and require long time; so an alternative process may render the use profitable and applicable for industrial use in the food and feed sectors. So, an alternative method which is green, economical and eco-friendly is required for lipid extraction from mealworm. De-oiled mealworms have a relatively high protein content and can be used directly, but hydrolysis is an effective way to increase their value as a food product. Usually, the hydrolysate is obtained by acid or alkali treatment, hydrothermal treatment and conventional methods. In this study, supercritical carbon dioxide extraction (SCO_2) and subcritical water hydrolysis (SWH) technique which is an eco-friendly and alternative extraction technology was used. SC-CO_2 and SWH have advantages such as short extraction time, non-toxic, good selectivity. The mealworm has high protein and lipid content and is highly available as a food, and it needs a proper characterization of its components to make it potential as food ingredient. Mealworm contains more than 70% of unsaturated fatty acids, which are highly valuable in terms of linoleic acid, palmitic

acid and oleic acid. Unsaturated fatty acids consist of two or three double bonds in long chain form and are a healthy fat [3]. The intake of lipids is a source of energy and essential fatty acid intake is important for various physiological functions. Mealworm oil can contribute to human nutrition by supplying energy and essential fatty acids [4] Oil content of mealworm is more than 35% which is highly likely to be used as food because of its high content of unsaturated fatty acids. It can be used as an alternative to vegetable oil and can be substitute of fish oil as it has no sensory difficulty to use as edible oil. Mealworm oil does not have trans fats, is good for using as food, and can be replaced with fish oil. Approximately 80% of the oil content in mealworm is in the form of triacylglycerol and the content of phospholipids in the oil is less than 20% and is widely consumed worldwide [5]. A new process is needed to utilize a variety of oil-rich mealworms. Currently, organic solvent extraction for extracting oil requires a new environmentally friendly and safe extraction method because of the risk of residual solvent and insecurity as a food.

A supercritical fluid is a fluid that exists at a temperature and pressure condition above a critical point. It exhibits a medium property between a liquid and a gas. Because it can change the density continuously through temperature and pressure changes, viscosity, and diffusion coefficient can be controlled easily. In addition, it exhibits properties of low viscosity, surface tension, and high diffusion coefficient,

and penetration into the solid material is effective. Therefore, it is advantageous to extract the active ingredient efficiently, and the extraction efficiency and purity are excellent. Among supercritical fluids, carbon dioxide is a natural solvent that has a relatively low critical point (73.8 bar, 31°C) and is nonflammable, non-toxic and recyclable. It has the advantage of little reactivity or corrosiveness with the extract and the price is low. Supercritical carbon dioxide has a dielectric constant similar to that of hexane and is a nonpolar solvent, so it can replace the process of extracting oil with hexane, and it has less loss because it can selectively extract oil. Therefore, the extracted oil does not contain the residue of solvent and considered as preferable technique for oil extraction. Compared to the organic solvent extraction method, the extraction time is shorter in SC-CO_2 extraction. The supercritical carbon dioxide extraction method can also extract polar substances by using a polar co-solvent as an auxiliary solvent. In general, it is possible to extract phospholipids in mealworm powder by using ethanol, which is a polar solvent, as an auxiliary solvent.

The mealworm contains 40 to 45% protein and nutritionally attracts attention as a substitute for protein source. Cattle farming cause environmental destruction due to meat production, which is currently a protein source. Figures for greenhouse gas emissions show a higher greenhouse gas emission than bee (2 ~ 122 g / kg mass) with beef cattle (2850 g / kg mass) and pork (80 ~ 1130 g / kg mass). Between 2012

and 2050, demand for meat protein is expected to increase by 70-80%, and in this respect, mealworms are proposed as a sustainable source of protein [5]. Several authors have proposed mealworm protein as an eco-friendly alternative to meat [6-9]. From the environmental point of view, the breeding system of mealworms has been recognized as an eco-friendly food source by emitting less greenhouse gas compared to livestock [10][11]. Processing methods for using the proteins of such a source include hydrolysis using acids or bases, hydrolysis using heating, and hydrolysis using enzymes. Hydrolysis with acid/ bases results in poor yields of the product and may result in the presence of harmful compounds for human and environmental contamination. Hydrolysis by heat treatment is low in selectivity and efficiency, and hydrolysis by enzyme treatment is costly due to high unit cost of enzyme. Additionally, there is a disadvantage that it is difficult to control the enzyme activity at the operation process. In order to solve such problems and to efficiently process hydrolysis, an efficient treatment process is required. A sub-critical hydrolysis process, which is one of the most eco-friendly processes in recent years, may be suitable. We have investigated the properties of hydrolysates containing peptides and amino acids by increasing the absorption of the hydrolysate by hydrolysis of the residue protein using sub-critical water hydrolysis and by lowering the protein to low molecular weight. The critical point of water is 374˚C and the pressure is 220 bar. Water existing above the critical point is called

supercritical water, and water of high temperature (100-374˚C) and high pressure to maintain water in liquid state is called subcritical water (Fig 2). Refers to hot water that maintains a liquid state with pressure applied between the boiling temperature of water (100˚C) and the critical temperature (374˚C). In the subcritical state, water has special characteristics such as dielectric constant, surface tension, viscosity, and weak intermolecular hydrogen bonding. The dielectric constant of water at 25˚C is about 27 at 80 to 250˚C, which is significantly lower as the temperature increases. This is similar to the dielectric constant of methanol ($\varepsilon = 32.6$), ethanol ($\varepsilon = 24.3$) and acetone ($\varepsilon = 20.7$) at room temperature and has both polar and nonpolar properties. The ion product (Kw) of water is the product of hydrogen ion (H^+) and hydroxide ion (OH^-) concentration, which can be expressed as ion product of water or the dissociation constant for water.

Materials and Methods

1. Materials

Carbon dioxide (CO2) gas purity was 99.99% and it was supplied by KOSEM (Yangsan, Korea). *p*-Anisidine, DPPH, ABTS+ Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Gallic acid, Folin-Ciocalteu reagent and all other chemicals and reagents were purchased from Sigma-Aldrich, St. Luis, Mo., USA. All reagents used in this study were of HPLC grade.

(a) (b)

Fig. 1. The pictures of (a) Mealworm and (b) Mealworm powder.

2. Sample collection and preparation

The mealworms used in the experiment was collected from mealworm breeders in Damyang, Jeollanam-do, South Korea. The mealworms were for 3 days, they were washed and dried, and then they were vacuum microwave-dried at a temperature of 45˚C. The dried mealworms were crushed using a blender (PN

SMKA-4000 mixer, PN Co., Ltd., Korea) and made into powder of uniform particles using a 1 mm-sized sieve. The powder sample was sealed in plastic bag and stored at −40˚C.

3. Method

3.1. Proximate composition

Crude lipid content was measured by soxhlet apparatus using hexane as solvent for overnight and protein content was examined with an automatic Kjeldahl analyzer. Moisture and ash were estimated using the standard AOAC methods [12]. Moisture content was determined after drying in oven at 105ºC until a constant weight was obtained. After moisture removed, ash content was estimated for overnight heating at 550ºC. Carbohydrate content was calculated by the following equation:

Carbohydrate (%) = 100 - (moisture + crude ash + crude lipid + crude protein)

3.2. Supercritical Fluid Extraction (SFE)

A laboratory-scale supercritical fluid extraction process was used. The supercritical fluid extraction device consists of a high pressure pump, an extractor, a collector and a flow controller. This apparatus can be operated at pressure up to 30 MPa. Firstly, 80 g mealworm samples were filled into the stainless steel

extraction vessel (extractor) which was 200 mL in volume. A thin layer of cotton was placed at the bottom of the extractor. Before plugging with cap another layer of cotton was used at the top of the sample. The liquefied carbon dioxide pressurized through the high-pressure pump passes through a heat exchanger and is heated to the desired extraction temperature and injected into the extractor. The extraction temperature was maintained by connecting the extractor to a water bath. Flow rates and accumulated gas volume passing through the apparatus were measured using a gas flow meter. The effects of temperature and pressure on lipid extraction from mealworm were studied at 40 to 50°C and 15 to 30 MPa. Extraction curves were obtained by setting the temperature (40 ~ 50°C) and the pressure (150 ~ 300 bar) for 2 hours. The extracted oil and mealworm residues were then stored at −40°C until further use and analysis. Mealworm residues obtained from the highest oil yield by SC-CO2 extraction were used for phospholipid isolation.

3.3. Organic solvent Extraction

To compare the characteristics of mealworm oil by SC-CO2 extraction and conventional organic solvent extraction, Soxhlet extraction techniques was applied as a conventional extraction. Three gram of the mealworm powder was placed into the extraction thimble and the extraction was run 12 h until the color of the condensed solvent at the top of the apparatus was clear. Oil was extracted with 150

mL of hexane for 24 h. Soxhlet extractions were conducted in triplicate. The extracted oil and mealworm residues were then stored at −20 °C until further use and analysis.

3.4. Characterization of Mealworm Oil

3.4.1. Acid value

The AV was determined according to AOCS official method (AOCS, 1998). 1 g of sample was dissolved in 100 mL ether:ethanol (1:1) with shaking. 4 drops of the indicator phenolphthalein were then added. The solution was titrated with 0.1 N KOH-ethanol until it becomes a pink color and the acid value was expressed as mg of KOH per g of sample.

$$\text{Acid value (AV)} = 56.11 * A * F/S \quad (1)$$

Where A is the volume of KOH-ethanol solution used in the titration (mL), F is the concentration of KOH-ethanol factor, S is the mass of the oil (g) and 56.11 is the molecular weight of KOH.

3.4.2. Peroxide value

The peroxide value was determined according to the AOCS official method (AOCS, 1998). One g of sample was dissolved in 6 mL acetic acid-chloroform (3:2)

solution. Then 0.1 mL saturated potassium iodide solution was added to the mixture, which was then allowed to stand with occasional shaking for 1 min. Distilled water (6 mL) was immediately added to the solution. The solution was titrated with 0.01 N of sodium thiosulfate until the yellow iodine color had almost disappeared. Next 0.4 mL starch indicator solution was added and the solution was titrated again until the blue color disappeared. A blank control was obtained following the same procedure. The peroxide value was expressed as milliequivalents peroxide/1,000 g sample.

$$\text{Peroxide value (POV)} = \frac{(S-B)\times N\times 1000}{M} \quad (2)$$

Where, S is volume of sample titrant (mL), B is the volume of blank titrant (mL), N is the normality of the sodium thiosulfate solution and M is the mass of sample (g).

3.4.3. Free fatty acid value

The free fatty acid value (%) was determine according to AOCS official method. 285 μL of 1% phenolphthalein was to 11 mL of ethanol. After mixing the mixer was boiled and 0.1 N NaOH was addd to get mild pink color. Then 0.5 g of sample oil added and titrated with 0.1 N NaOH to get back the previous pink color. The free fatty acid was calculate as follows:

$$\text{FFA}(\%) = \frac{\text{NaOH (mol)} \times \text{N} \times \text{factor}}{\text{sample weight (g)}} \quad (3)$$

3.4.4. *p*-Anicidine value

The *p*-anisidine value was determined by AOCS official method (AOCS, 2006). First of all, test oil samples were filtered through a Whatman No. 40 filter paper to remove moisture and impurities. 1.0g of oil sample was accurately weighed in a 50 mL volumetric flask. The oil samples were dissolved and diluted with 25 mL 2,2,4-Trimethylpentane (iso-octane). The absorbance of the oil sample was measured at 350 nm using a spectrophotometer (UVmini-1240 UV-Vis Spectrophotometer, USA). A 5 mL sample of oil was pipetted into one test tube and 1 mL of *p*-anisidine reagent was added. A 5 mL of iso-octane was added to another test tube and 1 mL of p-anisidine reagent was added to it and used as a blank. The p-anisidine reagent was prepared by adding 0.25 g p-anisidine to 100 mL of glacial acetic acid. After 10 minutes, the absorbance of the oil sample with the p-anisidine reagent was measured at 350 nm. The *p*-anisidine value was calculated by using following equation.

$$p\text{-anisidine value} = \frac{25*(1.2As - Ab)}{W} \quad (4)$$

Where, As = Absorbance of the fat solution after reaction with the *p*-anisidine reagent; Ab = Absorbance of the fat solution; W = Weight of oil (g).

3.4.5. Phospholipid content

The phospholipid content of lecithin from anchovies was determined by colorimetric based on complex formation between phospholipids and ammonium ferrothiocyanate according to Stewart (1980) [13]. First 0.35 mg of phospholipid was dissolved in 2 mL of chloroform. Subsequently, 1 mL of a solution prepared from ferric chloride (27 g/L) and ammonium thiocyanate (30 g/L) was added. After vortexing, the mixture was centrifuged at 1000 × g for 15 min. The lower phase was collected and the absorbance was recorded at 488 nm by a UV-spectrophotometer (BioTek Instruments, Winooski, VT, USA). The phospholipid content was calculated by constructing a calibration curve of the standard PC.

