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Effects of *Neurospora sp* Mold on the Nutritive Values, Bioactive Compounds, Fatty Acids and Amino Acids Profile of Solid Decanter of Palm Oil Sludge

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ABSTRACT

Background: Solid decanter is a by-product biomass of palm oil plants and it could be used as animal feed, particularly poultry. Solid decanter is however high in crude fiber content and needs further treatment before being given to the birds. One such treatment is a fermentation process using *Neurospora* mold.

Methods: Mixture of solid decanter of palm oil sludge, rice bran and urea were fermented using *Neurospora sp* mold. The experiment was designed according to a completely randomized design (CRD) with six concentrations of mold as the treatments with four replicates. Therefore, there were 24 numbers of the experimental units. The mold concentrations were 0, 2, 4, 6, 8 and 10% of the solid decanter palm oil sludge-based substrate. The mixtures were subjected to proximate, bioactive compounds, fatty acid and amino acid profile analyses after aerobic incubation for 72 hours at room temperature.

Result: Mold concentration in the mixture significantly increased in nutritive values, antioxidant activity, flavonoid, polyphenol, beta-carotene, fatty acids and amino acids, in which 8% of mold produced the best values of measured parameters. In conclusion, nutritive values, bioactive compounds, fatty acids and amino acids profiles of solid decanter of palm oil sludge improved by providing 8% of *Neurospora sp* mold in the substrate.

Key words: Amino acid, Bioactive, Decanter, Fatty acid, Mold.

INTRODUCTION

Palm oil plant of Indonesia produces annually a lot of by-product biomass such as palm oil frond, solid decanter and palm kernel cake. These can be used as an alternative feed since they are readily available and sustainable feed resources for animal production. Inclusion of these in the diets of livestock can be an effective way to overcome the lack of feed sources and to substitute the conventional concentrate ingredients such as rice bran. In fact, the use of palm oil by-product biomass can reduce the cost of feed. Solid decanter (SD) is generated from palm oil mill effluent (POME) by dehydration process of POME (Seephueak *et al.,* 2011). Solid decanter (SD) has potential as a feed source because its crude protein content is equal to rice bran (around 11-14%) and SD has been classified as a moderate feed ingredient with total digestible nutrients of 62.5% and metabolisable energy of 8.37MJ/kg (Kum and Zaharin, 2011). The limitation for using this ingredient as feed is its high fibre (Pasaribu, 2007), crude fibre content of 21.15 to 29.76% has been reported (Lekito, 2002). Therefore, efforts to overcome this problem is needed, one such option is bioconversion with *Neurospora sp* mold.

Fenita *et al.* (2015) stated that *Neurospora sp*-fermented palm sludge increased the crude protein content from 13.57% to 23.45% and reduced crude fibre from 28.03% to 17.34%. Meanwhile, Hasanuddin and Rusdi (2023) found an increase in the protein content of palm mud fermented with *Neurospora crassa* from 13.08% to 21.97% and the beta-carotene content also increased from 88 mg/kg to 315 mg/kg with an incubation period of 96 hours. The improved nutrient content of fermented palm oil sludge, will satisfy the protein requirements and serve as functional feed source for poultry.

Furthermore, Nuraini *et al.* (2016) stated that in addition to protein source, fermented-SD of palm oil acts as a source of functional feed because it contains significant amount of beta-carotene and minerals. The beta-carotene content of fermented SD of palm oil sludge is a reliable source of carotenoids for chicken rations. Elevation of carotenoid in the poultry diet is expected to produce meat or eggs that high in antioxidants and low in cholesterol levels. The ability of carotenoids to reduce cholesterol can be in two ways, namely (1) beta-carotene is an antioxidant which can prevent

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lipid oxidation and (2) beta-carotene is able to inhibit the activity of the HMG CoA reductase enzyme so that mevalonate is formed which is needed for cholesterol synthesis (Einsenbrand, 2005). This has been proven by Nuraini *et al.* (2005), who reported that feeding layer at the rate of 21% a mixture of fermented sago dregs and tofu dregs with *Neurospora crassa*, containing beta-carotene in the ration of 80.00 mg/kg can reduce chicken egg cholesterol by 33%. Based on the potentials of fermented SD as functional feed, SD of palm oil sludge fermented with *Neurospora sp* mold is expected to elevate its biological values by boosting nutritive values, bioactive compounds, fatty acid and amino acids. Therefore, the objectives of this study was to find out an optimum dose rate of *Neurospora sp* mold in SD substrate in order to produce a highest or best values for nutritives value, bioactive compounds, fatty acid and amino acid profile.

