

Chemical composition and antioxidant activities of black sesame (*Sesamum indicum*) seed pressed-cake

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Abstract

The aims of this research were to determine the chemical composition of black sesame seed pressed-cake (BSPC) and to study the effect of different ethanol concentrations (0, 40, 60, 80 and 100%) on total phenolic content (TPC) and antioxidant properties of extracts from BSPC. BSPC contained high content of fat (35.04%) and protein (22.61%). It had high levels of essential amino acid, in particular leucine, phenylalanine, valine, and isoleucine. Extract from BSPC using 60 % ethanol had highest TPC (0.90 mg GAE/g DW) and also showed high antioxidant activities determined by DPPH radical scavenging activity (0.20 $\mu\text{mol TE/g sample}$) and ABTS radical scavenging activity (9.19 $\mu\text{mol TE/g sample}$) methods. These results demonstrated that BSPC can be a good source of nutrient and have potential for being used as functional food ingredients.

Keywords: Amino acid, antioxidant, Black sesame cake, proximate, total phenolic content

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1. Introduction

Lipid oxidation is an important chemical deterioration in lipid-containing food (Tokur and Korkmaz, 2007). Lipid oxidation results in the formation of reactive oxygen species and free radicals; which are reportedly associated with carcinogenesis, mutagenesis, inflammation, DNA changes, aging and cardiovascular diseases (Wasowicz et al., 2004). To prevent the lipid oxidation in muscle food and food products containing lipid, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ter-butyl hydroquinone (TBHQ) have been used because they are the most effective method to prevent lipid oxidation and development of off-colors and flavors (Wasowicz et al., 2004). Though they are more effective and less expensive than natural antioxidants, but their safety has been questioned. BHA, BHT and TBHQ are banded from Japan and European countries because they are reported to be carcinogenic (Mousakhani-Ganjeh, Hamdami, and Soltanizadeh, 2016). Hence research into natural materials based on fruit and vegetable origin have received much attention as sources of biological active substances including antioxidants and anticarcinogens (Mousakhani-Ganjeh, Hamdami and Soltanizadeh, 2015). Natural phenolic compounds with antioxidant activity from various plant sources, such as rosemary extract, tea catechin, tannins, etc. have been gaining increasing attention due to their safety.

Sesame (*Sesamum indicum* L.) seed is one of the most important oil seed crops in the world. Besides providing highly stable oil, sesame seed is also a source of protein-rich meals and is used in sweetmeats and confectionery foods, and has varieties of medicinal properties. Black sesame cake, the byproduct obtained after the removal of oil is presently used as cattle field (Espinoza Rodezno et al., 2013). Most of the studies reported so far are on antioxidant activities of sesame seed. Nevertheless, little work regarding on chemical composition and antioxidant activities of sesame seed pressed-cake as affected by various ethanol concentrations have been reported. Therefore, the aims this study were to determine the proximate compositions and amino acid composition of black sesame seed pressed-cake and to evaluate total phenolic content and antioxidant activities of extracts from black sesame seed pressed-cake as affected by ethanol concentrations.

2. Material and Methods

2.1 Sample

Black sesame (*Sesamum indicum*) seed pressed-cakes (BSPC) were obtained from sesame research center, Ubon Ratchathani University, Ubon Ratchathani province, Thailand. The samples were collected and air-dried at 60 °C overnight. They were then ground, sieved and kept at -20 °C until used for analyses.

2.2 Chemicals

2,2'-diphenyl-picrylhydrazyl (DPPH.), 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and gallic acid were purchased from Sigma (St. Louis, MO). 2,4,6-Tri (2-pyridyl)-S-triazine (TPTZ), casein from bovine milk, bovine serum albumin (BSA) and Folin-Ciocalteu phenol reagent were procured from Fluka Chemica-Biochemika (Buchs, Switzerland). Tris and ethanol were obtained from Ajax Fine chem Pty. Ltd. (Auckland, NZ).