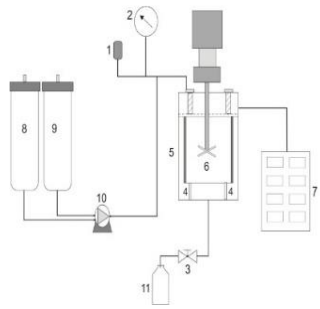
3.5. Analysis of fatty acid (Gas chromatography)

The fatty acid composition of mealworm oil obtained by SC-CO2 and organic solvent hexane extraction were determined by GC-flame ionization detector (FID) using a Agilent Technologies 6890N gas chromatograph (Agilent Technologies, CA, USA). The fatty acid methyl esters (FAMEs) were prepared according to official method and recommended practices of the AOCS (Ce 2-66) and then separated using an Agilent DB-Wax capillary column (30m length x 0.250 mm internal diameter, 0.25 μm of film). Helium at a flow rate 0.9 mL/min was used as a carrier gas of fatty acid methyl esters. The split ratio was fixed at 50:1. The oven

temperature was programmed starting at a constant temperature of 130°C for 3 min, and then increased to 240°C at a rate of 4°C/min and hold at 240°C for 10 min. Injector and detector temperatures were 250°C. Fatty acid methyl esters were identified by comparison of retention time with standard fatty acid methyl esters mixture (Supleco, Bellefonte, Pa., USA).

3.6. Subcritical water hydrolysis

The subcritical water hydrolysis was performed using a high-temperature, high-pressure reactor of 200 cm3 volume made of 276 Hastelloy with thermometer, and a device including electric heater, agitator, and cooling device. Eight g of deoiled mealworm powder and 160 mL of distilled water (w/v, 1:20) were mixed and placed in the reactor. Used distilled water was adjusted pH 7.4. After the reactor was sealed well, the heater was turned on and heated. After the initial pressure of 30 bar was injected, the hydrolysis reaction was carried out for 15 minutes when the temperature reached 150°C, 180°C and 210°C. The rotational speed of the stirrer was 200 rpm. The obtained hydrolysate was separated by centrifugation (1000 rpm, 15 min) for experiment and stored at 4°C. The precipitated residues were dried and weighed to calculate the hydrolysis efficiency.



1. Safety valve
2. Pressure gauge
3. Needle valve
4. Electric heater
5. High pressure reactor
6. Impeler / Stirrer
7. Stirring speed & temperature controller
8. Water tank
9. Reactant / Catalyst tank
10. Pump
11. Sample collector

Fig. 2. The picture of used subcritical water hydrolysis machine.

3.7. Yield of different extraction condition

The hydrolysis yield was calculated using the following equation.

$$\text{Yield of Hydrolysis } (\%) = \frac{\text{Sample (g)} - \text{Residue of hydrolysate (g)}}{\text{Sample (g)}} \times 100 \quad (5)$$

3.8. Conventional Hydrolysis

For comparative experiments, hydrolysate was obtained using conventional methods. Enzymatic hydrolysis and water hydrolysis with room temperature and normal pressure. Enzymatic hydrolysis was performed using flavourzyme, which is a typical protease. Eight g of the sample was mixed with 160 mL of distilled water adjusted to pH 6.2, and add 2 mL enzyme then the temperature was set at 50°C and the mixture was stirred for 30 hours for enzymatic hydrolysate [14].

3.9. Antioxidant activity

3.9.1. DPPH radical scavenging activity

The DPPH radical scavenging capacity of hydrolysate was estimated based on the method described by Miliauskas et al. (2004), Saha et al. (2004), and Cai et al. (2006) with slight modification [15-17]. 3.9 mL of ethanolic DPPH (60 μM) was firstly mixed with 0.1 mL of hydrolysate or ethanol (as control) and they were stored in dark room at ambient temperature for 30 min. Subsequently, the absorbance of hydrolysate and control was measured against ethanol (as blank) at 517 nm using UV-spectrophotometer (BioTek Instruments, Winooski, VT, USA). The absorbance measurements of hydrolysate and control were done in triplicate. Trolox was used for calibration of standard curve and the results were expressed as mg trolox equivalent antioxidant capacity per g of sample (mg trolox/g of sample). The calibration equation for trolox was $y=-0.0013x + 0.7706$ ($R^2=0.9975$).

3.9.2. $ABTS^+$

$ABTS^+$ radical scavenging capacity assay was carried out according to the procedures described by Cai et al. (2006), Wetwitayaklung et al. (2006), Guimarãs et al. (2007) and Surveswaran et al. (2007) [18-20]. ABTS radical solution was firstly prepared by mixing 10 mL of 7 mM ABTS solution with 10 mL of 2.45 mM potassium persulphate solution in a 250 mL amber bottle. Subsequently, the ABTS

radical solution was allowed to stand in a dark room at ambient temperature for 12-16 hours to give a dark blue solution. The $ABTS^+$ radical solution was diluted with denature ethanol until its absorbance was equilibrated to 0.7±0.02 at 734 nm before using. 3.9 mL of $ABTS^+$ radical solution was firstly mixed with 0.1 mL of undiluted hydrolysate or ethanol (as control) and they were allowed to store in dark room at ambient temperature for 6 min. Subsequently, the absorbance of hydrolysate and control was measured against ethanol (as blank) at 734 nm using UV-spectrophotometer (BioTek Instruments, Winooski, VT, USA). The absorbance measurements of hydrolysate and control were done in triplicate. Trolox was used for calibration of standard curve and the results were expressed as mg trolox equivalent antioxidant capacity per g of sample (mg trolox/g of sample). The calibration equation for trolox was $y = -0.0015x + 0.7841$ (R^2=0.9979).

3.9.3. FRAP

The FRAP assay was determined by Benzie and Strain (1996) with some modifications [21]. The stock solutions included pH 3.6, 250 mM acetate buffer, 10 mM TPTZ (2. 4. 6-tripyridyl-*s*-triazine) solution in acetate buffer, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3.6H_2O$ solution and then warmed at 37 °C before using. Mealworm hydrolysate (150 µL) were allowed to

react with 2850 μL of the FRAP reagent for 30 min in the dark room. The absorbance value of the reaction solution was measured at 593 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA), and a calibration curve was prepared using trolox (Sigma Chemical Co., USA). The antioxidant content of the extract was expressed as mg TE/g dry weight. Additional dilution was needed if the FRAP value measured was over the linear range of the standard.

3.10. Phenolic compound content

3.10.1. Total Polyphenol content (TPC)

The total polyphenol content (TPC) of hydrolysate was determines by the Folin-Ciocalteau colorimetric method according to C.R.N. Gereniu (2017) with slight modification [22]. 0.5 mL of 10 times diluted (v/v) hydrolysate was mixed 2.5 mL of 1:10 (v/v, in deionized water) diluted Folin-Ciocalteu reagent (FCR). After 4 min, 2.5 mL sodium carbonate solution (7.5%, w/v) was added into the mixture. Then, the mixture was vortexed for 5 sec and stored at room temperature in dark room for 2 hours. Blank was also prepared by replacing 0.5 mL of deionized water. The absorbance of mixture was also measured at 765 nm against blank using UV-spectrophotometer (BioTek Instruments, Winooski, VT, USA). The measurements were carried out in triplicate. Gallic acid (Sigma Chemical Co., USA) was used for calibration of standard curve. Result were expressed as mg gallic acid equivalent

per 100 g of dry weight sample (mg GAE/100 g DW). The calibration curve equation for gallic acid was y = 0.0056x + 0.0551 (R^2=0.9994).

3.10.2. Total Flavonoid content (TFC)

Total flavonoid content (TFC) of hydrolysate was estimated using procedures described by C. Chang et al [23]. 125 µL of undiluted hydrolysate was mixed 75 µL of 5 % (w/v) Sodium nitrite solution. The mixture was allowed to stand for 6min and 150 µL of 10 % (w/v) aluminum chloride solution was then added. The mixture was allowed to stand for another 5min and 750 µL of 1 M sodium hydroxide solution and 1400 µL of deionized water were added, accordingly. Subsequently, the mixture was vortexed for 5 sec and its absorbance was determined at 510 nm against blank using UV-spectrophotometer (BioTek Instruments, Winooski, VT, USA). The measurement were carried out in triplicate. Blank was prepared by replacing 125 µL of deionized water. Catechin (Sigma Chemical Co., USA) was used for used for calibration of standard curve. The result were expressed as mg catechin equivalent per 100 g dry weight sample (mg CE/100 g DW). The calibration curve equation for catechin was y = 0.0013x – 0.0584 (R^2=0.9946)

3.11. Protein content

3.11.1. Protein content analysis by Lowy's assay

Soluble proteins were measured using Lowry method [24]. 2 g of sodium hydroxide and 10 g of sodium carbonate were completely dissolved in 400 mL of distilled water, and the final volume was adjusted to 500 mL. 0.1 g of potassium sodium-tartrate and 50 mg of cupric sulfate were added to 8 mL of deionized water and completely dissolved. The final volume was adjusted to 10 mL. The above solutions were mixed in a ratio of 50:1. 0.6 mL of 10 times diluted (v/v) hydrolysate and 3 mL Lowry's solution, and vortex for 5 seconds and store in the darkroom for 20 minutes. Add 0.3 mL of 10 times diluted FC reagent solution and store in a dark room for 35 minutes. Blank was prepared by replacing 0.6mL of deionized water. Measure the absorbance of the reaction solution at 750 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA) ($y = 0.0012x + 0.2083$, $R^2 = 0.9943$) using bovin serum albumin (Sigma Chemical Co., USA). The soluble protein content of the extract was expressed as g bovine serum albumin equivalent per 100 g dry weight (mg BSAE / 100 g DW).

3.11.2. Amino acid content

Total and free amino acid quantification of the recovered mealworm hydrolysates were accimplished with an HPLC (Agilent LC system, Agilent technologies, Inc.,

CA, USA) fitted with a PDA detector. To quantify the free amino acids in the hydrolysate sample, it was mixed with water and directly derived using O-phthaldialdehyde. Sample separations were determined using 5um Capcellpak UG120 C18 column (4.6 x 250 mm). The derivative samples were eluted using a 40mmol/L NaH_2PO_4 (pH 7.8) as solvent A and acetonitrile: methanol: water (45:45:10, v/v/v) as solvent B and a total time of 40 min, at 40˚C, and flow rate was maintained 1.5 mL/min. Each standard amino acid solution (2.5 μmol/mL) was used as an internal standard, while a-amino-*n*-butyric acid (25 μmol/mL) was used as an internal standard for each sample. Total amino acid was determined following the similar method of free amino acids quantification [25].

3.12. Sugar content

3.12.1. Total sugar content

Measurement of total sugar content was carried out using colorimetric method to date for determination of carbohydrate concentration in aqueous solutions (A. Meillisa et al., 2015) with slight modification [26]. 0.5 mL of 10 times diluted hydrolysate was mixed with 0.5 mL of 5% (v/v) phenol solution in a test tube. Subsequently, 2.5 mL of sulfuric acid was added to the mixture. After vortexed for 10 seconds and placed for 20 min in a water bath at 40°C for 20 minutes. Blank was prepared by replacing 0.6 mL of deionized water. The absorbance of mixture

was also measured at 490 nm against blank using UV-spectrophotometer (BioTek Instruments, Winooski, VT, USA). The measurements were carried out in triplicate. D-Glucose (Sigma Chemical Co., USA) was used for calibration of standard curve. Result were expressed as mg glucose equivalent per 100 g of dry weight sample (mg GE/100 g DW). The calibration curve equation for glucose was $y = 0.0061x + 0.1961$ (R^2=0.9996)

3.12.2. Reducing sugar content

The Reducing sugar content was analyzed by the dinitrosalicylic (DNS) colorimetric method used by Saqib AAN. (2011) using D-glucose as a standard [27]. 10 g of dinitrosalicylic acid and 300 g of sodium potassium tartrate (Rochelle salt) were completely dissolved in 800 mL of 0.5 N sodium hydroxide solution, and the final volume was adjusted to 1000 mL with distilled water. 1 mL of undiluted hydrolysate and 4 mL DNS solution, and vortex for 5 seconds and store in boiling water bath for 5 minutes. After which the mixture was cooled to room temperature in a water bath. The absorbance was then measured with a spectrophotometer at 540 nm (BioTek Instruments, Winooski, VT, USA).

3.13. Physical characterization of mealworm hydrolysate

3.13.1. pH

The pH of the hydrolysates were measured using a pH meter (Mettler Toledo Five Easy Plus, Switzerland) at room temperature. The equipment was calibrated using technical buffer solutions of pH 4, 7, 10 prior to the measurements.