MATERIALS AND METHODS

Fresh solid decanter were taken from the palm oil processing industry (PT. Awana Sawit Lestari) and then it was allowed to stand for 24 hours at room temperature and then sun dried for about three days. The dried solid decanter of palm oil sludge was blended until it became sludge flour. Palm oil sludge flour was mixed with rice bran as much as 2.5% of the weight of sludge flour (4:1). The mixture was then sterilized in an autoclave at 121°C for 30 minutes, then cooled. After cooling, the inoculum and urea were put into the mix and stirred until homogenous. The amount of *Neurospora sp* mold used was by the treatment composition (w/w), namely 0% (NC0), 2% (NC2), 4% (NC4), 6% (NC6), 8% (NC8) and 10% (NC10). The dough was put in the plastic bag with a 1 mm hole and 2 cm distance between the holes and incubated aerobically for 72 hours at room temperature. After 72 hours, it was harvested and kept in the laboratory for proximate, phytochemical, fatty acids and amino acids profile analyses. Experiment was carried out on October, 2023 for six months period at the Livestock Technology Laboratory, Faculty of Animal Husbandry and Fishery, Tadulako University. Some chemical analysis were performing in the Chemistry Laboratory, Faculty of Natural Sciences, Tadulako University and Saraswanti Indogenetech Laboratory, Bogor Indonesia.

Antioxidant activity

The sample extract of 25 mg was put into a 25 mL measuring flask, then matched with ethanol solvent so that the concentration of 1000 ppm solution was obtained. Serial dilutions were carried out to obtain a solution of 10, 30, 50, 70 and 90 ppm. To 0.2 mL of the prepared solution was added 3.8 mL DPPH 50 µM solution. The mixture was homogenized and left for 30 minutes in dark place. Then the absorption was measured at a wavelength of 517 nm (Molyneux, 2004).

Beta-carotene

Samples were subjected to carotenoids analysis using high performance liquid chromatographic (HPLC) assay with diode-array detection at 450 nm, as described by Bidura *et al.* (2020). In brief, beta-carotene content was determined by placing 0.10-0.50 in a centrifuge tube, then adding 5 mL of acetone and 5 mL of pure petroleum ether (PE), shaking evenly, then centrifuging for 5 minutes at 3000 rpm. The supernatant was collected and stored in a test tube, while the sediment was mixed with 5 mL of acetone and centrifuged until the supernatant was colorless (clear). The collected supernatant was then transferred to a separator tube, washed with 15 mL of distilled water and repeated three times. The rinsing water was then removed and the clear top of the tube was put into the test tube, followed by 1 g of $NaSO_4$ and a vortex. The clear part was taken and the PE solution was added till the volume becomes 10 mL and then read on the spectrometry absorbent (abs) at λ = 450 ηm.

$$\text{Total carotene (ug/100 g)} = \frac{(\text{Total volume} \times \text{abs} \times 100)}{(0.2 \times \text{sample weight})}$$

Flavonoid

Total flavonoid content was determined by the spectrophotometric method using quarcetin as the standard sample as described in Guo *et al.* (2020). All samples were mixed with one milliliter of 5% sodium nitrite solution and shaken for 6 minutes at room temperature. Then, 1.0 mL of 10% aluminum nitrate solution was added and shaken for 6 minutes. Finally, 10.0 mL of 4% sodium hydroxide solution was added and shaken for 12 minutes. The solution without samples served as the blank reference. Absorbance was measured at 510 nm.

Total polyphenols

Total polyphenol was analyzed based on the procedure of AOAC (2005), to 1 mL of sample was added Na_2CO_3 solution 75 g/L 4 mL and Follin-Ciocalteau reagent (diluted 1:10) 5 mL then mixed. Subsequently incubated for 1 hour at room temperature in black conditions, 2 mL of extract was taken and put in a cuvette. Measured absorbance at wavelength (λ) 765 nm. Then it is calibrated with a standard curve to get the total phenol in mg GAE/g.

