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Variation of Sesamin, Sesamolin, and Sesamol Contents in Sesame (*Sesamum indicum* L.) Cultivars from Indonesia

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Abstract:
Sesame is a source of food oil which have high antioxidant activity. Sesame oil is widely used as a good source of natural antioxidants in food, medicine, and oil industry. Indonesia developed some sesame varieties which are named as Sumberrejo (SBR) 1, 2, 3 4 and Wijen Nasional (Winas) 1 and Winas 2. Sesame oil which derived from sesame seed has an antioxidant activity of 23.15%,20.37%, 18.93%,15.32%, 17.81%, 18.14%, RSA (Radical Scavenging Activity) on test DPPH (2,2-diphenyl-1-picrylhydrazyl) 0.1 mMol, respectively for Winas 1, Winas 2, SBR-1, 2,3, and 4 varieties. The highest content of sesamolin lignan was in Winas 1 varieties of 126.66 mg / 100 g, followed by Winas 2 of 121.47 and SBR-1 of 120.18 mg / 100 g of seeds Indonesian sesame variety potentially can be use as a source of natural antioxidant due to its lignan content which have high antioxidant activity.

Keywords: *Antioxidants, sesamol, sesamolin, sesame seed*

1. Introduction

Sesame (*Sesamum indicum*, sp.) has known as a producer of vegetable oils (sesame oil) which has many benefits, both as an antioxidant and its effect on human health. Sesame oil is widely used as an antioxidant due to the presence of lignan compounds (sesamin, sesamolin and sesamol) in sesame seed (Wright et al., 2001; Joshi et al., 2001; Joshi et al., 2002; McPhail et al., 2003). In 100 g of sesame seeds from roasted sesame seeds contain 638 mg sesamin, 292 mg sesamolin, and 11.5 to 16.1 mg sesamol (Mohamed and Awatif, 1998). Lignans are often the focus of research as a source of antioxidants in sesame oil is sesamol and sesamolin. Sesamol is naturally present in sesame oil from raw sesame seeds and can increase if the sesame seeds are roasted at temperatures up to 200° C (Yen and Tsai, 1990; Yoshida et al., 2000). Sesamol arises from the breakdown of sesamolin compounds in raw sesame seeds which are roasted to a temperature of 200° C (Kikugawa et al., 1983). Increasing the content of some lignans in sesame oil after heating process has been widely used as a source of natural antioxidants, either as antioxidants in food systems or natural sources of antioxidants in an effort to improve the health of the human body.

Indonesia has several varieties of sesame plants that can grow and adapt well. The majority of Indonesian sesame farmers, particularly in East Java, West Nusa Tenggara, and some areas in Central Java and South Sulawesi, planted sesame seeds after the rice planting process, this mean that sesame seeds were used as temporary replacement crops before the next rice crop. Crops Research Institute Sweeteners and Fiber of Indonesia (*Balai Penelitian Tanaman dan Serat* / Balittas) under the Ministry of Agriculture of the Republic of Indonesia issued several varieties, including Sumberrejo-1 (SBR-1), SBR-2, SBR-3, SBR-4, National Sesas (Winas 1), and Winas 2, to support the existence of the sesame plant in Indonesia and as an effort to support the utilization of paddy fields after rice(Directorate General of Plantation of Indonesia, 2010 and Balittas, 2012). Utilization of sesame in Indonesia is still within the limits of the use of sesame as food and in consumption is still in the form of whole sesame seeds after going through the roasting

process. In Indonesia, sesame is usually used as a complement to snacks and snack foods, but some of them also have been producing sesame oil although still on a small scale.
Given the high potential of sesame oil as a source of antioxidants and its use in the food industry in Indonesia, it is necessary to know how the lignans content in sesame seeds of sesame varieties that grow and develop in Indonesia. Therefore, this study aims to find out how much lignan content in sesame seeds of official varieties that exist in Indonesia and how much its antioxidant activity.

2. Material and Methods
Research conducted at the Laboratory of Chemistry and Biochemistry, Faculty of Agricultural Technology, Gadjah Mada University in 2015, most of the analysis carried out in the Laboratory of Biotechnology, Puspitek LIPI Serpong.