3.13.2. Color measurement

The color of the hydrolysates was measured means of reflectance spectra in a UV-spectrophotometer. For measurement, samples were placed in a clear tube. CIE L*, a*, b* color coordinates (considering standard illuminant D65 and observer 10) were then calculated. The color of the hydrolysates was calculated in the CIELab color space using a Minolta CM- 2600d (Minolta Camera Co., Osaka, Japan). The following color coordinates were measured: lightness (L*), redness (a*, ± red – green), and yellowness (b*, ± yellow–blue).

Results and Discussion

1. Proximate composition

The proximate composition of dried mealworm is shown in Table 1. The proximate compositions of mealworm (*Trebrio molitor*) were 1.58±0.07 for moisture, 2.87±0.80 for ash, 35.80±0.18 for lipid, and 42.90±0.52 for carbohydrate. All the data represented in percentage.

Table 1. Proximate composition of dried mealworm

Composition	Percentage (%)
Moisture	1.58±0.07
Ash	2.87±0.80
Lipid	35.80±0.18
Protein	42.90±0.52
Carbohydrate	17.23 ±1.08

1) Values are expressed as mean ± Standard deviation of triplicates.

2. Supercritical carbon dioxide extract yield

Solid-liquid extraction is a separation process involving the transfer of solutes from a solid matrix to a solvent. Solvents were chosen based on solubility characteristics of the desired solute. Ideally to achieve as a pure substance as possible, the solute should have high solubility in the solvent while other components in the solid matrix should not. Cost and safety are always a consideration and indeed, safer and less harmful solvents that are easy to remove,

or recover, are gaining more popularity. There has been much interest in the field of pressurized fluids, especially supercritical fluid extraction (SFE) with CO_2 is gaining more interest. The versatility of pressurized solvents is excellent due to the physicochemical properties of the solvent, including density, diffusivity, viscosity, and dielectric constant, which can be controlled by varying the pressure and temperature of the extraction system. Optimization of the experimental conditions is a critical step in development of a successful SFE method due to the effect of various parameters on the extraction yield. Generally, extraction pressure and temperature is considered as the most important factors. In this study, extraction pressure and temperature was selected as a factors were used to optimize. Table 2 shows the variation of the extraction yields obtained under different experimental conditions. The best condition for extraction of oil and phospholipid is 250 bar/ 45℃.

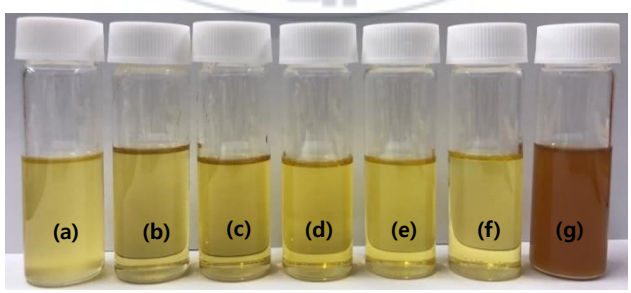


Fig. 3. Extracted mealworm oil by different extraction conditions; 45˚C/150bar(a), 45˚C/200bar(b), 45˚C/250bar(c), 45˚C/300bar(d), 40˚C/250bar(e), 50˚C/150bar(f), Soxhlet/*n*-hexane(g)

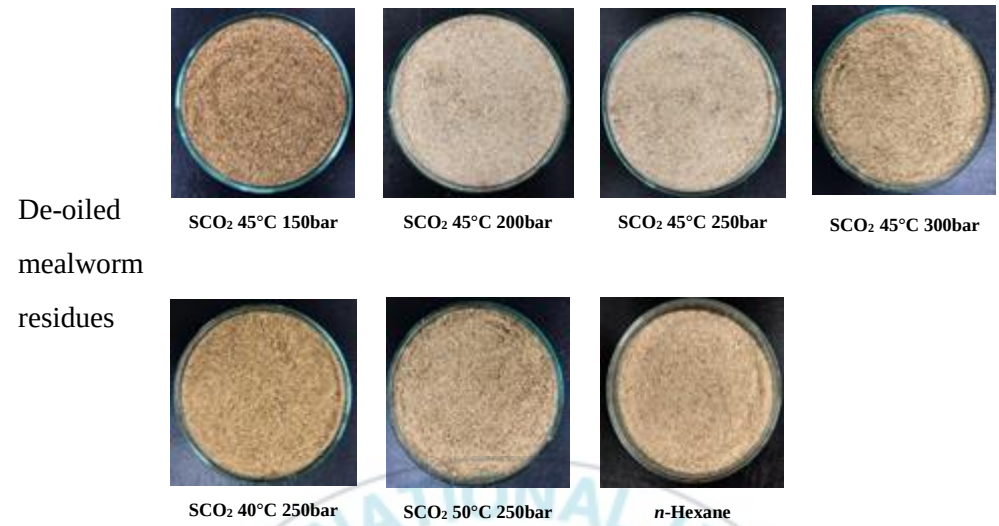


Fig. 4. De-oiled mealworm residue by different conditions.

Table 2. Lipid extraction efficiency and phospholipid content of mealworm powder obtained at different conditions

	Extraction method	Extraction condition		Yield of oil (%)	Phospholipid content (g PCE/ 100 g)
		Pressure (bar)	Temperature (°C)		
Lipid	SCO_2	150	45	5.25±0.05	0.8±0.01
		200	45	28.9±0.52	0.2±0.01
		250	45	34.53±0.64	0.7±0.01
		300	45	27.82±0.12	0.4±0.02
		250	40	16.75±0.09	0.6±0.02
		250	50	20.62±0.15	0.5±0.01
	Soxhlet	N/P	47	32.74±0.75	0.5±0.01
Phospholipid	SCO_2 (EtOH)	250	45	10.12±0.64	86.52±1.42
	Normal	N/P	R/T	10.08±1.03	73.88±0.10

1) Values are expressed as mean ± Standard deviation of triplicates.
2) N.*P*: Normal pressure, *R.T*: Room temperature, *PCE*: Phospatidyl choline equivalent.

3. Characterization of Mealworm Oil

3.1. Acid value and Peroxide value

As you can see in Table 3. Acid value and the Peroxide value of the oil extracted from the mealworm (*Tenebrio molitor*) using supercritical carbon dioxide are relatively low compared with the acid value of the oil extracted with the soxhlet and the result is that the oxidation stability of the oil extracted with supercritical carbon dioxide is high. It appeared. Here also the best condition was 250 bar/ 45℃. There is no significant difference in oxidation stability between hexane extraction and oil extracted with SCO_2 [28].

Table 3. Acid value and Peroxide value of SCO_2 and Soxhlet extracted mealworm lipids and phospholipids

	Extraction method	Extraction condition		Acid value (mg KOH/g)	Peroxide value (meq/kg)
		Pressure (bar)	Temperature (℃)		
Lipid	SCO_2	150	45	6.83±1.02	2.16±0.27
		200	45	6.16±0.52	3.20±0.19
		250	45	0.65±0.03	4.10±0.58
		300	45	4.13±0.12	3.56±0.23
		250	40	6.26±0.05	3.85±0.12
		250	50	6.58±0.06	3.19±0.02
	Soxhlet	N/P	47	7.61±0.56	7.47±0.21
Phospholipid	SCO_2 (EtOH)	250	45	2.92±0.05	1.18±0.02
	Normal	EtOH	R/T	5.61±0.02	4.41±0.18

1) Values are expressed as mean ± Standard deviation of triplicates.
2) N.*P*: Normal pressure, *R.T*: Room temperature.

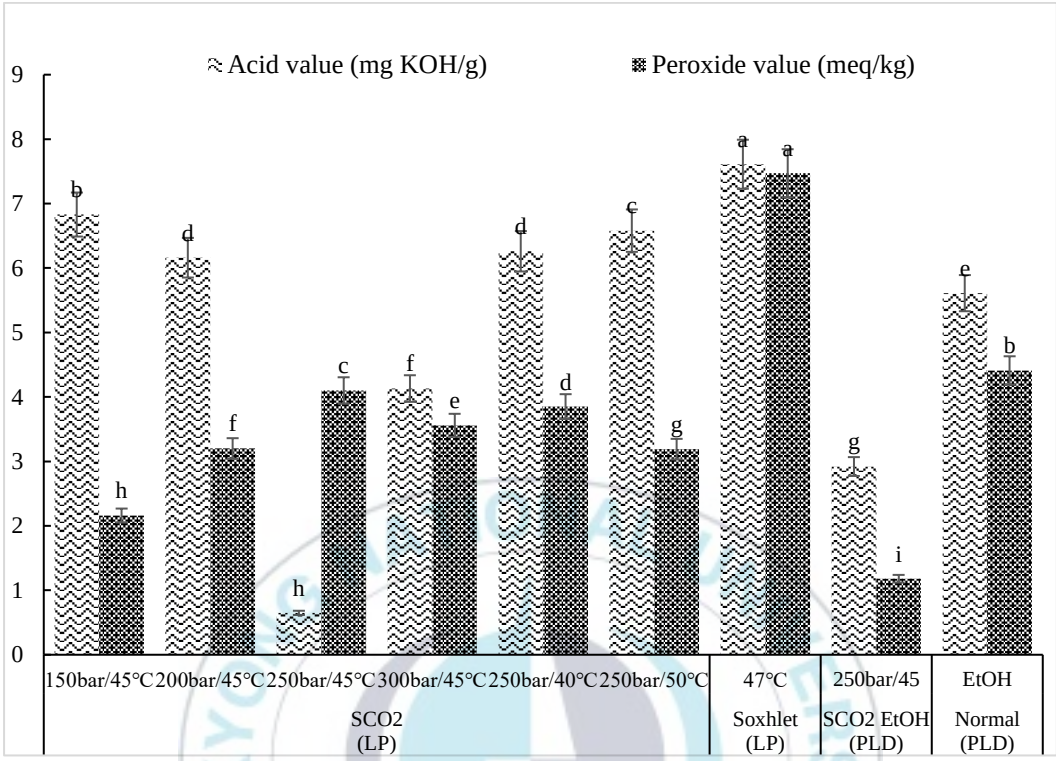


Fig. 5. Acid value and Peroxide value of SCO_2 and Soxhlet extracted mealworm lipids and phospholipids.

3.2. Free fatty acid value and *p*-Anicidine value

The result of free fatty acid value and *p*-anisidine value confirming the existence value of oxidation product in oil through free fatty acid and *p*-anisidine of oil extracted from mealworm. As you can see in Table 4. The value of oil extracted with SCO_2 was lower than that of Soxhlet method. The oils are not significantly different when the main physi-cochemical parameters are considered, but saponification, peroxide and iodine values showed a high concentration of triglycerides in the oil extracted by SFE owing to the higher selectivity of the supercritical solvent. The main differences between oils are related to the free fatty acid concentration and the unsaponifiable fraction, whose values are much lower for carbon-dioxide extracted oil than for hexane-extracted oil [29].

Table 4. Free fatty acid value and *p*-Anisidine value of SCO_2 and Soxhlet extracted mealworm lipids and phospholipids

	Extraction method	Extraction condition		FFA value (g/100g)	*p*-Anisidine value
		Pressure (bar)	Temperature (℃)		
Lipid	SCO_2	150	45	1.76±0.05	1.07±0.05
		200	45	3.14±0.01	0.33±0.01
		250	45	3.42±0.09	0.72±0.01
		300	45	3.13±0.11	1.37±0.08
		250	40	2.12±0.02	0.84±0.06
		250	50	2.92±0.07	1.35±0.04
	Soxhlet	N/P	47	3.71±0.09	4.12±0.21
Phospholipid	SCO_2 (EtOH)	250	45	1.03±0.04	0.75±0.02
	Normal	EtOH	R/T	1.93±0.02	6.15±0.12

1) Values are expressed as mean ± Standard deviation of triplicates.
2) N.*P*: Normal pressure, *R.T*: Room temperature.