Fatty acids profile

Fatty acid profile was evaluated according to AOAC (2005). Samples (0.3 mL) was methylated using 1.5 mL of Na-Metanolic and heated at 65°C for 15 minutes in the waterbath. A total of 1.5 mL of BF3-Methanol were added to the mixture, then heated at the same condition and the solution was allowed to cool down. The solution was extracted with 0.5 mL of N-Heptane and 1 mL of saturated NaCl and the top-layer of solution (1 µl) was injected to the Gas Chromatography mass spectrophotometry.

Amino acid profile

Amino acid profile was determined using a HPLC (AOAC, 2005). To 0.1 g of sample in a 20 mL headspace vial was added with 1 mL NaOH solution. Then, is was hydrolyzed at 110°C for 20 hours. The sample was transferred into a 50 mL glass beaker and 3 mL of citrate buffer solution was added. Solution was adjusted the pH to 4.25 with HCl or NaOH solution. It was transferred quantitatively into a 50 mL volumetric flask and added distilled water until the mark then homogenize. The solution was centrifuged at 14000 rpm for 3 minutes and filtered the supernatant with a 0.45 µm filter syringe into a 2 mL vial and then injected into HPLC.

Parameters observed were dry matter content, crude protein and crude fiber and phytochemical screening of bioactive components, fatty acid and amino acid profiles. Proximate analysis was based on AOAC (2005). Data were analysed using ANOVA and Duncan test for comparison (Steel and Torrie, 1991).

RESULTS AND DISCUSSION

Nutritive values

The improvement in nutritive values reported in the present study (Table 1) are corresponding to the results of Hasanuddin and Rusdi (2023). In general, inclusion rate of mold produced significant water content of the substrate ($P<0.05$). The water content decreased as the concentration of *Neurospora sp* mold increased, but there was no a regular pattern. This is in contrast with the theory that change in water content can be resulting from substrate hydrolysis and production of metabolic water.

Microorganisms in fermentation process, use and convert carbohydrate to produce water and carbon dioxide molecules (Fardiaz, 1989; Sathe and Mandal, 2016). In this case, only mold at the rate of 10% produced a non-significant improvement in the water content of the substrate media compared to the control and 4% group ($P>0.05$) and significantly higher than others one ($P<0.05$).

Interestingly, mold concentration had no significant effect on the crude fat of the substrate ($P>0.05$). The highest value of crude fat was achieved in the rate of 10% and the lowest value was in the rate of 6%. This fact is in line with Saono and Budiman (1981) reported that *Neurospora* is superior to other molds because of its complete enzymatic activity, namely amylase, protease and lipase enzymes. This lipase enzyme plays a key role in the degradation of substrate fat into glycerol and free fatty acids which are used as an energy source and therefore, at the end of fermentation the fat is reduced. Ardhana (1982) found that the organic materials lost during the fermentation process were starch and fat which were used to meet energy needs to support microbial growth.

The statistical analysis results showed that the mold had a significant effect ($P<0.05$) on the crude protein of fermented solid decanter of palm oil sludge. This corresponds with Hasanuddin and Rusdi, (2023) reported that mold elevated the crude protein of fermented palm oil sludge. Current study demonstrated that the highest crude protein content is achieved at the rate of 8% and the lowest one was in zero mold groups. Meanwhile, protein content at the rate 10% was lower than 6 and 8%, but it was higher than other ones. The improvement of crude protein occurs due to additional protein contributed by microbial cells during microbial growth, producing single cell protein products or biomass containing around 40-65% protein (Krishna and Devi, 2005). Similarly, fermentation of cotton seed meal with *Saccharomyces cerevisiae* elevated essential nutrients (Dharmakar *et al.,* 2023). Furthermore, the mold used may release metabolites in the form of proteins and enzymes during the biotransformation process and the microorganisms themselves are also a source of protein for individual cells. According to Kurniati (2012) that in the bioconversion process using *Neurospora sp*, protease enzymes will digest protein and produce into amino acids and lipase which will digest fat, triglycerides and turns into free fatty acids. Furthermore, according to Indrayanti and Rakhmawati (2013), the increase in protein during the fermentation process is because the mold itself contains nucleic acids which can supply nitrogen as a source of single cell protein. According to Garraway and Evans (1984) that fungal cell walls contain 6.3% protein, while the cell membranes of hyphal fungi contain 25-45% protein and 25-30% carbohydrates.