2.1. Research Materials and Reagent
Four varieties of sesame seeds (Sumberrejo-1 or SBR-1, SBR-2, SBR-3, and SBR-4) obtained from Crops Research Institute Sweeteners and Fiber (Balittas) Malang Indonesia, sorted, cleaned, polished, and dried. Chemicals for proximate analysis, methanol, ethanol, diethyl ether and chloroform, acetic acid, sesamin and sesamol (purity = 99%), D-saccharic acid-1,4-lactone, hesperetin, 4-hydroxycoumarin, sulfatase (Helix pomatia, S-9626) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were obtained from the official supplier of chemicals in Yogyakarta (Aldrich Lab, General Lab, BRATACO Chemical, and Chemix).

2.2. Proximate Analysis Method
The method of analysis includes proximate analysis method using the AOAC (1995), includes water content, fat content, protein content, ash content. Methods of heating (roasting) refers to Lee et. al., (2008).

2.3. Determination of DPPH Radical Scavenging Activity
The method described by Brand-Williams et al. (1995) was used to assess antioxidant activity of sesame seed. The assay mixture contained 2.5 mL of 0,1 mM DPPH dissolved in absolute methanol. Sample at different concentrations was added to DPPH and the final volume was adjusted to 4 mL. The mixture was shaken vigorously on a vortex mixer and then incubated for 90 min at ambient temperature in dark. A control run was also performed by taking 2.5 mL of DPPH and 0.5 mL methanol under the same reaction condition. Absorbance was measured spectrophotometrically at 517 nm. DPPH radical scavenging activity of the sesame seed was calculated as follows and expressed as a percentage of Radical Scavenging Activity (RSA).

DPPH radical scavenging activity absorbance of sample (%)=(1 - absorbance of sample/absorbance of control) x 100

2.4. Total Phenolic Content (TPC)
The TPC of sesame seed extracts was determined using Folin–Ciocalteu reagent (Singleton and Rossi 1965). For every 100 - 500 mL of samples, distilled water, 0.5 mL of Folin–Ciocalteu reagent and 1 mL of 20% sodium Carbonate were added. The content was mixed and allowed to stand for 90 min. The absorbance of the solution was recorded at 760 nm using Shimadzu ultraviolet (UV) visible spectrophotometer (Kyoto, Japan). The TPC was expressed as gallic acid equivalence in mg/100 g of the sample, using a standard curve plotted by taking 0–1,000 mg gallic acid/mL.

2.5. Sample extraction for sesamin and sesamolin determination (Rangkadilok, et. al., 2010)
Air-dried sesame seeds were ground into powder and weighed (0.4 - 0.5 g) into 15 mL plastic tubes (two replicates per sample). Of 80% methanol (5.0 mL) was added and the whole extracted for 30 min. The samples were then centrifuged at 2000g, for 3 min at 25oC. The supernatant was transferred into a 10-mL volumetric flask. The residues were then re-extracted with 4.0 mL of 80% methanol. All extracts were combined, volume adjusted with 80% methanol, and filtered through a 0.45 mL PVDF membrane (Chrom Tech, Apple Valley, MN) prior to HPLC analysis. For extraction of sesame oil, the oil sample (4.0 mL) was weighed (3.5 - 4.5 g) into 15 mL plastic tubes (two replicates per sample), and the whole extracted with 80% methanol by the same process as in the previous seed extraction.

2.6. HPLC analysis for sesamol, sesamin and sesamolin (Rangkadilok, et. al., 2010)
The HPLC analysis of sesamin and sesamolin was performed using the external standard method by Agilent 1100 high-performance liquid chromatography (Agilent Technologies, Baudrats, Germany) with a thermostatically controlled column oven, a binary pump, and a diode-array detector. A reversed-phase column, Hypersil BDS C5 lm, 150 _ 4 mm i.d. (Thermo Electron Co., Southend-on-Sea, UK), was used in this study. The mobile phase consisted of water (solvent A) and methanol (Merck, Darmstadt, Germany) (solvent B) with a gradient system: 0 min, 5%B; 0 - 5 min, 5 - 18%B; 5 - 10 min, 18 - 35%B; 10 - 15 min, 35 - 62%B; 15 - 18 min, 62 - 80%B, 18 - 22 min, 80%B; 22 - 23 min, 80 - 5%B, and 18 equilibrated at this condition (5%B) for 3 min at 25oC. The flow rate was 1.0 mL/min (injection volume 20 mL) with detection at 280 nm. Total run time was 26 min. Sesame lignan was identified by collecting the peaks from HPLC and analysis using a mass spectrometer (MicroTOF, Bruker Daltonics, Bremen, Germany).