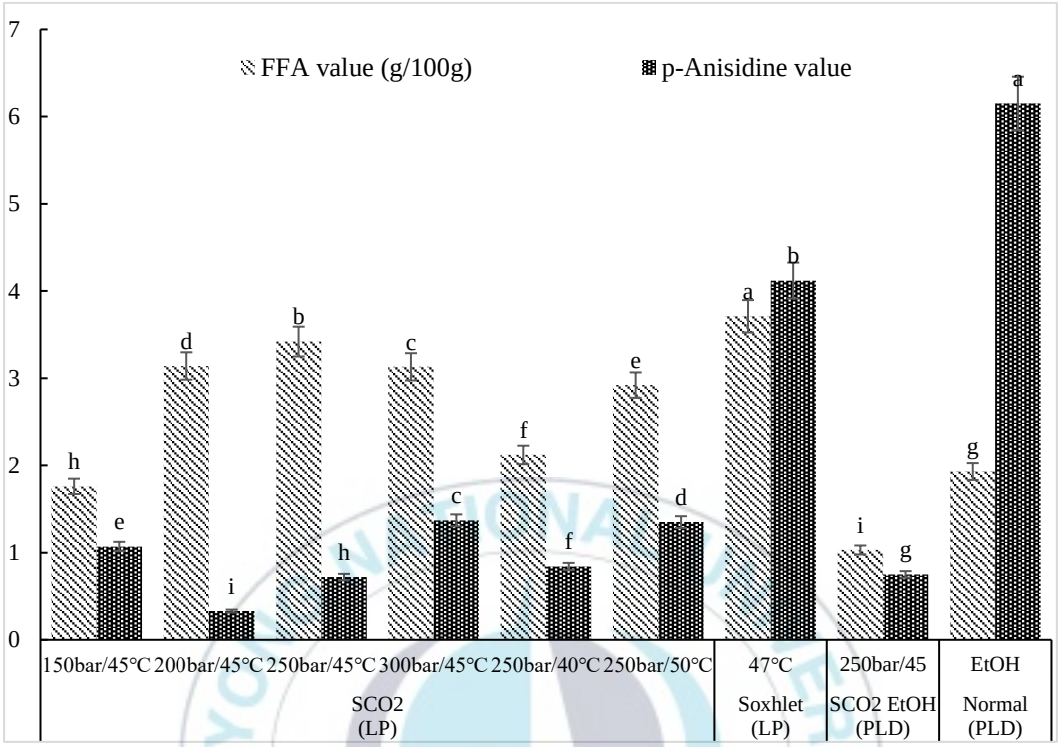


Fig. 6. Free fatty acid value and *p*-Anisidine value of SCO_2 and Soxhlet extracted mealworm lipids and phospholipids.

4. Analysis of fatty acid (GC)

The content of palmitic acid, oleic acid and linoleic acid in the composition of the mealworm (*Tenebrio molitor*) oil was higher. You can see in Table 5. Among the fatty acid composition of mealworm oil, palmitic acid was the most measured at 18.30 ± 0.16% at 45℃ at 20 MPa pressure, and 45.53 ± 2.86% at 45℃ temperature at 30 MPa pressure. Linoleic acid was the highest measured at 28.65 ± 0.04% at 45°C under 20 MPa pressure. Compared with the previously reported results of the fatty acid composition of the mealworm, the fatty acids of the wheat worm were oleic acid (39.71-41.82%), linoleic acid (26.45-29.80%), palmitic acid (17.59-17.77%), stearic acid (3.20%-3.45%). The content of unsaturated and saturated fatty acids is 71.14-73.45% and 23.22-24.71%, respectively, similar to the results of the previously reported in mealworm [30].

Table 5. Fatty acids composition of mealworm lipids extracted by SCO_2 and Soxhlet extraction

Fatty acid	Lipid						
	SCO_2 Extraction						Soxhlet
	15 MPa / 45˚C	20 MPa / 45˚C	25 MPa / 45˚C	30 MPa / 45˚C	25 MPa / 40˚C	25 MPa / 50˚C	*n*-Hexane
Lauric Acid (C12:0)	0.50±0.02^{fghi}	0.21±0.00^{g}	1.50±0.03^{g}	0.32±0.03^{f}	0.32±0.00^{i}	0.33±0.00^{i}	0.3±0.01^{i}
Tridecanoic Acid (C13:0)	0.11±0.01^{ghi}	ND^{g}	ND^{h}	0.82±0.08^{f}	ND^{l}	ND^{l}	ND^{m}
Mystric Acid (C14:0)	4.81±0.19^{d}	2.44±0.01^{f}	2.26±0.04^{f}	3.80±0.25^{de}	3.78±0.22^{e}	3.68±0.01^{e}	3.3±0.00^{e}
Myristoleic Acid (C14:1)	0.31±0.01^{fghi}	ND^{g}	ND^{h}	0.22±0.02^{f}	0.20±0.00^{j}	0.21±0.00^{j}	0.2±0.00^{k}
Pentadaecanoic Acid (C15:0)	0.16±0.01^{ghi}	0.16±0.00^{g}	0.16±0.00^{h}	0.15±0.01^{f}	0.13±0.00^{k}	0.14±0.00^{k}	0.1±0.00^{l}
Palmitic Acid (C16:0)	16.04±0.61^{c}	18.30±0.16^{c}	17.64±0.23^{c}	9.20±6.26^{c}	15.31±0.01^{c}	15.50±0.01^{c}	14.9±0.00^{c}
Palmitoleic Acid (C16:1)	1.41±0.05^{efg}	1.56±0.04^{f}	1.48±0.03^{g}	2.26±0.19^{def}	2.37±0.01^{f}	2.39±0.00^{f}	2.3±0.00^{f}
Heptadecanoic Acid (C17:1)	0.18±0.01^{e}	0.15±0.00^{d}	0.15±0.00^{d}	0.17±0.01^{d}	0.15±0.00^{d}	0.15±0.00^{d}	0.2±0.00^{d}
Stearic Acid (C18:0)	1.50±0.06^{ef}	2.93±0.01^{e}	2.97±0.05^{e}	1.96±0.14^{ef}	1.73±0.01^{g}	1.70±0.00^{g}	2.0±0.00^{g}

1) Values are expressed as mean ± Standard deviation of triplicates.
2) *ND* Not detected.

Table 5. Fatty acids composition of mealworm lipids extracted by SC-CO_2 and Soxhlet extraction (continue)

Fatty acid	Lipid						
	SCO_2 Extraction						Soxhlet
	15 MPa / 45˚C	20 MPa / 45˚C	25 MPa / 45˚C	30 MPa / 45˚C	25 MPa / 40˚C	25 MPa / 50˚C	*n*-Hexane
Oleic Acid (C18:1n9C)	43.02±0.09^{a}	39.57±0.04^{a}	39.67±0.50^{a}	45.53±2.86^{a}	42.61±0.07^{a}	42.50±0.04^{a}	42.6±0.01^{a}
Linolelaidic Acid (C18:2n6t)	0.10±0.01^{ghi}	0.11±0.00^{g}	0.11±0.00^{h}	0.23±0.02^{f}	0.21±0.01^{j}	0.21±0.01^{j}	0.2±0.00^{j}
Linoleic Acid (C18:2n6c)	27.99±1.01^{b}	28.65±0.04^{b}	28.33±0.36^{b}	28.62±1.81^{b}	27.55±0.10^{b}	27.45±0.06^{b}	28.1±0.02^{b}
Arachidic Acid (C20:0)	ND^{i}	0.12±0.00^{g}	0.12±0.00^{h}	ND^{f}	0.09±0.01^{kl}	ND^{l}	ND^{m}
Eicosenoic Acid (C20:1)	ND^{efgh}	0.15±0.00^{g}	0.16±0.00^{hf}	0.14±0.01^{e}	0.12±0.00^{jk}	0.11±0.00^{k}	0.1±0.00^{m}
Linolenic Acid (C18:3n3)	1.36±0.06^{hi}	1.59±0.00^{f}	1.57±0.03^{g}	1.42±0.10^{f}	1.36±0.00^{h}	1.34±0.00^{h}	1.3±0.00^{h}
Heneicosanoic Acid (C21:0)	0.06±0.06^{ghi}	0.05±0.00^{g}	ND^{h}	ND^{f}	ND^{l}	ND^{l}	ND^{m}
Eicosadienoic Acid (C20:2)	0.13±0.02^{hi}	ND^{g}	ND^{h}	0.18±0.01^{f}	ND^{l}	ND^{l}	ND^{m}
Behenic Acid (C22:0)	0.19±0.18^{ghi}	0.22±0.01^{g}	ND^{h}	ND^{f}	ND^{l}	ND^{l}	ND^{m}
Tricosanoic Acid (C23:0)	0.14±0.01^{ghi}	ND^{g}	ND^{h}	ND^{f}	ND^{l}	ND^{l}	ND^{m}

1) Values are expressed as mean ± Standard deviation of triplicates.
2) *ND* Not detected.

Table 5. Fatty acids composition of mealworm lipids extracted by SCO_2 and Soxhlet extraction (continue)

Fatty acid	Lipid						
	SCO_2 Extraction						Soxhlet
	15 MPa / 45˚C	20 MPa / 45˚C	25 MPa / 45˚C	30 MPa / 45˚C	25 MPa / 40˚C	25 MPa / 50˚C	*n*-Hexane
Docosadienoic Acid (C22:2)	0.12±0.01^{i}	ND^{g}	ND^{h}	ND^{f}	ND^{l}	ND^{l}	ND^{m}
Docosahexanoic Acid (C22:6n3)	ND^{i}	ND^{g}	ND^{h}	0.14±0.01^{f}	ND^{l}	ND^{l}	ND^{m}
Total	100	100	100	100	100	100	100

1) Values are expressed as mean ± Standard deviation of triplicates.
2) *ND* Not detected.

Table 6. Fatty acids composition of mealworm Phospholipid extracted by SCO_2 and Soxhlet extraction

Fatty acid	Phospholipid	
	SCO_2 (EtOH)	Solvent extract
Butyric Acid (C4:0)	ND	2.12±0.02
Caproic Acid (C6:0)	ND	0.44±0.13
Lauric Acid (C12:0)	0.15±0.01	3.86±0.24
Tridecanoic Acid (C13:0)	ND	0.44±0.03
Mystric Acid (C14:0)	2.05±0.02	ND
Myristoleic Acid (C14:1)	ND	0.73±0.06
Pentadaecanoic Acid (C15:0)	ND	ND
Palmitic Acid (C16:0)	14.74±0.06	11.56±0.26
Palmitoleic Acid (C16:1)	1.23±0.02	0.51±0.25
Heptadecanoic Acid (C17:0)	5.95±0.03	10.53±0.65
Stearic Acid (C18:0)	3.85±0.01	7.16±0.27
Elaidic Acid (C18:1n9t)	ND	ND
Oleic Acid (C18:1n9C)	35.39±0.12	24.43±1.02
Linolelaidic Acid (C18:2n6t)	0.27±0.01	0.26±0.06
Linoleic Acid (C18:2n6c)	32.81±0.06	36.77±2.03
Arachidic Acid (C20:0)	0.16±0.00	ND
Eicosenoic Acid (C20:1)	0.16±0.00	ND
Linolenic Acid (C18:3n3)	1.22±0.00	0.98±0.23
Heneicosanoic Acid (C21:0)	0.37±0.00	ND
Eicosadienoic Acid (C20:2)	0.15±0.00	0.22±0.05
Behenic Acid (C22:0)	0.25±0.01	ND
Tricosanoic Acid (C23:0)	0.38±0.02	ND
Docosadienoic Acid (C22:2)	0.34±0.01	ND
Eicosapentanoic Acid (C20:5n3)	0.14±0.02	ND
Nervonic Acid (C24:1)	0.17±0.01	ND
Docosahexanoic Acid (C22:6n3)	ND	ND
Total	100	100

1) Values are expressed as mean ± Standard deviation of triplicates.
2) *ND* Not detected.

5. Subcritical water hydrolysis

5.1. Yield of subcritical water hydrolysis (with conventional method)

The hydrolysis efficiency of mealworm (*T. molitor*) at different temperatures after SWE is shown in Table 7. The residual mealworm (*T. molitor*) powder recovered after SWH was weighed [W (g)], Conversion yield of mealworm (*T. molitor*), X, was evaluated from the weight change of mealworm (*T. molitor*), as:

$$X = \frac{Wo - W}{Wo} \times 100 \quad (6)$$

Where, W_0 is the total amount of mealworm (*T. molitor*) introduced in reactor, which is approximately 8g. As you can see in Table 7. It was found that the hydrolysis yield increased with the increase in temperature in the vessel. The highest hydrolysis yield in mealworm (*T. molitor*) was 82.90±19 % after hydrolysis of the mealworm protein with SWE at 210°C after removing the oil with SCO_2. However, the solubility of new substances in water, such as water-soluble proteins except monosaccharides, increases at higher temperature. In addition, hydrolysis yield was increased due to increased hydrolysis rate as the water ionization constant increased at high temperature.

Table 7. Hydrolysis efficiency of mealworm hydrolysates obtained at different SWE conditions

De-oil method	Extraction method	Recovery Condition		Yield of hydrolysate (%)
		Pressure (bar)	Temperature (℃)	
Soxhlet	SWE	30	150	65.62±1.03
	SWE	30	180	70.38±0.59
	SWE	30	210	72.09±2.03
	Water extract	N.P	R.T	19.15±0.56
	Enzyme	N.P	R.T	30.59±1.21
SCO_2	SWE	30	150	67.67±2.03
	SWE	30	180	75.83±1.36
	SWE	30	210	82.90±1.89
	Water extract	N.P	R.T	23.03±0.68
	Enzyme	N.P	R.T	33.93±0.64
Raw	SWE	30	150	63.46±0.96
	SWE	30	180	65.01±0.35
	SWE	30	210	74.10±1.36
	Water extract	N.P	R.T	28.67±0.24
	Enzyme	N.P	R.T	31.40±1.21

1) Values are expressed as mean ± Standard deviation of triplicates.
2) *N.P*: Normal pressure, *R.T*: Room temperature.