Inclusion of *Neurospora sp* had a significant effect ($P<0.05$) on the crude fibre content of fermented solid decanter of palm oil sludge. The crude fibre content of fermented solid decanter of palm oil sludge was in the

Table 1: Nutritive values of fermented solid decanter of palm oil sludge.

Variables (%)	Treatments					
	NC0	NC2	NC4	NC6	NC8	NC10
Water content	12.50±1.50^{a}	9.53±1.28^{bc}	11.57±0.13^{ab}	8.59±0.36^{c}	10.05±1.30^{bc}	13.21±0.15^{a}
Crude fat	4.48±0.94	3.99±1.46	4.77±0.11	3.47±0.62	3.78±0.42	4.55±0.63
Crude protein	16.27±0.36^{e}	17.89±0.79^{d}	18.14±0.38^{d}	22.60±0.71^{b}	24.02±0.38^{a}	21.24±0.36^{c}
Crude fibre	17.89±0.48^{b}	15.01±0.15^{c}	14.56±0.21^{c}	17.70±0.26^{b}	18.14±0.23^{b}	20.80±0.83^{a}
Ash	18.41±0.50^{d}	20.31±0.44^{ab}	20.21±0.37^{ab}	21.07±0.79^{a}	19.59±0.37^{bc}	18.52±0.48^{cd}

Description: Mean values (mean±sd) with different alphabetical superscripts within the rows are significantly different ($P<0.05$); NC0: Without *Neorospora sp*; NC2: *Neurospora sp* 2% ; NC4: *Neurospora sp* 4%; NC6: *Neurospora* sp 6%; NC8: *Neurospora sp* 8%; NC10: *Neurospora sp* 10%.

range of 14.56-20.80% (Table 1). The highest value of crude fibre was in NC10 and it was significantly higher than the others group (P<0.05). This agrees with Hasanuddin and Rusdi (2023), who revealed a linear increase in the crude fibre as an increase mold in the substrate. In general, the addition of mold to a medium will increase the number of mold cell walls in the medium (substrate) itself, but in this study the concentration of crude fibre decreased when using 2-4% mold, which is thought to be a higher rate of carbohydrate hydrolysis from the substrate than in the formation of the mold biomass itself. This is in line with Mahfudz (2006), who found that *Neurospora sp* causes the degradation of cellulose, hemicellulose and its polymers into simple sugars or their derivatives and can increase the nutrition of the substrate material. Moreover, Nuraini *et al.* (2009) stated that the decrease in crude fibre content in each treatment after fermentation was due to the enzyme produced by *Neurospora sp* being able to break down cellulose into glucose in the fermentation process. The cellulase enzyme is a complex enzyme that works in stages to break down cellulose into glucose and then the glucose produced from the substrate will be used as a source of carbon and energy. Addition more *Neurospora sp*, the crude fibre content of fermented solid decanter of palm oil sludge increased (Table 1). This condition is thought to be due to the higher growth of mold, leading to increase in the mycelial walls of mold cells. They are cellulose and parts of the crude fibre such as hemicellulose that have not been digested by *Neurospora sp*.

The ash content was affected by *Neurospora sp* inclusion rate (P<0.05) and there was no a specific characteristics trend of change. This research shows that the bioconversion process has a major influence on the ash content of palm oil sludge. Similar result has been reported by Mhalaskar *et al.* (2017), who found an increase in ash content of fermented broken rice using *Monascus purpureus*. The NC6 had the most ash, which is assumed to be attributable to the activity of the *Neurospora sp* mold, which relies on organic components to survive. The pattern of increased ash content is the inverse of the trend of increased protein content, which is one source of organic material production. This is assumed to be due to the creation of diverse inorganic materials during the bioconversion process, resulting in an increase in inorganic compound components, which is consistent with the increase in crude fibre content.