3. Result and Discussion
From the result of the proximate analysis, it can be seen that the oil content of the largest oil content to the smallest is the Winas 1 varieties about 45.12%, Winas 2 at 43.8%, SBR-1 at 42.75%, SBR-2 at 41.65%, SBR-4 at 41.21%, and SBR-3 40.18%. The statistical

tests show that Winas 1, Winas 2, and SBR-1 varieties have the highest oil content that is significantly different from other varieties. All of these proximate data are displayed in dry base data. This difference in oil content is due to differences in varieties affecting morphology and the size of sesame seeds which will affect the size of the cell vacuoles in cotyledons of sesame seeds where sesame oil is stored (Marliani, 2005). The results of the proximal analysis of Indonesian sesame varieties can be seen in Table 1.

Variable (% db)	Indonesian Sesame Varieties					
	SBR-1	SBR-2	SBR-3	SBR-4	Winas 1	Winas 2
Oil	$42,75^{c}$±1,4	$41,65^{d}$±0,56	$40,18^{e}$±0,52	$41,21^{d}$±1,21	$45,12^{a}$±0,19	$43,8^{b}$±0,25
Protein	$19,26^{c}$±0,87	$19,98^{cb}$±1,14	$19,18^{c}$±0,79	$20,65^{b}$±0,61	$22,61^{a}$±0,87	22^{a}±0,33
Carbohydrate	$29,07^{c}$±1,14	$30,23^{b}$±0,23	$31,54^{a}$±0,33	$30,04^{b}$±1,27	$24,60^{d}$±0,21	$25,27^{d}$±0,69
Water	$4,91^{a}$±0,19	$3,79^{b}$±0,62	$4,53^{a}$±0,55	$3,77^{b}$±0,31	$3,28^{b}$±0,31	$5,28^{a}$±0,86
Ash	$4,01^{ab}$±0,39	$4,35^{a}$±0,37	$4,57^{a}$±0,15	$4,33^{a}$±0,76	$4,39^{a}$±0,49	$3,65^{b}$±0,51

Table 1: Proximate analysis results of six varieties of Indonesian sesame.

Data is shown in ± StDev, the same letter on the same line shows no significant difference, with P> 0.05

Differences in oil content present in each of these varieties leads to differences in the content of polyphenolic compounds and differences in antioxidant activity. This is because most of the compounds that have antioxidant activity in the form of sesame lignans which are in the sesame oil fraction (Jan et. al., 2008). Lignans sesame included in the class of polyphenolic compounds that are derivatives of compounds of phenylalanine through dimerization of a class of compounds alcohol cinnamic, called monolignols, which serves as a framework forming the structure dibenzilbutan or propilbenzen in lignans, this reaction is assisted by the catalyst of oxidative enzymes of bio-peptide acid derivatives amino (Axelson, et al., 1982). TPC analysisshow that the highest content of phenolic compounds contained in sesame seed varieties Winas 1 that is equal to 23.78 mg GAE / g extract, followed by varieties Winas 2 at 23.18, SBR-1 at 22.05, SBR-4 at 21.43, SBR-3 at 20.87, and SBR-2 at 20.17 mg GAE / g extract. TPC content data and antioxidant activity of each variety can be seen in Figure 1.