2.3 Chemical compositions analyses

2.3.1 Determination of proximate composition

The proximate compositions of BSPC, including moisture, ash, fat, fiber and protein contents were determined according to the methods of AOAC 1999 (AOAC, 1999). Moisture content was determined by drying to a constant weight at 105°C. The crude lipid content was determined by extracting the sample with petroleum ether with a Soxhlet apparatus. The protein content was determined by the micro-Kjeldahl method. Available carbohydrates were calculated by difference.

2.3.2 Amino acid profiles

The amino acid composition of the BSPC was determined according to the AOAC method number 994.12 (AOAC, 2000). Amino acids were liberated from the pressed-cakes by hydrolysis with 6 M HCl. Hydrolysates were diluted with a sodium citrate buffer, and the pH was adjusted to 2.2. Individual amino acid components were separated and identified by using gas chromatography-mass spectrophotometry. The content of each amino acid was reported as mg per 100 g sample.

2.4 The study on effect of various concentrations of ethanol on total phenolic content and antioxidant activities of extracts from BSPC.

2.4.1 Sample extraction

BSPC samples were used as the starting materials for extraction according to the method described in Atala et al. (Atala, Vásquez, Speisky, Lissi, & López-Alarcón, 2009). Briefly, 10 g of BSPC powdered sample was extracted with 100 ml of different ethanol concentrations (0, 40, 60, 80 and 100%). The extracts were shaken in a water bath at 25 °C for 90 min and centrifuged at 950 \times g for 15 min. The supernatant was then stored at -20 °C until further analysis

2.4.2 Determination of total phenolic content (TPC)

TPC was determined using the Folin-Ciocalteu reagent as described by Sato et al. (Sato, Ramarathnam, Suzuki, Ohkubo, Takeuchi, & Ochi, 1996) with a slight modification. The extracts were dissolved in distilled water to obtain a final concentration of 0.2 mg/ml. An aliquot of 100 μ l was mixed with 0.75 ml of Folin-Ciocalteu reagent. After 3 min, 0.75 ml of 6% (w/v) sodium carbonate was added. The mixture was allowed to stand for 1 h at room temperature. The absorbance at 760 nm was measured by UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). Gallic acid (0-500 mg/ml) was used as the standard. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g sample.

2.4.3 Antioxidant determinations

DPPH radical scavenging activity

DPPH radical scavenging activity was determined as described by Wu et al. (Wu, Chen, & Shiau, 2003) with a slight modification. Samples (1.5 ml) with a concentration range of 0.5–10 mg/l were added to 1.5 ml of 0.15 mM 2, 2-diphenyl-1-picryl hydrazyl (DPPH) in 95% ethanol. The mixture was mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of the resulting solution was measured at 517 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The sample blank at each concentration was prepared in the same manner except that ethanol was used instead of DPPH solution. A standard curve was prepared using Trolox in the range of 10-60 μ M. The activity was calculated after the sample blank subtraction and expressed as μ mol Trolox equivalents (TE)/ g sample.

ABTS radical scavenging activity

ABTS radical scavenging activity was assayed as per the method of Arnao et al. (Arnao, Cano and Acosta, 2001) with a slight modification. The stock solutions

included 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS solution with 50 ml methanol in order to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). A fresh ABTS solution was prepared for each assay. Samples (150 μ l) with a concentration range of 0.5–10 mg/l were mixed with 2850 μ l of ABTS solution and the mixture was left at room temperature for 2 h in dark. The absorbance was then measured at 734 nm using the spectrophotometer. A sample blank at each concentration was prepared in the same manner except that methanol was used instead of ABTS solution. A standard curve of Trolox ranging from 50 to 600 μ M was prepared. The activity was expressed as μ mol Trolox equivalents (TE)/ g sample.