Antioxidant activity

In general, fermentation process allows an improvement of antioxidant activity (Erskine *et al.,* 2023). This is in accordance with the present fermentation study that the antioxidant activity was affected by the level of *Neurospora sp* in the substrate in which the antioxidant activity was significantly elevated by increasing the level of *Neurospora sp* (P<0.05). The data in Table 2 demonstrated that the highest values of antioxidant activity is 133.02 ppm in the treatment without *Neurospora sp* and the lowest value is 75.14 ppm found in treatment of 8.0% mold. This means that the highest antioxidant activity was achieved in the dose rate of 8% mold. This improvement is assumed to be due to biochemical changes in FSD caused by *Neurospora sp* activity, which causes an increase in the bioactive components of palm oil sludge such as polyphenols, total acids and total vitamins. According to Rodriguez *et al.* (2009), the fermentation process, might boost antioxidant activity due to the presence of phenolic components, where these compounds are chemicals that contribute to the antioxidant activity of palm oil sludge. Furthermore, Pratiwi and Dina (2013), the antioxidant activity of polyphenolic compounds is facilitated by -OH group. This OH group attached to the aromatic ring, where this hydrogen atom works as a hydrogen donor, allowing DPPH radicals to be reduced to non-radicals and become more stable. The more hydrogen atoms are in chemical, the more effective it is at reducing free radicals.

In this study revealed that the antioxidant activity of FSD is higher than the antioxidant activity (IC_{50}) of moringa leaves (Komang *et al.,* 2016), which was 427.49 ppm. This antioxidant activity of FSD was categorized as a weak to moderate (Phongpaichit *et al.,* 2007). Therefore, FSD is potentially to be used as antioxidant feed ingredient sources.

Beta-carotene

The beta-carotene content increased as the concentration of mold used increased, where the optimum concentration

Table 2: Bioactive compounds for fermented of solid decanter of palm oil sludge.

Variables	Treatments					
	NC0	NC2	NC4	NC6	NC8	NC10
Antioxidant activity, (ppm)	133.02±0.42^{a}	120.51±0.01^{b}	111.54±0.18^{c}	94.22±0.01^{d}	75.26±0.12^{e}	81.93±0.17^{f}
Beta carotene, (mg/100 g)	865.70±0.68^{a}	1343.04±1.49^{b}	1458.34±0.36^{c}	1543.44±1.44^{d}	1656.87±0.59^{e}	1481.11±0.35^{c}
Total polyphenol, (mg GAE/g)	16.96±0.13^{a}	17.92±0.17^{b}	18.67±0.08^{c}	20.38±0.21^{d}	21.29±0.21^{e}	19.08±0.17^{c}
Flavonoid, (mg QE/g)	4.06±0.08^{a}	4.62±0.06^{b}	5.12±0.07^{c}	5.74±0.14^{d}	6.36±0.06^{e}	4.84±0.07^{b}

Description: Mean values (mean±sd) with different alphabetical superscripts within the row are significantly different (P<0.05); NC0: Without *Neorospora sp*; NC2: *Neurospora sp* 2%; NC4: *Neurospora sp* 4%; NC6: *Neurospora sp* 6%; NC8: *Neurospora sp* 8%; NC10: *Neurospora sp* 10%.

was achieved at the concentration of 8% (P<0.05). This is thought to be beta-carotene produced by *Neurospora sp* which is carotegenic, producing yellow and orange pigments. The higher the concentration of mold used, the more the oxidation reaction increases so that many of the oxidation by-products are in the form of free radicals such as singlet oxygen. The beta-carotene present then acts as an antioxidant to reduce the side products of the oxidation reaction and interacts with free radicals to become a more stable compound. Another possibility is that the increase in beta-carotene occurs because the carotenogenic of *Neurospora sp* can synthesize geranyl geranyl diphosphate (GGDP) to finally become beta-carotene (Díaz-Sánchez *et al.,* 2011). The biosynthesis of beta-carotene starts from the GGPP molecule producing carotenphytoene which is catalysed by the al-2 enzyme. Initially, up to five double bonds are conjugated to phytoene mediated by al-1, resulting in the formation of 3,4-didehydrolycopene which starts from the formation of phytofluene, ζ-carotene, lycopene and neurosporene. The al-2 enzyme will then convert it to beta-carotene (Díaz-Sánchez *et al.,* 2011). In this study, the beta-carotene content of FSD was higher than the beta-carotene of crude palm oil reported by Saputra *et al.* (2020), namely 330-533.73 ppm.