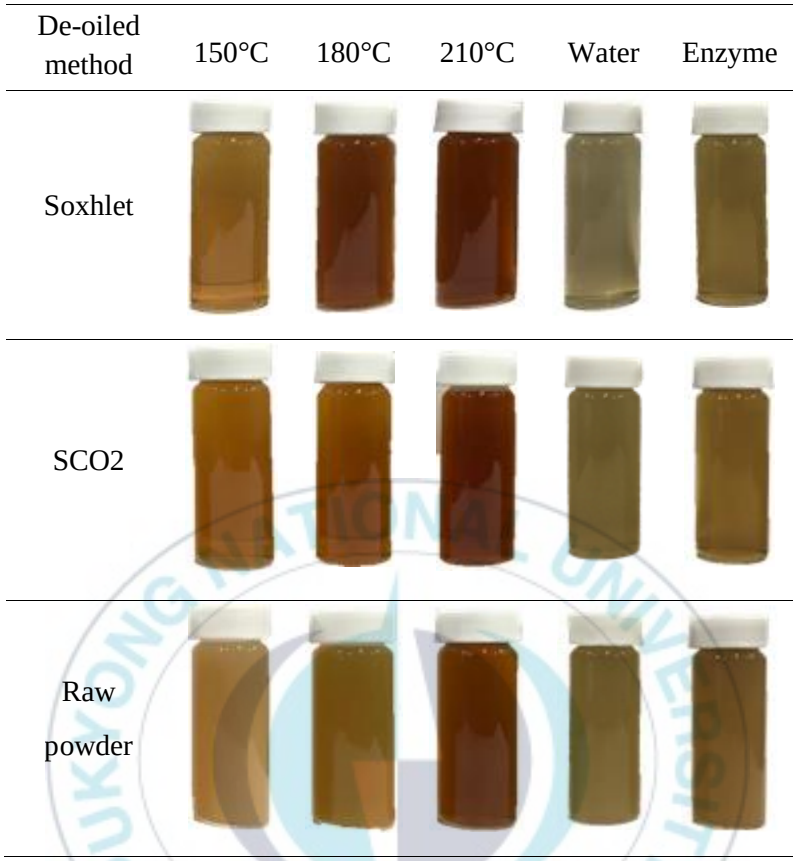


Fig. 7. Mealworm hydrolysates obtained at different hydrolysis conditions.

7. Antioxidant activity

The antioxidant values of hydrolysates extracted from mealworms are represented in Table 8. Antioxidant activity was measured by DPPH, ABTS +, FRAP method, and the results were compared using Trolox as a reference material. As a result of the measurement, subcritical hydrolyzed extracts showed the highest activity at high temperature. Especially, de-oiled with SCO2 showed higher antioxidant. On the other hand, the high antioxidant activity of the 210°C water extracts is likely related to the presence of Maillard reaction compounds produced by thermal degradation of the sample. Moreover, the contribution of other compounds such as caramelization and Maillard reaction products on the antioxidant activity of the SWE extracts cannot be ruled out [31].

Table 8. Antioxidant activity of mealworm hydrolysates obtained at different SWE conditions

De-oil method	Extraction method	Recovery Condition		DPPH (mg TE / g dried mass)	ABTS (mg TE / g dried mass)	FRAP (mg TE / g dried mass)
		Pressure (bar)	Temperature (°C)			
Soxhlet	SWE	30	150	51.40 ± 0.23^{g}	54.28 ± 0.4^{f}	4.38 ± 0.01^{k}
	SWE	30	180	$60.17\pm.0.38^{b}$	89.88 ± 0.07^{b}	8.74 ± 0.06^{c}
	SWE	30	210	62.17 ± 0.69^{a}	94.55 ± 0.07^{a}	9.32 ± 0.05^{b}
	Water extract	N.P	R.T	53.68 ± 0.24^{ef}	26.55 ± 4.73^{k}	4.93 ± 0.01^{j}
	Enzyme	N.P	R.T	54.09 ± 0.31^{def}	24.28 ± 0.20^{j}	5.14 ± 0.02^{i}
SCO_2	SWE	30	150	54.86 ± 0.15^{de}	66.28 ± 0.10^{e}	6.03 ± 0.03^{g}
	SWE	30	180	57.78 ± 0.92^{c}	80.28 ± 0.10^{c}	6.86 ± 0.02^{e}
	SWE	30	210	63.78 ± 1.08^{a}	93.61 ± 0.27^{a}	9.92 ± 0.01^{a}
	Water extract	N.P	R.T	55.37 ± 1.77^{de}	31.75 ± 0.08^{h}	4.88 ± 0.06^{j}
	Enzyme	N.P	R.T	55.78 ± 1.38^{d}	53.21 ± 0.20^{g}	4.91 ± 0.02^{j}
Raw	SWE	30	150	52.48 ± 0.08^{fg}	56.68 ± 0.80^{f}	5.35 ± 0.01^{h}
	SWE	30	180	55.55 ± 1.46^{d}	71.21 ± 0.20^{d}	6.39 ± 0.05^{f}
	SWE	30	210	59.63 ± 0.01^{b}	92.68 ± 0.13^{a}	8.27 ± 0.08^{d}
	Water extract	N.P	R.T	55.40 ± 1.38^{de}	29.48 ± 1.00^{i}	4.10 ± 0.02^{l}
	Enzyme	N.P	R.T	55.07 ± 1.46^{de}	52.01 ± 0.13^{g}	3.48 ± 0.02^{m}

1) Values are expressed as mean ± Standard deviation of triplicates.
2) N.*P*: Normal pressure, *R.T*: Room temperature, *TE*: Trolox Equivalent.

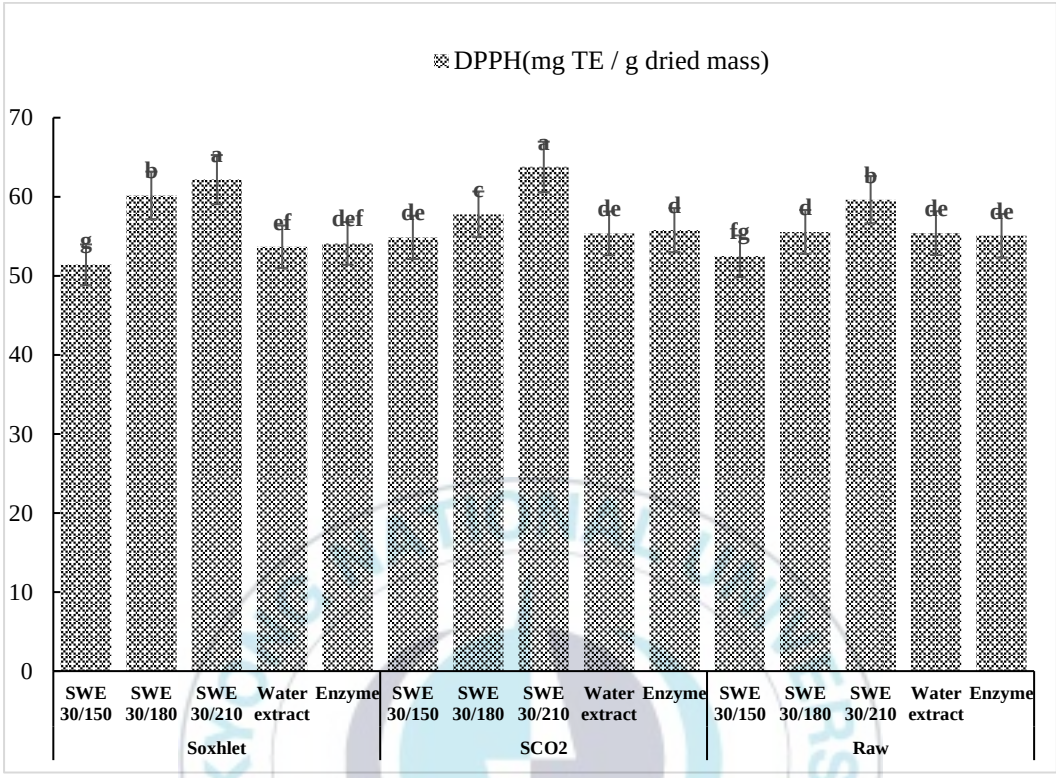


Fig. 8. DPPH of mealworm hydrolysates obtained at different SWE conditions.

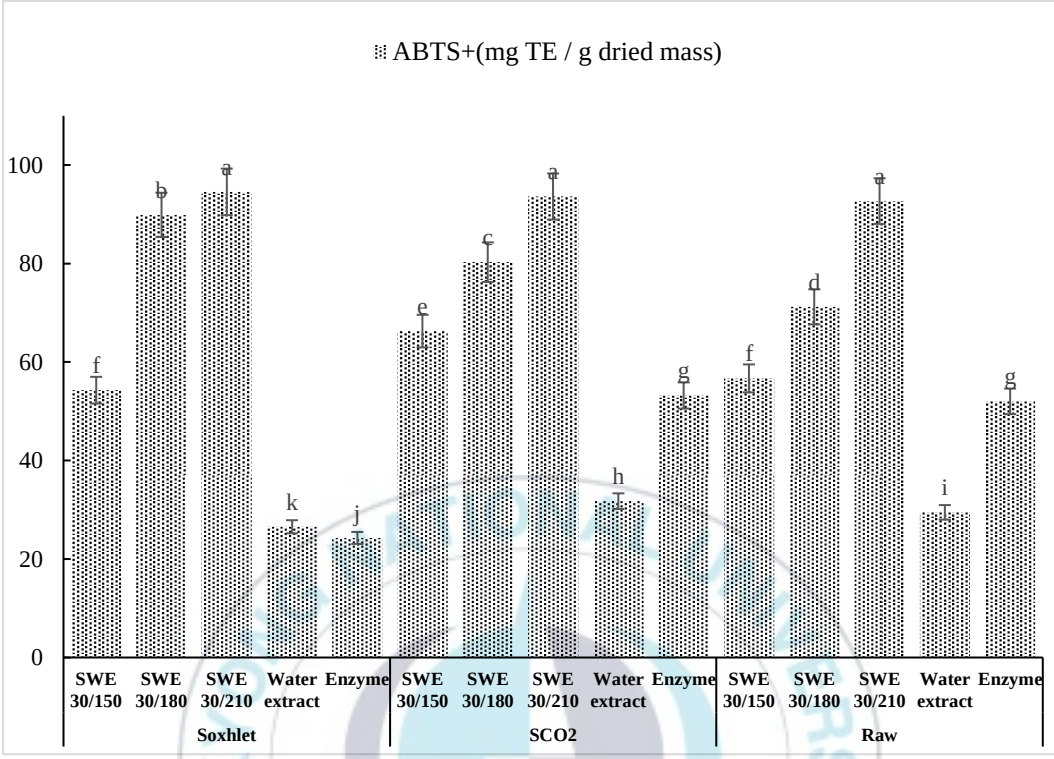


Fig. 9. ABTS^{+} of mealworm hydrolysates obtained at different SWE conditions.

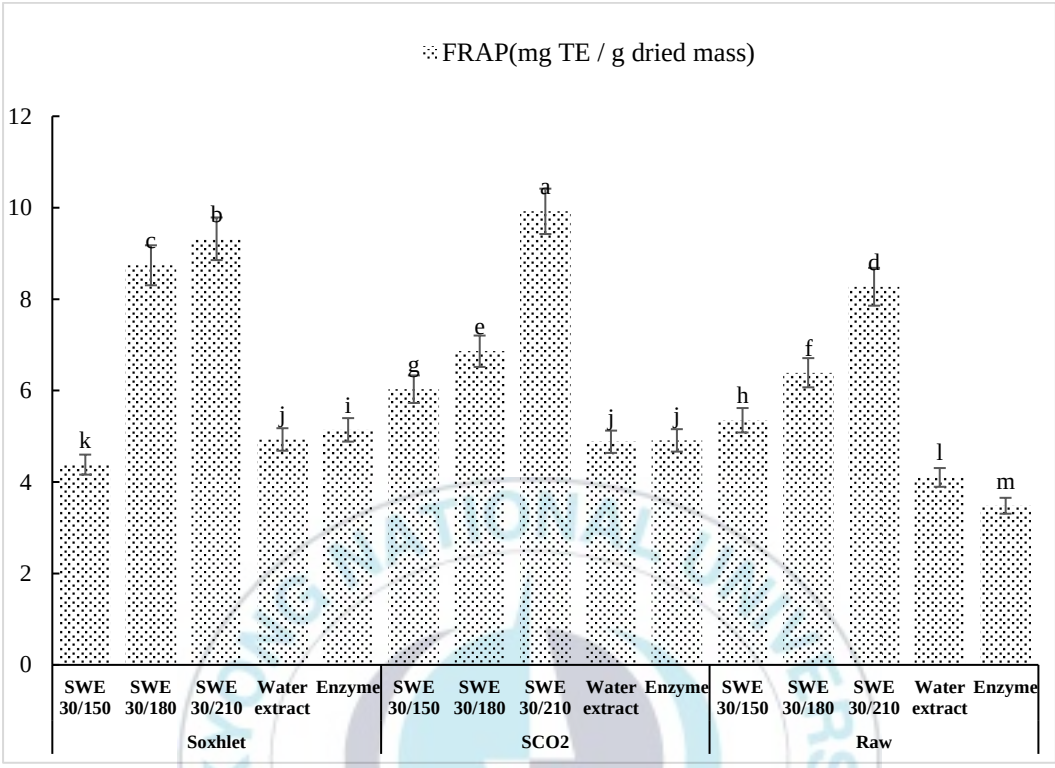


Fig. 10. FRAP of mealworm hydrolysates obtained at different SWE conditions.