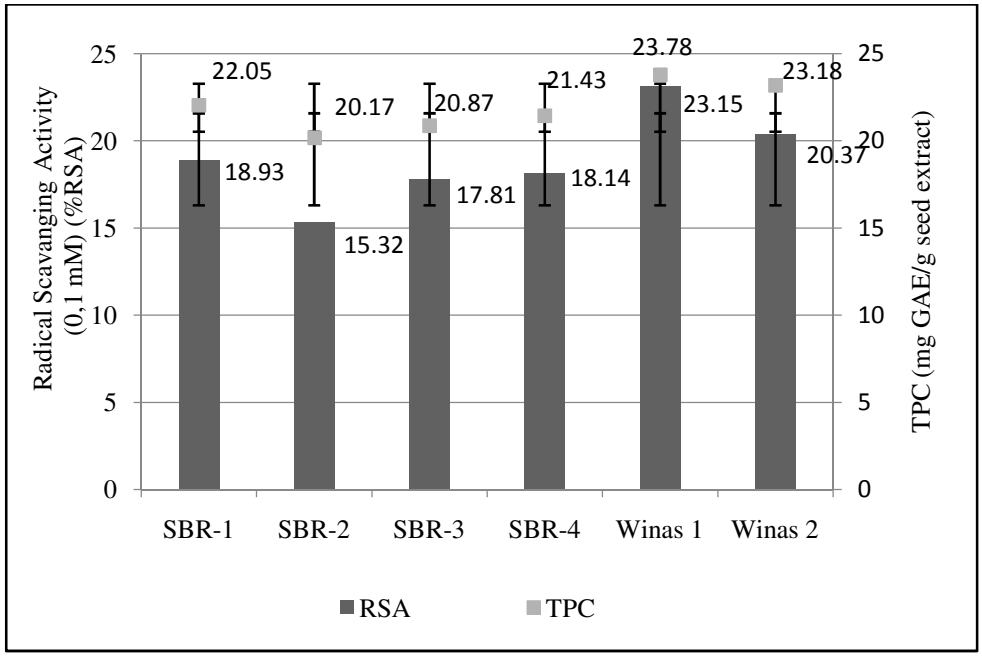

Figure 1: TPC (Total Polyphenol Content) and antioxidant activity of each Indonesian sesame varieties.

In addition to the proximate test and antioxidant content, the three varieties containing the highest oil content, TPC, and antioxidant activity, i.e. Winas 1, Winas 2, and SBR-1 varieties will then test Lignan content to see the level of each lignan in each variety. Analysis of lignan content of sesame varieties was done by HPLC method (Rangkadilok, et al, 2010). This analysis uses standard compounds as a comparative time for each lignan retention of each sesame variety. Seamin and sesamol standards are available in large quantities, whereas for sesamolin standards a retention time approach is used by purifying sesamolin compounds from sesame seed extraction (Shyu and Hwang, 2002). This method of extraction was performed by maceration of sesame seeds with N-hexane solvent and methanol, the extraction result gave extracts containing sesamin and sesamolin. Lignan sesamol had retention time at ± 14.16 min, while lignan sesamin and sesamolin, had a retention time of ± 22.59 and ± 23.09 min. Data on lignan content of some Indonesian sesame varieties can be seen in Table 2. HPLC chromatogram of standard compounds of sesame lignans (sesamin, sesamolin, and sesamol) can be seen in Figure 2.