Total polyphenol

Inclusion of *Neurospora sp* resulted in significant effect on the polyphenol content of FSD (P<0.05). Total polyphenol levels were found in the range of 16.96-21.29 mg GAE/g, where the lowest value was in without *Neurospora sp and* the highest value was in the rate of 8% *Neurospora sp*. Total polyphenol however decreased when the concentration of *Neurospora sp* was at the rate of 10%. This revealed that optimum level of *Neurospora sp* is 8% in the substrate. The increase in total polyphenols in FSD is closely related to the metabolic activity of *Neurospora sp* during the fermentation process which can modify bioactive components such as polyphenol groups, tannins and flavonoids. The presence of *Neurospora sp* in the fermentation process contributes to the conversion of complex polyphenols into simple ones and the depolymerisation of high molecular weight polyphenols (Othman *et al.,* 2009).

Supriyono *et al.* (2014) demonstrated an increased in total polyphenols when microbial application was at the rate of 10% and decreased at the rate of 15%. This is related to an increase in acidic conditions, as well as an increase in the number of molds and glucose concentrations. *Neurospora sp* produces the acid by converting glucose into organic acids such as citric acid and lactic acid. Furthermore, the oxidation reaction produced by this fermentation process generates by-products in the form of free radicals such as singlet oxygen. Polyphenols, like beta-carotene, will also acts as antioxidants to counteract the by-products of the oxidation reaction.

Flavonoids

Based on Table 2 shown that flavonoids content of FSD increase linearly with elevation of *Neurospora sp* in the substrate (P<0.05), but it decreased when *Neurospora sp* was at the rate of 10%. The highest value was 6.36 mg QE/g and was achieved at the rate 8%, while the lowest value was 4.06 mg QE/g in without *Neurospora sp*. The increase in total flavonoid during the fermentation process is

Table 3: Fatty acids profile for fermented of solid decanter of palm oil sludge.

Fatty acid (%)	Treatments					
	NC0	NC2	NC4	NC6	NC8	NC10
Caprylic acid (C8:0)	0	0.09±0,10	0	0	0	0
Capric acid (C10:0)	0.11±0,07^{b}	0.24±0.02^{ab}	0.36±0.06^{a}	0.21±0.14^{ab}	0.07±0.08^{b}	0.07±0.15^{b}
Lauric acid (C12:0)	0.87±0.23^{ab}	1.27±0.72^{a}	0.68±0.05^{ab}	0.89±0.16^{ab}	0.48±0.08^{b}	0.76±0.12^{ab}
Myristic acid (C14:0)	1.33±0.56^{bc}	1.56±0.15^{ab}	1.90±0.33^{a}	1.62±0.10$_{ab}$	1.06±0.01^{c}	1.40±0.10^{bc}
Pentadecanoic (C15:0)	0.15±0.03^{a}	0.23±0.23^{a}	0^{b}	0^{b}	0^{b}	0b
Palmitic acid (C16:0)	49.66±1.39^{a}	40.39±0.47^{bc}	38.37±0.83^{c}	38.09±1.26^{c}	39.24±0.74^{c}	42.49±2.23^{b}
Heptadecanoic (C17:0)	0.15±0.03^{a}	0.23±0.25^{a}	0^{b}	0^{b}	0^{b}	0b
Stearic acid (C18:0)	5.33±0.27^{a}	4.74±0.34^{ab}	3.84±0.39^{c}	4.29±0.23^{bc}	4.25±0.07^{bc}	4.42±0.45^{bc}
Arachidoic acid (C20:0)	0.49±0.02^{a}	0.52±0.16^{b}	0.41±0.05^{c}	0.47±0.05^{a}	0.58±0.13^{d}	0.49±0.11^{a}
Lignoseric acid (C24:0)	0.58±0.05^{c}	0.85±0.14^{b}	0.97±0.09^{b}	1.18±0.08^{a}	0.88±0.04^{b}	0.94±0.10^{b}
Total saturated fatty acids	58.67	50.12	46.53	46.75	46.56	50.57
Palmitoleic acid (C16:1)	0.25 ±0.60^{b}	0.37±0.09^{b}	1.47±0.60^{a}	0.64±0.08^{c}	0.42±0.01^{c}	0.28±0.03^{b}
Oleic acid (C18:1) W9	29.48±1.17^{b}	34.98±1.76^{a}	29.79±1.10^{b}	32.58±0.513^{a}	33.91±0.68^{a}	32.82±0.15^{a}
Linoleic acid (C18:2) W 6	10.98±0.62^{c}	13.53±2.27^{bc}	17.66±0.70^{a}	16.27±0.70^{a}	17.40±0.07^{a}	15.20±1.54^{ab}
Linolenic acid (C18:3) W3	0.60±0.06^{a}	0.65±0.13^{b}	0.65±0.44^{b}	0.86±0.09^{c}	0.68±0.14^{bd}	0.72±0.08^{d}
Decosadienoic (C220:2)	0^{d}	0.16±0.18^{cd}	0.53±0.08^{ab}	0.62±0.05^{a}	0.31±0.14^{bc}	0^{d}
Total unsaturated fatty acids	41.31	49.32	48.63	50.33	52.72	49.02