FRAP (ferric reducing antioxidant power)

FRAP was assayed according to Benzie and Strain (Benzie and Strain, 1996). Stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution. A working solution was prepared freshly by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution. The mixed solution was incubated at 37 °C for 30 min in a water bath (Memmert, D-91126, Schwabach, Germany) and was referred to as FRAP solution. A sample (150 μ l) with the concentration range of 0.5–10 mg/l was mixed with 2850 μ l of FRAP solution and kept for 30 min in the dark at room temperature. The ferrous tripyridyltriazine complex (colored product) was measured by reading the absorbance at 593 nm. A sample blank at each concentration was prepared by omitting FeCl_3 from the FRAP solution and distilled water was used instead. The standard curve was prepared using Trolox ranging from 50 to 600 μ M. The activity was expressed as μ mol Trolox equivalents (TE)/ g sample.

Chelating activity

Ferrous ion chelating activity was measured by the method of Thiansilakul et al. (Thiansilakul, Benjakul, & Shahidi, 2007). Diluted sample (1 ml) was mixed with 20 μ l of 2 mM FeCl_2 and 40 μ l of 5 mM ferrozine. After 20 min of reaction at room temperature, the absorbance was then read at 562 nm. EDTA with the concentration range of 0-50 μ M was used as the standard. Ferrous ion

chelating activity was expressed as μmol EDTA equivalents/g sample.

Statistical analysis

Experiments were run in triplicate. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT) (Steel and Torrie, 1980). Statistical analyses were performed using the Statistical Package for Social Science (SPSS 13.0 for windows, SPSS Inc., Chicago, IL).

3. Results and discussion

3.1 Chemical composition of BSPC

The chemical composition of BSPC is shown in Table 1. The result showed that BSPC is the good source of protein (22.61%), fat (35.04%) and fiber (7.09%). This result was in agreement with Anilakumar et al. (2010) that sesame seed contained high protein (25.4%) and fat

(44.3%) contents. Apart from high protein, fat and fiber contents, high content of carbohydrate (27.60%) was also found (Table 1). High content of carbohydrate in perilla seed pressed-cake with 56.88% (Rawdkuen, Sriket, and Palakawong, 2018) and 69.15% Kongkeaw et al. (2015) was also reported. The reason for the high carbohydrates content in the sesame seed and perilla seed is that the company used the whole seed (kernel and shell) as a starting material when they did the cold press for oil recovery (Rawdkuen et al., 2016). Because of this, large amount of cellulose from the seed shell may be generated. However, BSPC had low moisture content (7.72%) (Table 1). So, it could be stored for a long time. High moisture content could cause decomposition of fatty acids by microbial action (Bozan and Temelli, 2008). Nevertheless, proximate composition of oil seed plants depends on many factors including species, season, environment, harvest time and oil extraction process (Longvah and Deosthale, 1991).

Table 1 Chemical compositions of black sesame seed pressed-cake (BSPC)

Compositions	Content (%)
Moisture	7.72±0.04 ^{*d**}
Protein	22.61±0.32 ^c
Fat	35.04±0.23 ^a
Ash	6.67±0.16 ^f
Carbohydrate	27.60±0.50 ^b
Fiber	7.09±0.02 ^e

* Means \pm Standard deviation (n=3).

** Different letters in the same column indicate significant differences ($p < 0.05$)

3.2 Amino acid composition of BSPC

The amino acid composition of BSPC expressed as mg/100 g of sample is depicted in Table 2. BSPC is rich in essential amino acids such as leucine (7,200 mg/100 g sample), phenylalanine (6,250 mg/100 g sample), valine (4,400 mg/100 g sample) and isoleucine (4,100 mg/100 g sample). Also, threonine, methionine and histidine were the major essential amino acids in SPC, while only average amounts of lysine and tryptophan were found. These results was in agreement with Anilakumar et al. (2010) who reported that sesame seed contained high content of phenylalanine (9,600 mg/100 g sample) and valine (4,600 mg/100 g sample).