8. Phenolic compound contents (TPC+TFC)

In the hydrolysis method using SWE, the results of phenolic and flavonoid contents in the hydrolysates of mealworm powder extracted under various conditions were higher than those of other hydrolysis methods in terms of phenol and flavonoid content as shown in Table 9. below. Phenol and flavonoid contents were higher in the mealworm protein powder removed by SCO_2 and higher in SWE hydrolysis products. The mealworm powder with oil removed with SCO_2 showed the highest content of phenolic compounds. Polyphenol (25.50 ± 0.07 mg / g) and flavonoid (1.63 ± 0.04 mg / g) were measured. 210 ℃/ 30 bar showed higher activity in hydrolyzed products by SWE. In high temperature, the phenolic compounds were produced in higher amount. At a high temperature, phenolic compounds could dissolve in subcritical water as much as they dissolve in the organic solvents [32].

Table 9. Phenolic and flavonoid contents of mealworm hydrolysates obtained at different SWE conditions

De-oil method	Extraction method	Recovery Condition		Polyphenol (mg GE/ g dried mass)	Flavonoid content (mg CE / g dried mass)
		Pressure (bar)	Temperature (℃)		
Soxhlet	SWE	30	150	10.82±0.01^{h}	0.42±0.02^{h}
	SWE	30	180	23.10±0.14^{c}	1.34±0.01^{c}
	SWE	30	210	23.69±0.02^{c}	1.53±0.03^{b}
	Water extract	N.P	R.T	8.48±0.16^{j}	0.42±0.04^{h}
	Enzyme	N.P	R.T	19.26±0.63^{d}	0.80±0.08^{f}
SCO_2	SWE	30	150	15.84±0.12^{i}	1.16±0.05^{d}
	SWE	30	180	19.48±0.13^{d}	0.99±0.02^{e}
	SWE	30	210	25.50±0.07^{a}	1.63±0.04^{a}
	Water extract	N.P	R.T	7.94±0.02^{k}	0.43±0.13^{h}
	Enzyme	N.P	R.T	18.68±0.29^{e}	0.54±0.05^{g}
Raw	SWE	30	150	11.71±0.11^{g}	0.64±0.03^{g}
	SWE	30	180	16.53±0.18^{f}	0.86±0.10^{f}
	SWE	30	210	24.01±0.20^{b}	1.36±0.02^{c}
	Water extract	N.P	R.T	6.12±0.13^{l}	0.58±0.06^{g}
	Enzyme	N.P	R.T	15.96±0.46^{f}	0.68±0.02^{g}

1) Values are expressed as mean ± Standard deviation of triplicates.
2) N.*P*: Normal pressure, *R.T*: Room temperature, *GE*: Gallic acid equivalent, *CE*: Catechin equivalent.

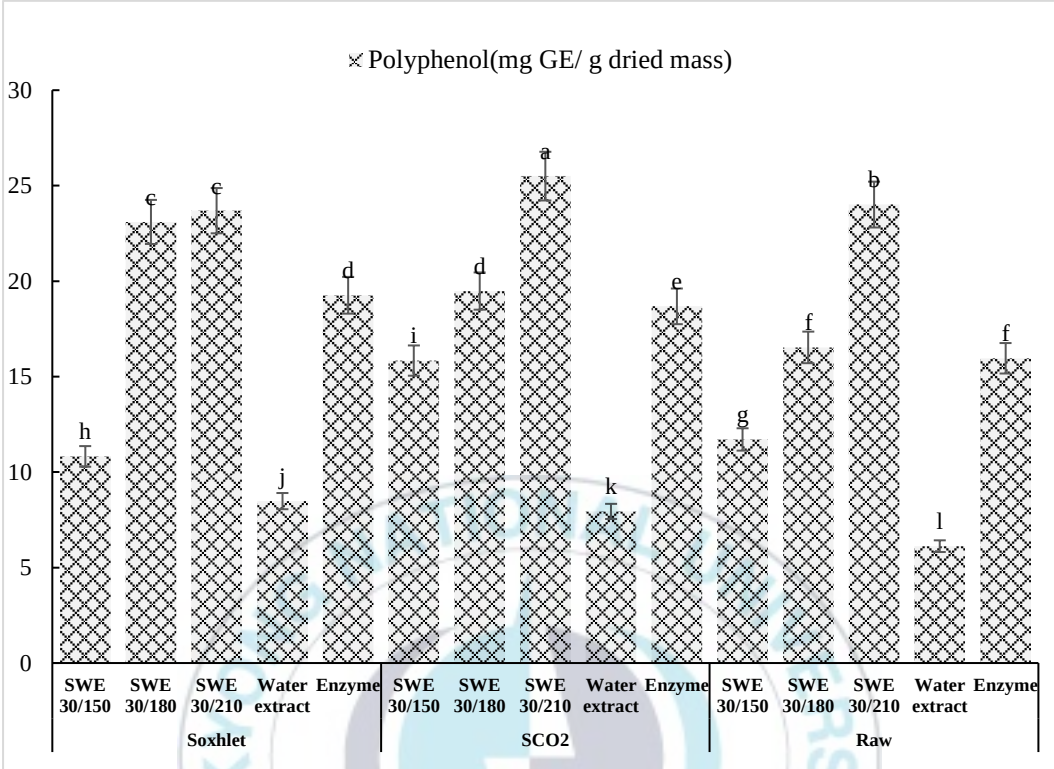


Fig. 11. Polyphenol contents of mealworm hydrolysates obtained at different SWE conditions.

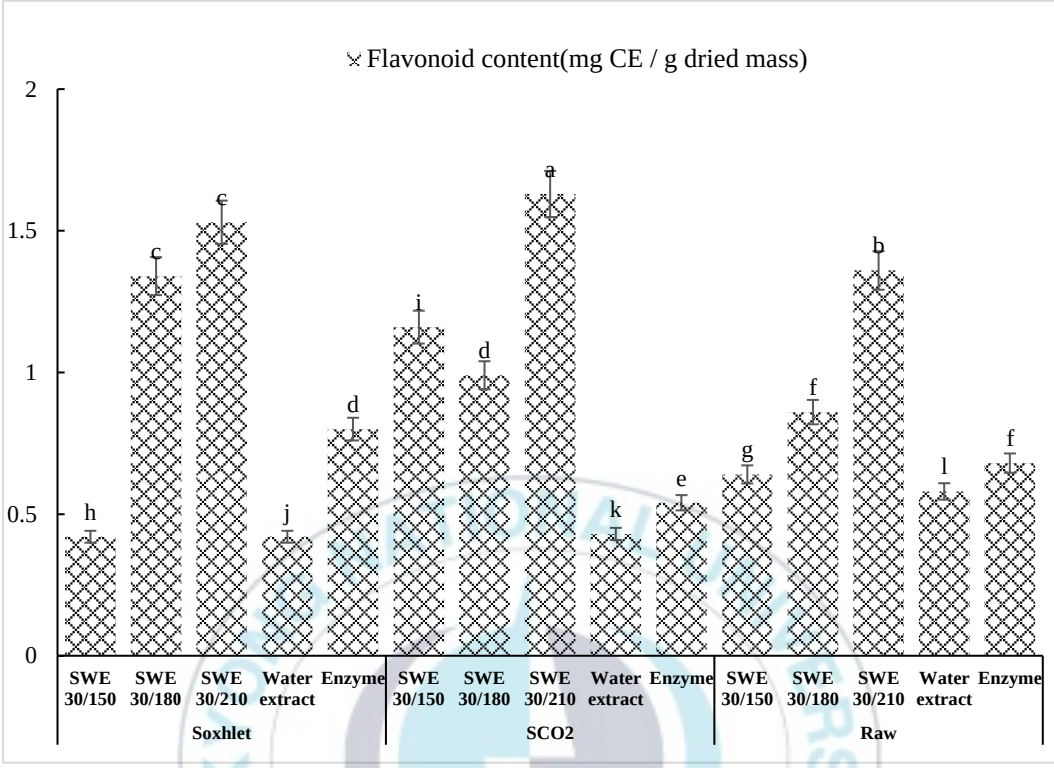


Fig. 12. Flavonoid contents of mealworm hydrolysates obtained at different SWE conditions.

9. Protein content

9.1. Lowry's assay

The water-soluble protein contents of the hydrolysates of the mealworms extracted under the various conditions is shown in Table 10. As you can see in Fig.13, The protein content of the hydrolysate gradually increased as the subcritical temperature increased. The highest content of hydrolysates extracted at high temperature of 210 ˚C was the highest in the hydrolysates by Subcritical hydrolysis. Generally, the increase in protein with increasing temperature in pressurized SWE is due to a change in polarity of water in the subcritical region [33]. This was in agreement with previous work [34] which reported that protein increased with increasing temperature up to 210 °C for rice bran by SWE. Hydrolysates of mealworm protein powder hydrolyzed with oil removed by SCO_2 showed a higher water soluble protein content than mealworm powder that did not remove oil and with oil removed by soxhlet.

Table 10. Protein content of mealworm hydrolysate obtained at different SWE conditions

De-oil method	Extraction method	Recovery Condition		Protein (mg BE / g dried mass)
		Pressure (bar)	Temperature (°C)	
Soxhlet	SWE	30	150	207.57±3.00^{f}
	SWE	30	180	282.57±11.00^{d}
	SWE	30	210	344.57±3.00^{a}
	Water extract	N.P	R.T	80.57±2.33^{h}
	Enzyme	N.P	R.T	236.57±3.33^{e}
SCO_2	SWE	30	150	290.57±2.00^{cd}
	SWE	30	180	325.23±0.67^{ab}
	SWE	30	210	341.57±8.33^{bc}
	Water extract	N.P	R.T	70.79±0.84^{h}
	Enzyme	N.P	R.T	215.23±1.33^{ef}
Raw	SWE	30	150	238.57±4.00^{e}
	SWE	30	180	275.90±1.00^{d}
	SWE	30	210	334.07±1.83^{a}
	Water extract	N.P	R.T	37.79±0.51^{i}
	Enzyme	N.P	R.T	179.23±2.00^{g}

1) Values are expressed as mean ± Standard deviation of triplicates.
2) N.*P*: Normal pressure, *R.T*: Room temperature, *BE:* Bovin serum equivalent.

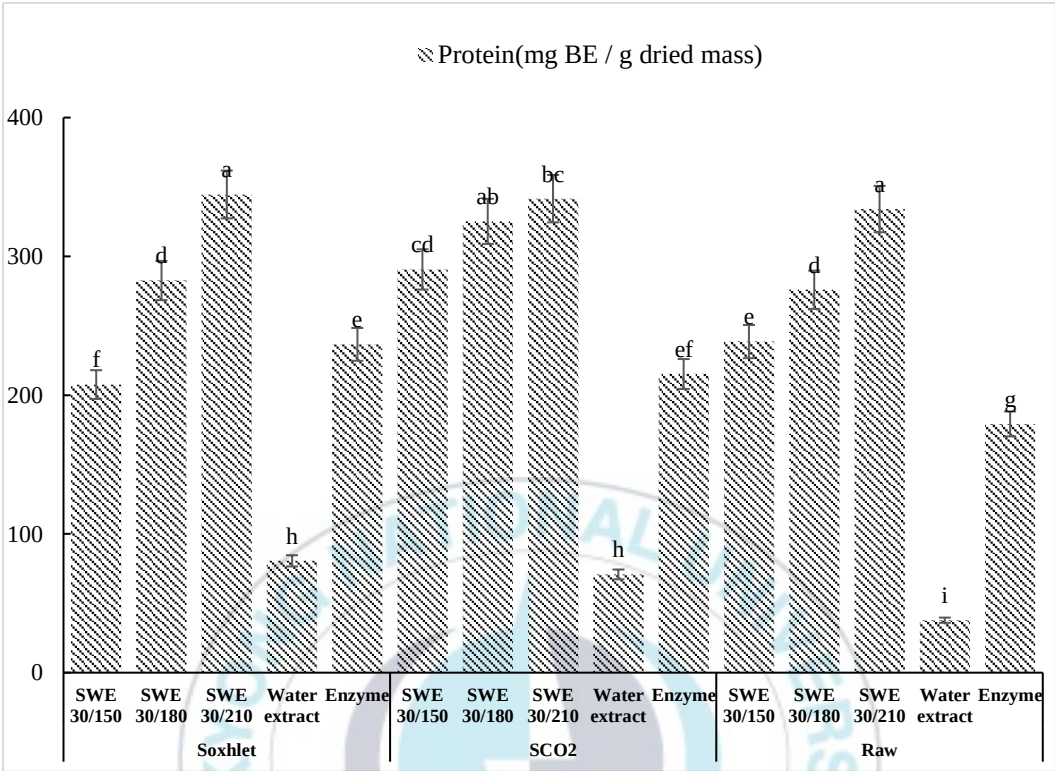


Fig. 13. Protein content of mealworm hydrolysate obtained at different SWE conditions.

