# STUDI PENDAHULUAN KADAR LEMAK, KADAR PROTEIN DAN KADAR KAROTENOID PADA SUBSTRAT AMPAS KELAPA YANG DIFERMENTASI DENGAN NEUROSPORA SPP

*A Preliminary Study Of Fat, Protein, and Carotenoids Content On Fermented Coconut Dregs By Neurospora Spp*

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**ABSTRAK**

*Proses fermentasi dengan kapang Neurospora mampu meningkatkan kualitas gizi bahan pangan. Ampas kelapa adalah limbah bahan pangan yang dapat difermentasi sehingga kualitas nilai gizinya meningkat. Tujuan dari penelitian ini adalah menguji kadar lemak dan kadar protein dan kadar karoten ampas kelapa yang difermentasi dengan jamur Neurospora sp. Ampas kelapa difermentasi selama 2, 3, 4 dan 5 hari dengan inokulum Neurospora sp. Ampas hasil fermentasi masing-masing kemudian diuji kadar protein dan kadar lemak mengacu pada metode SNI 01-2891-1992 dan SNI 01-2891-1992. Hasil pengukuran menunjukkan terjadinya peningkatan kadar protein dan penurunan kadar lemak pada ampas kelapa selama masa fermentasi. Kenaikan kadar protein paling tinggi sebesar 69.27% dicapai pada hari ke-2 fermentasi dan penurunan kadar lemak paling tinggi sebesar 26.21% dicapai pada hari ke-5 fermentasi. Proses fermentasi juga menaikan kadar karotenoid ampas dari tidak terdeteksi menjadi 1.95 µmol/L. Dari studi ini dapat disimpulkan bahwa kualitas gizi ampas kelapa dapat ditingkatkan melalui proses fermentasi menggunakan jamur Neurospora sp*

**Kata kunci:** ampas kelapa, *Neurospora sp.,* karotenoid, fermentasi padat

***ABSTRACT***

The fermentation process with Neurospora mold can improve the nutritional quality of food. Coconut dregs can be fermented *using neurospora sp* to increase its nutritional value. The purpose of this study was to test a fat, protein, and carotenoids content of coconut dregs fermented with Neurospora sp at different fermentation time 2, 3, 4 and 5 days). The protein and fat content of fermented coconut dregs was determined according to SNI (Standar Nasional Indonesia ) No. 01-2891-1992 and SNI 01-2891-1992 methods. The results show an increase in protein and carotenoids content and a decrease in fat content in coconut dregs during fermentation period. The highest increase in protein content by 69.27% was achieved on day 2 of fermentation, the highest decrease in fat content by 26.21% was achieved on day 5 of fermentation, meanwhile the carotenoids content become 1.95 (µmol/L) on day 5 of fermentation process. Results of this study confirmed the improvement of coconut dregs nutritional value after fermentation with *Neurospora sp*

**Keywords**: coconut dregs, *Neurospora sp*, carotenoids, solid fermentation

## INTRODUCTION

*Neurospora sp*, a member of carotenoid pigment-producing fungi group, commonly used in fermentation proses of peanut and tofu waste to produce Indonesian traditional food namely *oncom*. The oncom cake produced from this fermentation process has known for the bright orange color comes from its carotenoid content (Pahlevi *et al*., 2008). The fermentation process with the Neurospra sp fungi can improve the nutritional value of food. During the fermentation process, the fungi produce enzymes with high lipolytic activity which hydrolyzes triglycerides to free fatty acids, decreases the percentage of crude fiber and increases protein content and produce some vitamins such as riboflavin, vitamin B 12, provitamin A and other carotenoids compounds (Nurhaita *et al.,* 2012, Nurfaizin and Matitaputty, 2015).

Previous studies have shown the protein content of citrus peel fermented with *Neurospores sitophyla* increases between 15% to 18% (Shojaosadati *et al.,* 1999). Other type of *Neurospra* sp also known to produce phytase enzymes (Kanti and Sudiana, 2016), the enzymes of the phosphatase group which catalyze the hydrolysis of phytic acid -indigestible organic from phosphorus organic phosphate- into inorganic phosphate that is easily absorbed in the digestive system (Mullaney E.J., 2000).