For non-essential amino acid, the highest content was glutamic acid (13,567 mg/100g sample) in BSPC. Significant amounts of arginine (10,433 mg/100g

sample) and aspartic acid (7,150 mg/100g sample) were also found in BSPC, while cysteine was the lowest content, compared with other amino acids. Al-Surmi et al. (2016) reported that three species of flax seeds had high content of leucine, phenylalanine, valine, glutamic acid, arginine and aspartic acid, compared with other amino acids. Onsaard (2012) also reported low content of lysine in sesame flour which in accordance with low content of lysine (2,363 mg/100 g sample) found in this study (Table 2). Liu et al. (2015) found that sunflower seed cake lacks of lysine, methionine, threonine, and tryptophan. Hojilla-Evangelista et al. (2013) revealed that high contents of glutamic acid, aspartic acid, arginine, leucine and glycine were observed in pennycress cake but lacks of methionine, lysine and tryptophan contents were found.

Table 2 Amino acid composition of black sesame seed pressed-cake (BSPC)

Amino acid (mg/100 g)*	BSPC
<i>Essential amino acid</i>	
Leucine	7200±26.4
Phenylalanine	6250±217
Valine	4400±10.0
Isoleucine	4100±10.0
Threonine	3350±31.2
Methionine	3334±32.0
Histidine	2735±30.9
Lysine	2363±15.5
Tryptophan	1416±10.4
<i>Nonessential amino acid</i>	
Glutamic acid	13567±40.4
Arginine	10433±140
Aspartic acid	7150±15.0
Proline	5700±10.0
Glycine	4933±32.1
Alanine	4533 ±49.3
Tyrosine	4500± 60.0
Serine	4100±10.0
Cystine	2373±37.2

* In house method based on AOAC official method 994.12, 988.15 (2000) Detected by GC-MS

3.3 Effect of various ethanol concentrations (0, 40, 60, 80 and 100%) on total phenolic content and antioxidant activities of extracts from BSPC

3.3.1 Total phenolic content (TPC) of extracts from BSPC
 TPC of extracts from BSPC is shown in Figure 1. Phenolic compounds are bioactive substances widely distributed in plants and are important constituents of the human diet. The uses of plant phenolic compounds as antioxidant in food system have been gaining increasing attention due to their safety (Maqsood and Benjakul, 2010). Extracts from BSPC contained TPC ranging from 0.29, 0.60, 0.90, 0.66 and 0.64 mg GAE/g sample for 0, 40, 60, 80 and 100% ethanol, respectively (Figure 1). This was slightly different from TPC reported in black sesame (1.38 mg GAE/g sample), white sesame (2.88 mg GAE/g sample) (Ali, et al., 2016) and sesame cake (1.94 mg GAE/g sample) (Mohdaly, et al., 2011). It was noticed that among all concentrations used, 60% ethanol showed the highest TPC, compared with other concentrations ($p < 0.5$). However, no difference in TPC in extracts using ethanol with concentrations of 40, 80 and 100% was observed. Degree of polymerization of phenolics, the

interaction between phenolics and other plant constituents, as well as polarity of solvent influence the solubility of phenolic compounds (Cvetanović, et al., 2015). Ethanol is a low polar solvent, whereas water is a strong polar solvent. Ethanol at an appropriate concentration could extract phenolics with antioxidative activity to a high extent. With the addition of water to ethanol, the polarity of the mixed solvent increased continuously (Zhang et al., 2007).

Additionally, TPC content in all extracts using ethanol with concentrations ranging from 40 to 100% were higher than TPC content reported in Sacha inchi seed (0.51 mg GAE/g sample) (Rawdkuen et al., 2016). Phenolic content in plants varied with plant species, harvest season and environment has been reported (Jansomboon, Boontanon, Boontanon, Polprasert, & Thi Da, 2016). Campo-Deano, Tovar and Borderias (2010) reported that lignan glycosides including sesaminol glucosides, pinoresinol glucosides and sesamolol glucosides were the major phenolic acids in sesame cake. Therefore, BSPC could be used as an alternative source of natural antioxidant.