Description: Mean values (mean±sd) with different alphabetical superscripts within the row are significantly different (P<0.05); NC0: without *Neorospora sp*; NC2: *Neurospora sp* 2% ; NC4: *Neurospora sp* 4%; NC6: *Neurospora sp* 6%; NC8: *Neurospora sp* 8%; NC10: *Neurospora sp* 10%.

Table 4: Amino acids profiles for fermented solid decanter of palm oil sludge.

Amino acids (%)	Treatments					
	NC0	NC2	NC4	NC6	NC8	NC10
			Essential amino acids			
L-Serine	0.70	0.70	0.71	0.71	0.79	0.73
L-Phenylalanine	0.58	0.59	0.59	0.61	0.62	0.64
L-Isoleucine	0.66	0.67	0.69	0.71	0.71	0.72
L-Valine	0.81	0.81	0.84	0.86	0.9	0.89
L-Lysine	0.64	0.65	0.67	0.67	0.7	0.67
L-Leucine	1.07	1.07	1.15	1.16	1.2	1.2
L- Proline	0.77	0.77	0.87	1.11	1.21	1.03
L-Threonine	0.73	0.76	0.78	0.78	0.83	0.86
L-Histidine	0.45	0.46	0.46	0.48	0.48	0.51
Total essential amino acids	6.41	6.48	6.76	7.09	7.44	7.25
			Non essential amino acids			
L-Glutamate acid	1.38	1.49	1.57	1.71	1.79	1.66
L-Alanine	0.8	0.81	0.86	0.86	0.9	0.91
L-Arginine	0.49	0.5	0.53	0.55	0.57	0.54
Glycine	0.73	0.74	0.75	0.75	0.77	0.8
L-Aspartate acid	1.14	1.14	1.24	1.27	1.32	1.34
L-Tyrosine	1.3	0.31	0.31	0.34	0.31	0.31
Total non essential amino acids	5.84	4.99	5.26	5.48	5.66	5.56

Description: NC0: without *Neorospora sp*; NC2: *Neurospora sp* 2%; NC4: *Neurospora sp* 4%; NC6: *Neurospora sp* 6%; NC8: *Neurospora sp* 8%; NC10: *Neurospora sp* 10%.

believed to be caused by mold activity, where during the fermentation process *Neurospora sp* produces enzymes that can break down sugars and degrade complex phenolic compounds, thereby adding phenol groups to form flavonoid compounds (Dwiputri, 2018).