9.2. Amino acid content

Total Amino Acids and Free Amino Acids increased the amount of amino acids as the SWE hydrolysis temperature increased. As you can see in Table 11. In the de-oil method, total amino acid (858.65 mg / 100g) and free amino acid (167.76 mg / 100g) were found in the mealworm protein powder from which the oil was removed by SCO_2, and Total amino acid (375.95 mg / 100g) and free amino acid (6.40mg / 100g) were found in the mealworm protein powder without oil removal. In the hydrolysis method, especially the enzymatic hydrolysates more amino acids due to the added enzyme. The amino acid content of the mealworm hydrolysate was different depending on the type of mealworm oil removed. Moreover hydrolysates of mealworms with oil removed using SCO_2 had more amino acids measured at higher temperatures. In particular, the hydrolysates of mealworm using enzymes showed the highest amino acid measurement because of the protein component of the enzyme.

Table 11. Total amino acids profile of mealworm hydrolysate samples on different conditions

	Conditions (De-oil method / °C)						
	SCO_2 /150°C	SCO_2 /180°C	SCO_2 /210°C	Soxhlet /150°C	Raw /150°C	SCO_2 /Water Extraction	SCO_2 /Enzyme
Essential amino acids (EAA)							
Histidine	47.21	55.89	51.18	35.93	46.58	13.74	39.62
Isoleucine	44.50	52.17	55.81	38.96	16.52	20.61	131.79
Leucine	70.92	86.06	105.05	67.16	25.45	24.04	137.40
Lysine	59.06	60.95	49.84	41.33	27.04	14.08	54.05
Methionine	10.46	12.69	16.86	12.65	5.71	1.99	9.03
Phenylalanine	34.84	42.87	52.72	33.78	17.95	5.35	26.67
Threonine	41.40	46.97	27.82	34.05	17.76	12.07	79.77
Valine	65.12	79.89	92.96	58.37	20.90	32.15	135.88
Total	373.51	437.48	452.23	322.23	177.92	124.01	614.21
Non-essential amino acids (NEAA)							
Arginine	73.90	77.38	58.36	24.56	70.82	2.41	17.82
Aspartic acid	89.40	99.32	47.27	37.36	60.47	18.85	93.51
Serine	42.91	51.42	36.90	30.02	17.85	12.07	64.61
Glutamic acid	154.37	169.08	203.77	212.18	138.96	55.67	186.68
Proline	163.09	176.82	148.72	97.94	17.79	128.76	178.34
Gycine	52.56	67.88	109.87	45.81	23.42	15.34	69.81
Alanine	69.69	90.38	154.39	65.74	22.48	33.68	156.03
Tyrosine	64.36	77.61	96.54	67.14	21.93	15.10	99.95
Cystine	1.19	0.13	2.84	1.50	2.24	0.73	1.23
Total	711.49	810.00	858.65	582.24	375.95	282.61	867.97

Values are expressed as mean ± SD and in mg/100g. N.D, not detected.

Table 12. Free amino acids profile of mealworm hydrolysate samples on different conditions

	Conditions (De-oil method / SWE °C)						
	SCO_2 /150°C	SCO_2 /180°C	SCO_2 /210°C	Soxhlet /150°C	Raw /150°C	SCO_2 /Water Extraction	SCO_2 /Enzyme
Essential amino acids (EAA)							
Histidine	9.03	9.81	6.62	7.51	N. D.	N. D.	N. D.
Isoleucine	6.24	6.90	6.74	10.61	0.86	12.27	90.00
Leucine	6.96	8.76	13.30	18.97	N. D.	12.75	71.20
Lysine	6.35	6.28	0.11	N. D.	N. D.	N. D.	N. D.
Methionine	N. D.	N. D.	3.00	4.71	N. D.	N. D.	N. D.
Phenylalanine	4.10	5.83	9.21	10.24	4.88	N. D.	N. D.
Threonine	2.09	3.09	2.35	8.45	N. D.	4.15	40.76
Valine	12.00	15.57	14.25	16.64	N. D.	16.43	68.49
Total	46.76	56.23	55.58	77.13	5.74	45.59	270.45
Non-essential amino acids (NEAA)							
Arginine	24.32	22.73	13.62	N. D.	40.25	N. D.	3.66
Aspartic acid	3.61	13.34	11.43	1.34	6.27	3.57	29.20
Serine	2.64	5.05	8.51	1.49	N. D.	4.35	28.43
Glutamic acid	9.94	2.34	1.18	8.56	52.16	24.71	58.91
Proline	90.03	97.16	62.75	47.92	N. D.	82.21	91.68
Gycine	3.99	7.08	22.63	7.13	N. D.	4.24	20.45
Alanine	13.53	18.83	44.14	21.35	1.97	17.94	74.93
Tyrosine	14.68	16.06	17.12	26.79	N. D.	5.72	39.45
Total	138.41	159.87	167.76	114.58	60.40	142.74	343.04

Values are expressed as mean ± SD and in mg/100g. N.D, not detected.

10. Sugar content

The sugar content of the hydrolysates extracted from mealworms is described in Table 11. The content of polysaccharides decreased and the amount of reducing sugar monosaccharides increased with increasing temperature. Especially, the sugar degradation rate of subcritical hydrolysates was higher than general water extraction and enzyme hydrolysates of mealworm. The higher the temperature at the SWE extraction, the more reducing sugar production and the lowering of the glycogen content of polysaccharide as shown in Fig.14. As shown in Fig.15, the higher the hydrolysis temperature, the more the degradation into monosaccharide. These results may be attributed to the increase of ionization constant (also called dissociation constant or ion product constant) of water (KW) at elevated temperature, namely the concentrations of hydronium and hydroxide ions increase so as to break glycosidic bonds in glycogen. Glycogen can be hydrolyzed in pure water with attack by electrophilic hydrogen atoms on the glycosidic bonds, but this is a very slow reaction at ambient temperature and pressure. The rate of hydrolysis can be increased by the use of elevated temperatures and pressures or by acid catalysis. The acid hydrolysis of cellulose proceeds via the protonation of the polysaccharide, which slowly breaks down to give a cyclic carbenium ion. After rapid addition of water, free sugars (glucose) are liberated [35] [36].

Table 13. Glycogen and reducing sugar content of mealworm hydrolysate obtained at different SWE conditions

De-oil method	Extraction method	Recovery Condition		Glycogen (mg GE / g dried mass)	Reducing Sugar (mg GE / g dried mass)
		Pressure (bar)	Temperature (°C)		
Soxhlet	SWE	30	150	40.08±0.89^{f}	7.29±0.28^{j}
	SWE	30	180	72.20±1.09^{b}	19.82±0.80^{d}
	SWE	30	210	47.01±0.89^{d}	25.78±1.28^{b}
	Water extract	N.P	R.T	32.98±0.20^{h}	16.32±0.48^{f}
	Enzyme	N.P	R.T	40.84±0.04^{ef}	14.03±0.61^{gh}
SCO_2	SWE	30	150	75.36±0.85^{a}	13.13±0.32^{h}
	SWE	30	180	74.06±0.20^{a}	13.62±0.43^{h}
	SWE	30	210	41.26±0.12^{e}	24.58±1.41^{c}
	Water extract	N.P	R.T	29.45±0.05^{i}	15.03±0.01^{g}
	Enzyme	N.P	R.T	36.32±0.81^{g}	14.03±0.53^{h}
Raw	SWE	30	150	33.11±0.40^{h}	18.78±0.01^{a}
	SWE	30	180	53.36±0.41^{c}	17.64±0.56^{e}
	SWE	30	210	19.79±0.27^{k}	27.53±1.03^{de}
	Water extract	N.P	R.T	12.13±0.06^{l}	13.42±0.08^{h}
	Enzyme	N.P	R.T	22.79±0.81^{j}	11.93±0.37^{i}

1) Values are expressed as mean ± Standard deviation of triplicates.
2) N.*P*: Normal pressure, *R.T*: Room temperature, *GE*: Glucose equivalent.

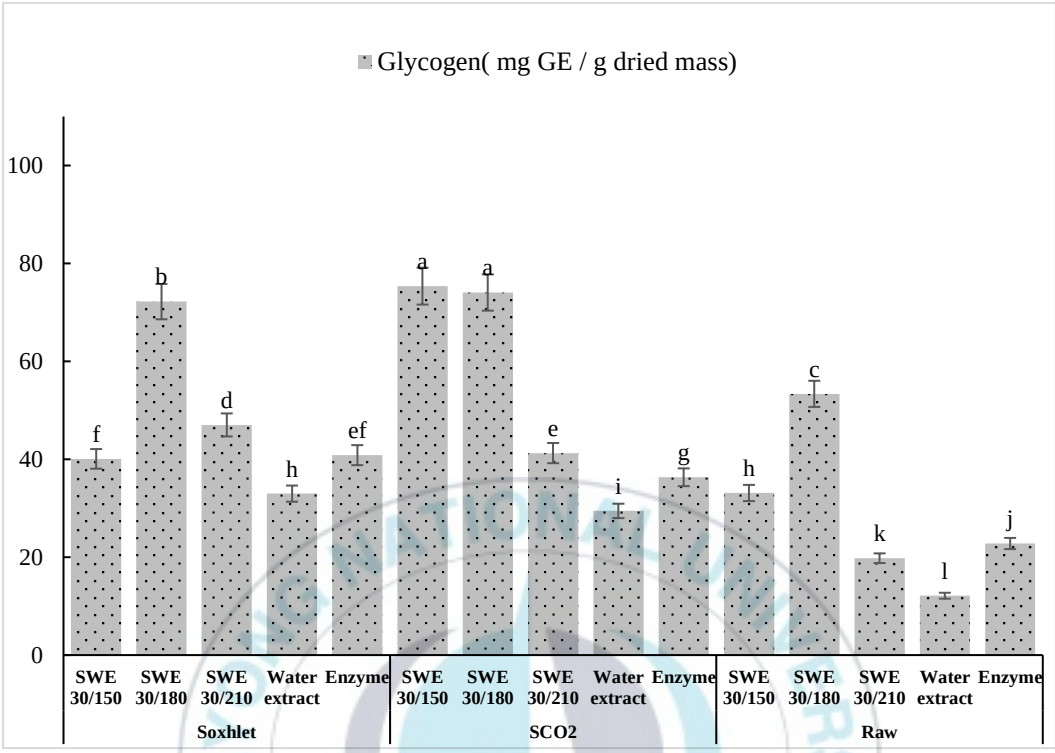


Fig. 14. Glycogen content of mealworm hydrolysate obtained at different SWE conditions.

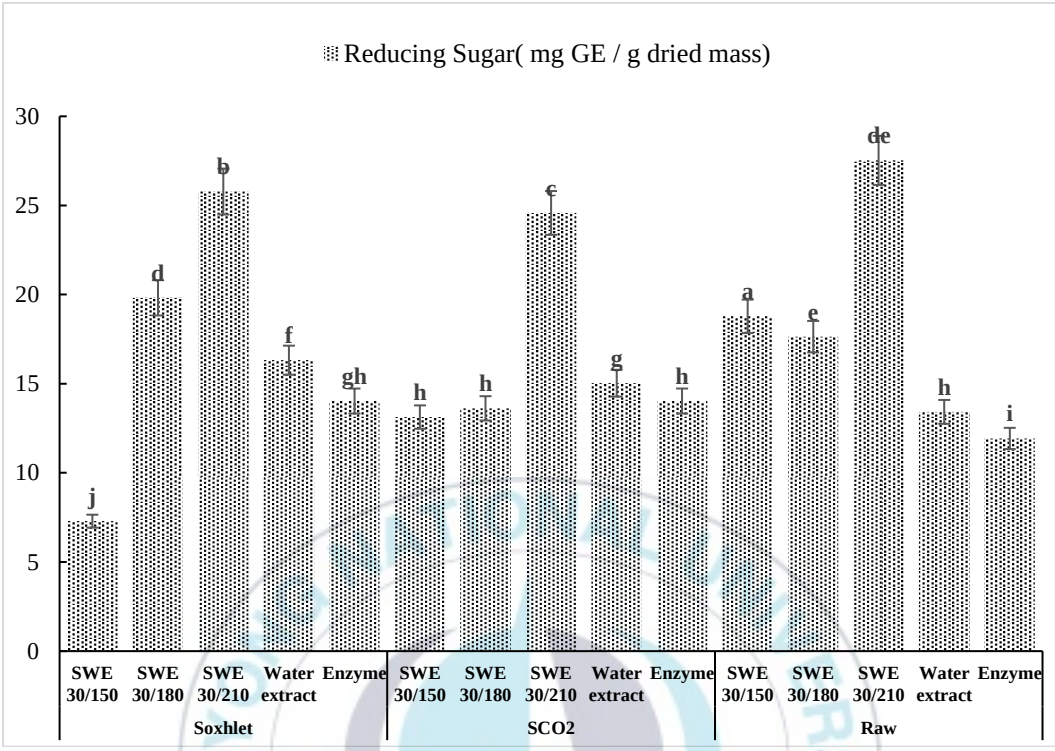


Fig. 15. Reducing Sugar content of mealworm hydrolysate obtained at different SWE conditions.