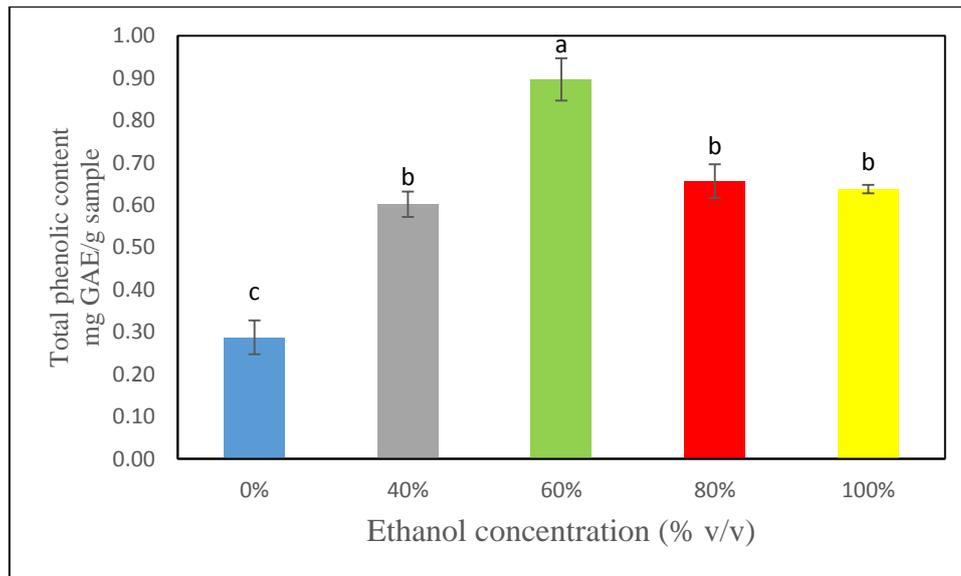


Figure 1. Total phenolic content (TPC) of black sesame seed pressed-cake extracted with different ethanol concentrations. Bars represent the standard deviation (n=3). Different letters indicate the significant differences ($p < 0.05$).

3.3.2 Antioxidant activities of extracts from BSPC

DPPH radical scavenging activity

The use of natural antioxidants for controlling rancidity and limiting its deleterious consequences has been gaining increasing attention due to its safety (Maqsood and Benjakul, 2010). DPPH radical scavenging activity of extracts from BSPC extracted with various concentrations (0-100%) of ethanol is shown in Figure 2. Extract from BSPC extracted with 60% ethanol showed the highest DPPH radical scavenging activity (0.20 $\mu\text{mol TE/g}$ sample). This result was in agreement with the highest TPC content in extract from BSPC with 60% ethanol (Figure 1). Plant phenolic compound has the aromatic ring bearing one or more hydroxyl (OH) substituents. It was reported to play a role as reducing agents, hydrogen donors and singlet oxygen quenchers (Proestos, Sereli and Komaitis, 2006). The different TPC and DPPH radical scavenging activity of BSPC extracts might be due to different ethanol concentrations used.

The above result was different from (Espinoza Rodezno, et al., 2013) who reported the highest DPPH radical scavenging activity in sesame cake extracted with 100% methanol. Mohdaly et al. (2011) also reported the higher DPPH radical scavenging activity in sesame cake extracted with methanol than did with ethanol. Teh and Birch (Ooizumi and Xiong, 2004) reported that extracts from different seed oil press cakes (flax seed cake, canola seed

cake, hemp seed cake) showed different antioxidant activity. Several parameters influenced the TPC content and antioxidant property including plants species, solvent type, polarity of solvent, concentration, etc. were reported (Dai and Mumper, 2010).