Fatty acid profiles

The results indicated that total saturated fatty acids decreased along with increasing concentrations of *Neurospora sp* up to 8% ($P<0.05$) and it decreased at the rate of 10% (Table 3). Meanwhile, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were elevated along with elevation of the mold. The higher the concentration of *Neurospora sp* utilized, the more enzymes and lipids produced. Furthermore, it can be seen that when the concentration of *Neurospora sp* use is increased, the amount of fatty acids decreases, which is believed to increase the concentration of mold use, the more nutrients are needed. Conversely, when nutrients are insufficient, *Neurospora sp* is unable to develope its cells, affecting the product in the form of enzymes. This is supported by Ratledge (1992) that the enzyme activity produced by oleaginous microorganisms will be greater when there are sufficient carbon and nitrogen sources in the substrate. Therefore, it can support mold cell growth, metabolism and can increase the ability of mold to produce fatty acids.

The type of saturated fatty acid found in significant amounts was palmitic fatty acid with a range of 38.09%-49.66%, the highest monounsaturated fatty acid was oleic fatty acid with a range of 29.48% - 34.98%, while the highest polyunsaturated fat is linoleic fatty acid with a range of 10.98% - 17.77%. Oleic and linoleic fatty acids are important fatty acids that can serve as a source of energy and antioxidants in livestock (Al-Saghir *et al.,* 2004). Furthermore, Muchtadi *et al.* (1993) stated that oleic acid, as a MUFA group, will have a function in protecting, lowering LDL cholesterol levels in the blood and increasing HDL cholesterol levels. According to the current findings, fermentation of solid decanter resulted in a 49% decrease in saturated fatty acids and a 38% increase in unsaturated fatty acids. Apart from the level of unsaturated fatty acids in FSD, this study also found omega-3, omega-6 and omega-9, indicating that it has the potential to be employed as a feed source for poultry, particularly laying hens. These three forms of fatty acids will create healthy eggs high in omega-3, omega-6 and omega-9 while inhibiting fat biosynthesis in livestock.

Amino acids profiles

The amino acid profile of FSD is presented in Table 4. Descriptively (Table 4) shows that increasing the use of *Neurospora sp* concentration in the bioconversion process increases the amino acid content of the FSD. The fermentation process of *Neurospora sp* increases every type of amino acid, both essential and non-essential. This increase in amino acid levels is believed to have occurred because the *Neurospora sp* mold can convert nitrogen elements from ammonium and carbon components from palm oil sludge into amino acids during the fermentation process. Furthermore, Nuraini (2006) stated that *Neurospora sp*

is a mold that can hydrolyse complex proteins into peptides and free amino acids and can produce amylase and hemicellulose enzymes. Similarly, amino acids increased in fermented-casava skin using *Trichoderma viridae* (Ezekiel *et al.,* 2010). Based on the type of amino acid content of FSD, the highest values in both essential amino acids and non-essential amino acids in general were achieved at the rate of 8%. Several types of amino acids in FSD in this study were higher than the amino acid levels obtained by Sinurat *et al.* (2012). Those amino acids are phenylalanine, valine, lysine, leucine, threonine and alanine at the level of 0.39%; 0.55%; 0.49%; 0.71%; 0.31% and 0.70%, respectively, compared to the values at 0.64%; 0.90%; 0.70%; 1.20%; 0.81% and 0.91%, respectively in the present study. The essential amino acids found were threonine and leucine, which were at the higher levels in the range of 0.73-0.86% and 1.07-1.2% compared to the fermentation results of cassava flour by Martono *et al.* (2016) found 0.3% and 0.65% for threonine and leucine, respectively.

CONCLUSION

Inoculation of *Neurospora sp* mold in the solid decanter of palm oil sludge as a growing medium improved its nutrient values. The use of *Neurospora sp* mold at the rate of 8% increase in antioxidant activity from 133.02 ppm to 75.14 ppm which was elevated by 56.49%. Beta-carotene concentration increased from 865.70 mg/100g to 1481,11 mg/100 g, an increase of 190.80%. Meanwhile, there was an increase of 25.53% and 56.65% for total polyphenols and flavonoids, respectively. Furthermore, the concentration of saturated fatty acids decreased by 32.08% and conversely the concentration of unsaturated fatty acids increased by 28.90%. Essential amino acids increased by 16.07% and non-essential amino acids decreased by 3.08%.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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