Other studies reported that plant-based products fermented with *N. sithophyila* and *N. intermedia* have shown higher antioxidant activity (Tapati *et al.,* 2016), reduce cholesterol levels in blood plasma (Matsuo M. 2000), significantly inhibits the formation of aflatoxin in peanut products (Husein *et. al.,* 2007) and inhibits the formation of toxoflavin and bongkrekic acid in fermented coconut dregs products (Setiarto, 2017). Some fermented food also show the beneficial effect on brain and cognitive function (Kim *et al*., 2016).

Coconut dregs could easily obtained in abundant quantities. Dry coconut dregs according to Balasubramanian analysis (1976) contain 93% carbohydrates consisting of 61% galactomannan, 26% mannose and 13% cellulose. Galactomannan had been reported to be very effective in reducing cholesterol (LDL) levels in blood plasma. Previous study confirmed confirm an increase the quality of coconut dregs after being fermented with the *Aspergillus niger* (Heri *et al.,* 2016), but there has been no research related to the fermentation of coconut dregs using Neurospora sp. Thus The purpose of this study was to determine changes in protein, fat and carotenoid content in coconut dregs fermented with the *Neurospora spp.* fungi.

## METHODOLOGY

### Material

The main materials used were coconut dregs and *Neurospora sp* derived from *oncom* mold. Both materials was obtained from local market in Depok City of West Java Province,

Indonesia. Other materials were wheat flour, aquadest, 70% ethanol, concentrated H2SO4, 30% NaOH, 2% boric acid, phenolphthalein indicator, HCl 0.05 N and n-hexane solvent. The randomized design using 5 different samples with two replications as follows was applied as follows:
Group 1 (G1): coconut dregs without fermentation
Group 2 (G2): 2 days fermented coconut dregs
Group 3 (G3): 3 days fermented coconut dregs
Group 4 (G4): 4 days fermented coconut dregs
Group 5 (G5): 5 days fermented coconut dregs

**Solid Fermentation Process**

The solid fermentation process was carried out based on the traditional *oncom* processing with a slight of modifications. A total of 1 kg of coconut dregs is washed and pressed with cheesecloth until no water drips. The dregs was then mixed evenly with 50 grams of wheat flour and steamed with boiling water for 40 minutes then cooled. The steamed coconut dregs were mixed with 100 grams neurospora mold taken from the surface of *oncom* cake then placed in a sterile fermentor box each100 grams. Fermentation process was carried out at a room temperature under aerobic conditions. Antioxidant activity, protein content, and fat content was tested on the second, third, fourth and fifth day of dregs fermentation. Prior to the test, the fermented coconut dregs is dried in an oven at 55 ° C, mashed, then filtered with 40 mesh sieve. Each treatment was conducted in 2 replications.

**Protein Content Test (01-2891-1992)**

The protein content of fermented dregs was tested using the semi-Kjedahl method reffering to SNI 01-2891-1992 of the Indonesian National Standardization Agency. Each dried sample (ca. 0.5 g) was added with 2 g of selenium reagen and 25 ml of concentrated H2SO4 in a100 ml Kjeldahl flask. The mixture was boiled for two hours to produce clear-greenish solution then transferred to 100 ml volumetric flask and made up to reach the mark line with distilled water. Five 5 ml of solution was added with 5 ml of 30% NaOH and a few drops of PP indicator then distilled for 10 minutes. The distillated solution was mixed with 10 ml of 2% boric acid with few drop of indicator followed by titration process using HCl 0.05 N. The titration was also carried out on blank sample which prepared in the same procedure. Protein content was expressed as a percentage (%) of sample was calculated as follows:

**Protein content (%) :**

$$\frac{[(V_1 - V_2) \times N \times 14{,}008 \times FK \times 100\ \%]}{W}$$

**Note**:
V1: volume of 0.01 N HCl for sample titration (mL),
V2: volume of 0.01 N HCl for blank titration (mL),
N : normality of HCl solution,
W : sample weight (mg),
14,007 is the weight of the nitrogen atom,
FK is a protein factor of 5.2.