ABTS radical scavenging activity

ABTS radical scavenging activity (ABTS) of extracts from BSPC using different concentrations of ethanol (0, 40, 60, 80 and 100%) is shown in Figure 3. Among all ethanol concentrations used, 60% ethanol showed the highest ABTS (9.19 $\mu\text{mol TE/g}$ sample), compared with other concentrations ($p < 0.05$). The result was coincidental with highest DPPH found in BSPC extracted with 60% ethanol (Figure 2). DPPH radical scavenging activity is used to determine the antioxidant activity in hydrophobic system, while ABTS radical scavenging activity is used to determine the antioxidant activity both hydrophilic and lipophilic system (Ooizumi and Xiong, 2004). This result was slightly different from Sarkis et al. (2013) who reported that extracts from sesame cake using 80% ethanol showed the highest ABTS (1,146 $\mu\text{mol/Lite TE}$). In addition, highest ABTS activity found in extract using 60% ethanol was concomitant with highest TPC content in sample extracted with 60% ethanol (Figure 1). Furthermore, Das et al. (2007) also reported that DPPH scavenging activity and ABTS activity of sesame honey were positively correlated to its phenolic content.

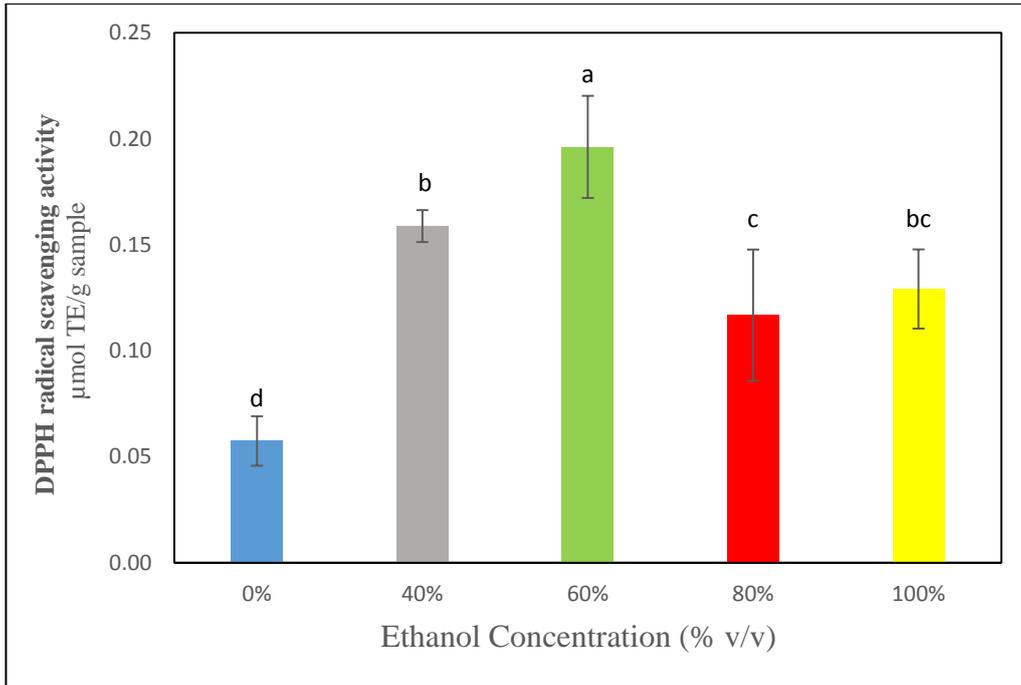


Figure 2. DPPH radical scavenging activity of black sesame seed pressed-cake extracted with different ethanol concentrations. Bars represent the standard deviation (n=3). Different letters indicate the significant differences ($p < 0.05$).