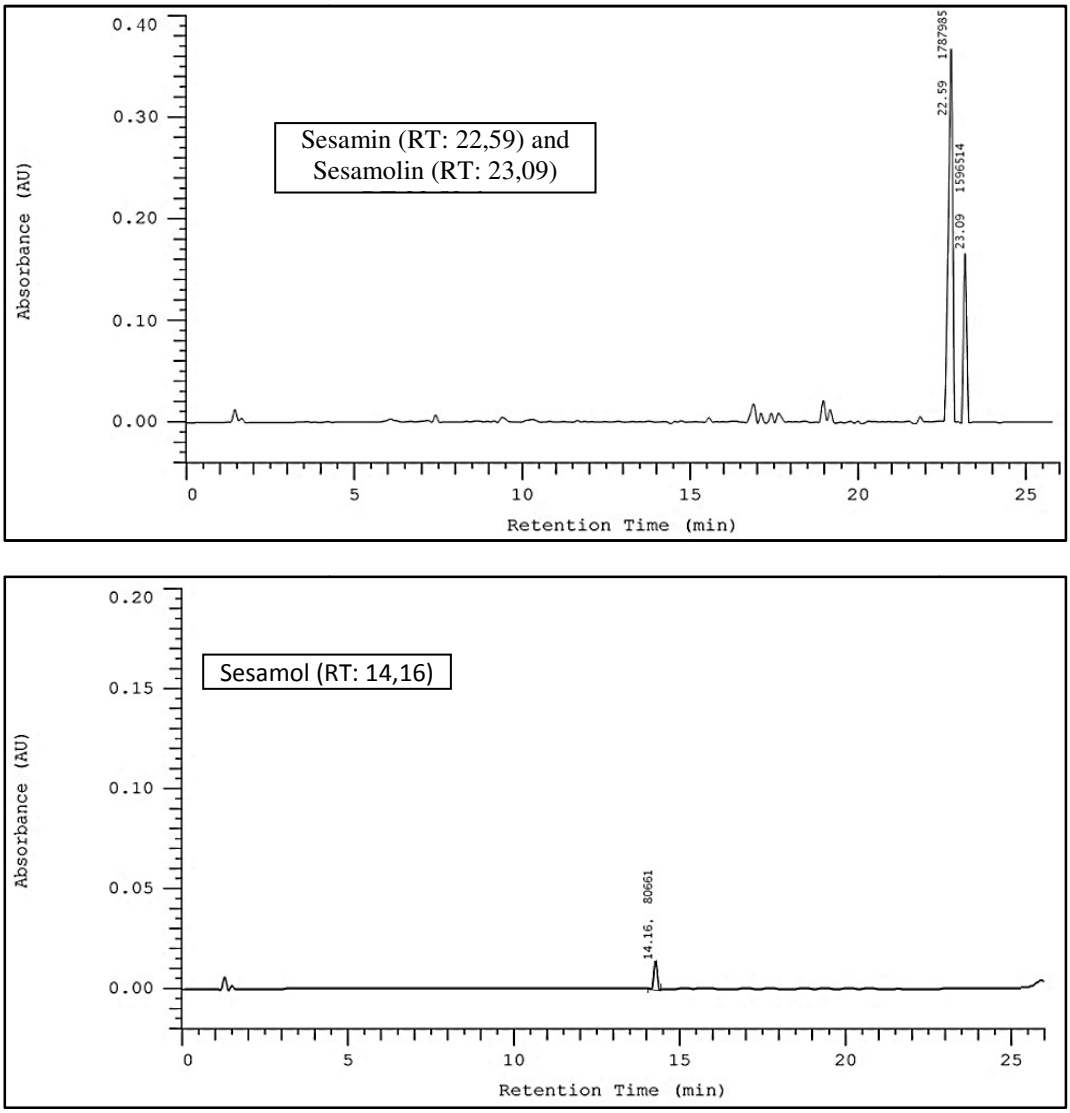

Figure 2: HPLC chromatogram of standard compound of sesamin, sesamolin and sesamol.

Indonesian Sesame Varieties	Lignan (mg/100g sesame seed)		
	Sesamin (RT: ±22.59 m)	Sesamolin (RT: ±23.09 m)	Sesamol (RT: ±14.16 m)
Winas 1	265.33	126.66	2.39
Winas 2	242.51	121.47	2.17
SBR-1	211.34	120.18	2.09
SBR-4	204.95	114.79	1.74
SBR-3	201.22	113.28	1.42
SBR-2	206.71	104.94	1.36

Table 2: Lignan contents of sesame seed of 6 varieties of sesame in Indonesia.

4. Conclusion

Indonesian sesame variety potentially can be use as a source of natural antioxidant due to its lignan content which have high antioxidant activity. Oil content, antioxidant content, and lignan content is responsible to the high of antioxidant activity of sesame seed. The highest content of sesamolin lignan was in Winas 1 varieties of 126.66 mg / 100 g, followed by Winas 2 of 121.47 and SBR-1 of 120.18 mg / 100 g of seeds. Therefore, Indonesian sesame variety can use as a natural source of antioxidants that can be used for various purposes in the food sector.

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