11. Physical characterization

11.1. pH

pH of the hydrolysates were measured using a pH meter (Mettler Toledo Five Easy Plus, Switzerland) at room temperature. The equipment was calibrated using technical buffer solutions of pH 4, 7, 10 prior to the measurements. As shown in Table 14. The pH of the hydrolysates extracted from various conditions was as high as pH 6-7 of hydrolysates using SWE as a whole. The hydrolysates with general water extraction and enzymes were at pH 5-6. The low pH at higher temperatures was due to break down of sugars to organic acids followed by chain reactions of these acids creating the acidity that enhanced the rate of subsequent reactions as an auto catalyst process.[37] However, the constant increase in pH with increasing temperature could be due to decomposition of acidic compounds to other substances. Moreover, it may also be attributed to the formation of salts and degradation of organic matter and most probably to the presence of acidic materials such as phenolic compounds.[38]

Table 14. pH of mealworm hydrolysates obtained at different SWE conditions

De-oil method	Extraction method	Recovery Condition		pH
		Pressure (bar)	Temperature (°C)	
Soxhlet	SWE	30	150	6.46±0.00^{de}
	SWE	30	180	6.66±0.02^{b}
	SWE	30	210	6.89±0.02^{a}
	Water extract	N.P	R.T	5.74±0.01^{h}
	Enzyme	N.P	R.T	5.65±0.02^{i}
SCO_2	SWE	30	150	6.44±0.02^{e}
	SWE	30	180	6.68±0.00^{b}
	SWE	30	210	6.89±0.00^{a}
	Water extract	N.P	R.T	6.05±0.01^{g}
	Enzyme	N.P	R.T	5.66±0.01^{i}
Raw	SWE	30	150	6.53±0.02^{c}
	SWE	30	180	6.49±0.01^{d}
	SWE	30	210	6.88±0.00^{a}
	Water extract	N.P	R.T	6.10±0.01^{f}
	Enzyme	N.P	R.T	5.28±0.01^{j}

1) Values are expressed as mean ± Standard deviation of triplicates.
2) N.*P*: Normal pressure, *R.T*: Room temperature.

11.2. Color

The difference in the lightness (L*), redness (a*), and yellowness (b*) over hydrolysates is shown in Fig. 7, and Table 13. As shown in Table15. The hydrolysates degraded under various conditions showed a tendency to decrease L *, increase a *, and decrease b *. The hydrolysates extracted by the general extraction method had higher values of L * and b * and lower values of a * than SWE hydrolysate. The SWE hydrolysate tended to have lower L * and b * values and higher a * values at higher temperatures. Browning at pH 7.5. The complexity of nonenzymatic browning reactions is known to be at least partly due to the sugar caramelization processes [39]. It therefore seemed to be of interest to determine the contribution of caramelization and that resulting from the interaction between amino acids and reducing sugars to the overall nonenzymatic browning [40].

Table 15. Color properties of mealworm hydrolysates obtained at different SWE conditions

De-oil method	Extraction method	Recovery Condition		L*	a*	b*
		Pressure (bar)	Temperature (℃)			
Soxhlet	SWE	30	150	44.56±0.31^{b}	5.16±0.02^{f}	35.50±0.24^{a}
	SWE	30	180	18.22±0.89^{h}	19.04±0.81^{b}	24.07±1.04^{c}
	SWE	30	210	11.03±2.24^{i}	12.37±2.16^{e}	11.30±1.89^{g}
	Water extract	N.P	R.T	46.44±0.82^{a}	-0.13±0.01^{i}	13.67±0.66^{f}
	Enzyme	N.P	R.T	39.28±1.32^{c}	2.42±0.06^{h}	22.58±0.33^{cd}
SCO_2	SWE	30	150	29.48±0.27^{f}	14.05±0.03^{d}	36.00±0.50^{a}
	SWE	30	180	26.01±0.69^{g}	16.35±0.46^{c}	28.29±1.94^{b}
	SWE	30	210	9.85±0.20^{i}	21.09±0.33^{a}	14.25±0.29^{f}
	Water extract	N.P	R.T	36.12±0.18^{de}	2.81±0.05^{gh}	22.02±0.79^{d}
	Enzyme	N.P	R.T	39.18±1.61^{c}	2.42±0.06^{h}	22.58±0.33^{cd}
Raw	SWE	30	150	43.36±1.11^{b}	-0.14±0.04^{i}	11.46±0.44^{g}
	SWE	30	180	28.84±0.69^{f}	3.03±0.20^{gh}	13.56±0.72^{f}
	SWE	30	210	25.00±0.51^{g}	3.67±0.05^{g}	14.15±0.72^{f}
	Water extract	N.P	R.T	37.40±0.38^{d}	-0.20±0.06^{i}	13.92±0.75^{f}
	Enzyme	N.P	R.T	34.85±0.34^{e}	2.63±0.10^{gh}	19.44±0.81^{e}

1) Values are expressed as mean ± Standard deviation of triplicates.
2) N.*P*: Normal pressure, *R.T*: Room temperature.

Conclusion

The lipids of mealworms was carried out by supercritical carbon dioxide at temperatures between 40 and 50°C and pressure ranges between 150 and 300 bar. Extraction of lipids showed the highest lipid mass extracted at 45°C and 250 bar (34.53±0.64 %). Analysis of the fatty acids of the oils extracted with supercritical carbon dioxide revealed that the contents of oleic acid, linoleic acid and palmitic acid were high. Oxidation products such as acid value and peroxide value measured to estimate oxidative stability of extracted lipid of mealworm, and the results showed that the oil of the mealworm extracted with SCO_2 was much higher than the oil extracted with organic solvent. Phospholipids was extracted from mealworm protein powder with lipid removal under the highest lipid extraction conditions (SCO_2, 250bar / 45℃) using ethanol as a co-solvent. Sub-critical hydrolysis was performed with the de-oiled mealworm protein by SCO_2, and carried out at a stirring speed of 200 rpm under 50 bar and temperature range of 150 to 200°C. For comparative experiments, enzyme hydrolysis and normal water extraction were performed at ambient temperature. The hydrolysate obtained different conditions were characterized with antioxidant activity, phenolic compound and flavonoid content, water-soluble protein content, sugar content, pH and color. As a result, the antioxidant activity, phenolic compound and flavonoid

content of the subcritical water hydrolysate (SWH) showed high value than enzyme hydrolysate (EH) and water extract (WH). At 210˚C, SWH showed highest antioxidant activity, phenolic compound, flavonoid, water-soluble protein, and reducing sugar content, but total sugar was decreased above 180˚C. This result verified subcritical water hydrolysis could be decompose high molecular compounds.

아임계 및 초임계 유체를 이용한 갈색거저리(*Tenebrio molitor*)로부터 회수된 추출물의 특성에 관한 연구

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요약

밀웜은 거저리과(***Tenebrio molitor***)의 곡물거저리속이며, 갈색거저리라고 한다. 전세계적으로 대량으로 양식되어 식품 또는 사료 이용의 목적으로 사용되거나 애완곤충으로 판매되고 있다. 밀웜은 지방 및 단백질의 함량이 높고 특히 지방중에서도 불포화지방산인 Palmitic Acid (C16), Oleic Acid (C18), Linoleic Acid (C18) 이 주로 이루고 있어 식품산업에서 다양하게 이용될 가능성이 높다. 밀웜은 유기용매 및 물리 화학적인 방법으로 지방과 단백질을 추출할 수 있지만 최근은 안전하고 환경친화적으로 분리할 수 있는 공정을 선호하고 있다. 이런 점에서 초임계 이산화탄소 (SCO_2) 추출법과 아임계수 가수분해 공정은 밀웜의 오일을 추출하고 잔류물을 이용하여 가수분해물을 얻을 수 있는 가장 훌륭한 방법이다. 본 연구의 목적은 초임계 이산화탄소 (SCO2)를 이용하여 압력과 온도에 따른 조건(40~50℃ / 15~30 MPa)을 제시하여 비극성 오일을 추출하고 각 조건에 따른 추출 곡선을 비교하여 최적 수율 조건

을 확인하고, SCO_2 추출법과 hexane을 이용한 유기용매 추출법으로 추출된 오일의 성분과 안정성을 비교하는 것이다. 오일의 최대 추출 수율은 온도 45℃, 압력 25MPa에서 최대 추출 수율을 나타내었다. 오일의 산화안정성은 산가, 과산화물가, *p*-Anicidine, 유리지방산으로 확인하였다. 산가, 과산화물가, *p*-Anisidine 및 유리지방산은 유기용매로 추출된 지질보다 SCO_2 추출 지질이 더 낮은 값의 결과를 나타내어 SCO2를 이용하여 추출한 오일이 유기용매를 이용하여 추출한 오일보다 높은 산화 안정성을 나타냈다. 특히 25 MPa / 45℃에서 추출되 오일의 산화안정성이 가장 높게 나타났으며 오일의 지방산 분석을 위해 Gas chromatography (GC)를 이용하였고 그 결과 SCO_2를 이용하여 추출한 오일과 유기용매를 이용하여 추출한 오일을 비교 하였을때 SCO2 추출 조건에서 30 MPa / 45℃ 에서 추출한 오일의 불포화지방산 (Palmitic Acid, Oleic Acid, Linoleic Acid) 이 평균적으로 높게 나타났다. 초임계 이산화탄소 (SCO_2) 추출법은 이산화탄소뿐만이 아니라 극성보조용매를 사용하여 극성 물질을 추출할 수 있다. 에탄올을 보조 용매로 이용하여 초임계 이산화탄소만으로 추출할 수 없는 극성의 물질을 추출하였다. 비극성인 오일이 제거된 밀웜 단백분에서 에탄올을 보조 용매로 추출을 진행하여 극성인 인지질을 추출하였고 인지질 (phospholipid)을 high performance liquid chromatography (HPLC)를 이용하여 정성 분석하였다. 지질이 완전히 제거된 밀웜단백분은 아임계수를 이용하여 가수분해물을 얻은 뒤 가수분해물의 특성을 확인하였다. 가수분해를 진행한 단백분은 초임계이산화탄소와 Shoxhlet으로 지질이 제거된 밀웜 단백분과 지질이 제거되지 않은 밀웜 분말을 사용하여 시료 비교를 하였다.

단백질을 주로 이루고 있는 밀웜단백분을 가수분해하면 압력과 온도로 인해 고분자의 단백질이 저분자 펩타이드 및 아미노산으로 분해되어 기능적인 면에서 뛰어난 가수분해물이 추출이 되며 다양한 기능적 특성을 알아볼 수 있다. 아임계수 가수분해의 조건은 압력 30 bar, 교반 속도 200 rpm, 온도 150 내지 225℃로 하여 가수분해물의 특성을 확인했다. 가수분해물의 수용성 단백질량, 당 함량, 항산화 능 등을 확인한 결과, 가수분해물내 함유된 수용성 단백질과 당류의 함량은 가수분해온도가 높아지면서 함량 또한 증가하는 것을 확인할 수 있었다. 다양한 조건에서 가수분해들중 특히 SCO_2로 지질을 추출한 후 아임계로 가수분해한 샘플에서 가장 높은 항산화능과 페놀화합물 등 높은 활성을 나타었고, 아임계수 가수분해의 온도가 높아질수록 활성또한 증가하는것으로 확인되었다. 아임계수 가수분해 온도가 상승할수록 단당류의 함량이 증가하였고 수용성단백질의 결과도 증가하였다. SCO_2로 지질을 추출한 밀웜 단백분으로 아임계가수분해한 조건 중 210℃ 에서 추출한 가수분해물에서 항산화 능과 그와 관련된 페놀화합물 함량이 가장 높은 결과가 나타났다.

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