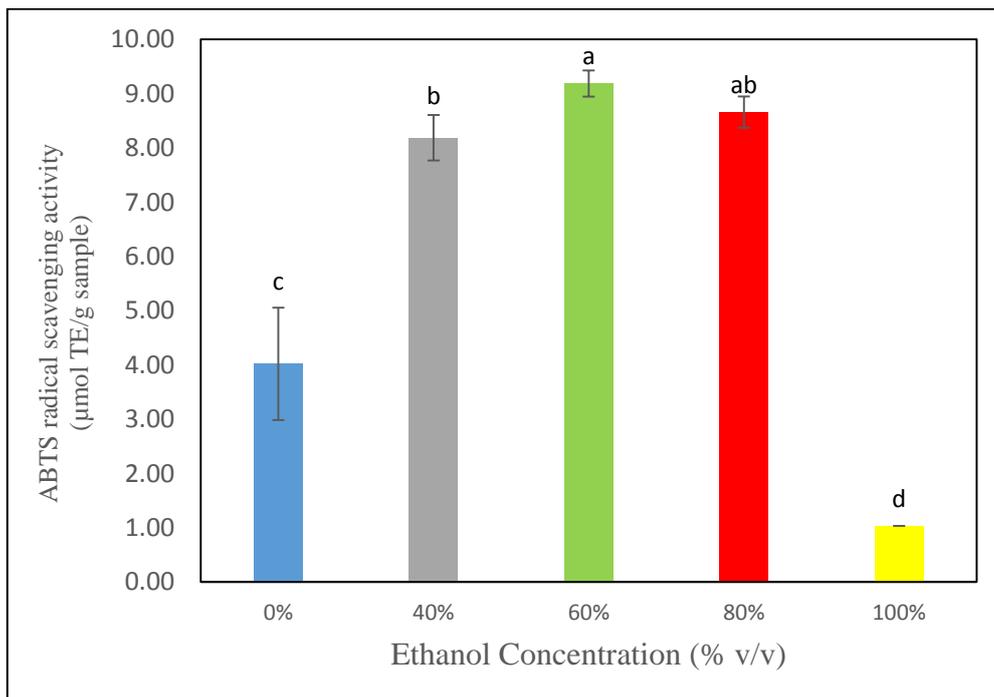


Figure 3. ABTS radical scavenging activity of black sesame seed pressed-cake extracted with different ethanol concentrations. Bars represent the standard deviation (n=3). Different letters indicate the significant differences ($p < 0.05$).

Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) of extracts from BSPPC using various concentrations of ethanol is depicted in Figure 4. Among all concentrations used, 80% ethanol exhibited the highest FRAP (11.57 μmol TE/g sample), compared with other concentrations

($p < 0.05$) (Figure 4). This result was slightly different from the highest DPPH (Figure 2) and ABTS (Figure 3) activities in BSPPC extracts using 60% ethanol. This was plausibly due to different mode of antioxidant action among them. FRAP is used to determine the ability of compound in reducing TPTZ-Fe (III) complex to TPTZ-Fe (II) complex (Benzie

and Strain, 1996). Chotphruethipong et al.(2017) revealed the highest FRAP found in cashew leaves extract using 80% ethanol. Ethanol at an appropriate concentration

could extract phenolics with antioxidant activity to a high extent.

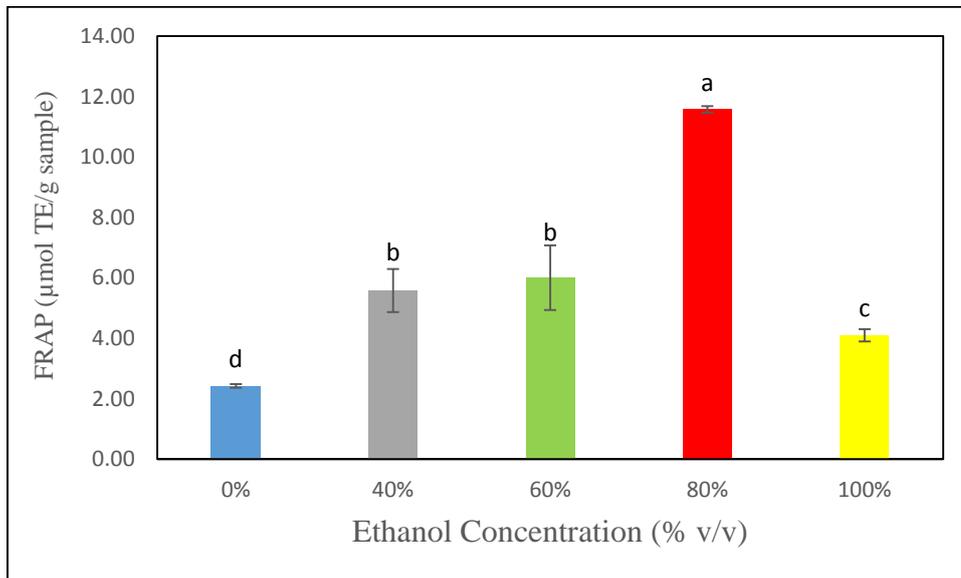


Figure 4. Ferric reducing antioxidant power (FRAP) of black sesame seed pressed-cake extracted with different ethanol concentrations. Bars represent the standard deviation (n=3). Different letters indicate the significant differences ($p<0.05$).

Metal chelating activity

Metal chelating activity of extracts from BSPC using different concentrations of ethanol is shown in Figure 5. The result showed that extracts from BSPC had 0.60, 1.21, 1.19, 1.32 and 1.23 µmol EDTA equivalent/g sample for BSPC extracted with 0, 40, 60, 80 and 100% ethanol, respectively. It was noticed that no different chelating activity was observed among all extracts using ethanol with concentrations ranging from 0-100% ($p>0.05$). No different chelating activity among all extracts (Figure 5) might be due to 40% ethanol used in this study is enough to extract phenolics that have chelating ability. The chelating activity method is based

on ability of tested samples in chelating or deactivating transition metals by inhibition Ferrozine- Fe^{2+} complex formation (Sofidiya and Familoni, 2012). However, BSPC extracted with 0% ethanol showed the lowest chelating activity ($p<0.05$) (Figure 5). Polarity of solvent influences the solubility of phenolic compounds (Cvetanović et al., 2015). Ethanol is a low polar solvent, whereas water is a strong polar solvent. Ethanol at an appropriate concentration could extract phenolics with antioxidative activity to a high extent. With the addition of water to ethanol, the polarity of the mixed solvent increased continuously (Zhang, et al., 2007)

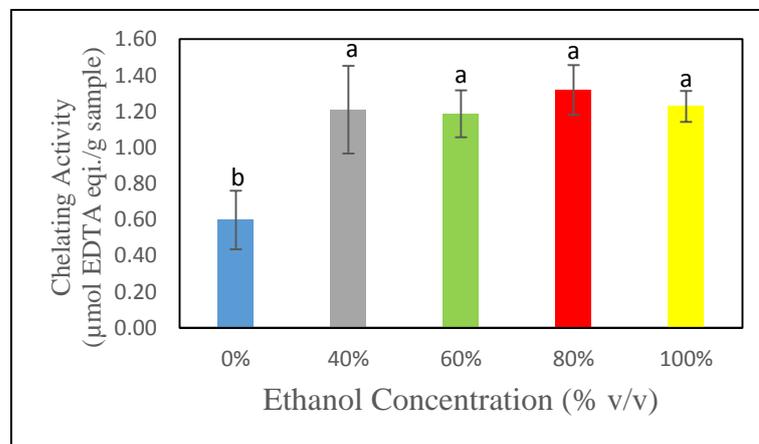


Figure 5. Metal chelating activity of black sesame seed pressed-cake extracted with different ethanol concentrations. Bars represent the standard deviation (n=3). Different letters indicate the significant differences ($p<0.05$).

4. Conclusion

Base on high content of fat (35.04%) and protein (22.61%) as well as essential amino acid (leucine, phenylalanine, valine, and isoleucine), BSPC could be a useful ingredient for human consumption. BSPC extracted with 60% ethanol showed the highest TPC and antioxidant activities determined by DPPH and ABTS methods. Extracts from BSPC, especially using 60% ethanol can be used as natural antioxidant for food application.

5. Conflicts of interest

The authors have declared that there is no conflict of interest regarding the publication of this article.

6. Acknowledgments

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