**Fat Content Test (01-2891-1992 )**

The fat content of fermented dregs was tested using direct-extraction method referring to SNI 01-2891-1992 of the Indonesian National Standardization Agency. Two grams of each dried sample was put in a paper sleeve, clogged and covered with cotton, dried in an oven at a temperature of 80°C for one hour. The paper sleeve containing sample was extracted in a soxhlet appr. with 500 ml of hexane for approximately 6 hours. The empty flask was weighed prior to extraction process. result of fat extraction in the flask is evaporated with a rotary evaporator until all hexane evaporates. The remaining fat is dried in a drying oven at 105oC to a constant weight. The fat content (%) was calculated as follows:

$$\textbf{Fat content} = \frac{\mathrm{W2} - \mathrm{W1}}{\mathrm{W}}\ x\ 100\%$$

**Note:**

W2: weight of flask after extraction (gr)

W1: weight of empty flask (gr).

W: weight of coconut dregs sample (gr)

**Carotenoids Content Test**

The carotenoids content was tested using spectrophotometric methods. One gram of each dried sample was extracted with 80 mL of acetone, stirred to dissolve the carotenoids the filtered using Whatmann paper no. 1. The absorbance of filtrate was measured using UV-VIS spectrophotometer (100DA-@B-One) at wavelength of 480 nm, 646 nm and 663 nm. The carotenoids content (µmol/L) was calculated as follow:

**Carotenoid (µmol/L)** :

$$\frac{(A480+(0{,}114\ x\ A663))-(0{,}638x\ A645x\ V.10^{3})}{112{,}5\ x\ W}$$

**Note**:

A480 : absorbance at a wavelength of 480 nm

A646 : absorbance at a wavelength of 646 nm

A663 : absorbance at a wavelength of 463 nm

V : volume of extract (mL)

W : weight of sample (g)

**RESULTS AND DISCUSSION**

Physical appearance of fermented coconut dregs can be seen in Figure 1. Its appears that the *Neurospora sp* was able to grow in coconut dregs substrate.

**Figure 1.** Physical appearance of 3 days fermented coconut dregs

**The Protein and Fat Content of Fermented Coconut Dregs**

The protein content of coconut dregs at different length of fermentation day can be seen in

table 1 and figure 2, while the percentage increase in protein content can be seen in table 2, table 3, table 4, and figure 3 describe the fat content of fermented coconut dregs.

**Tabel 1.** The protein content of fermented coconut dregs at different fermentation days

| Length of fermentation (day) | Average of protein content (%) |
|---|---|
| Unfermented coconut dregs (0 day) | 3.71 |
| 2 days fermented coconut dregs | 6.28 |
| 3 days fermented coconut dregs | 5.86 |
| 4 days fermented coconut dregs | 6.21 |
| 5 days fermented coconut dregs | 5.94 |

**Table 2.** The Increase of protein content of fermented coconut dregs at different fermentation days

| Length of fermentation (days) | Increase of protein content (%) |
|---|---|
| 2 | 69.27 |
| 3 | 57.95 |
| 4 | 67.34 |
| 5 | 60.11 |

**Table 3.** The fat content of fermented coconut dregs at different fermentation days

| Length of fermentation (day) | Average of fat content (%) |
|---|---|
| Unfermented coconut dregs (0 day) | 22.20 |
| 2 days fermented coconut dregs | 20.19 |
| 3 days fermented coconut dregs | 19.11 |
| 4 days fermented coconut dregs | 17.00 |
| 5 days fermented coconut dregs | 16.38 |

The overall results showed a significant increase in protein content with the highest protein content of 6,28% was achieved on the second day of fermentation process. The increase of protein content in fermented dregs could be due to the conversion of inorganic nitrogen into mold nucleic acids and cells protein during mold growth in coconut dregs (Laelasari and Purwadaria, 2004) and degradation of protein to release peptides and amino acids (Pranoto *el al*. 2013). Other possible cause of protein increase during the fermentation process is a formation of intermediate compounds during conversion of glucose to pyruvic acid through the glycolysis pathway. Four types of amino acids will be formed subsequently as intermediate compounds through the process of amination or transamination. Amino acid alanine was derived from pyruvic acid which is a product of glycolysis process and the serine was formed from the synthesis of 3-phosphoglycerate. Furthermore serine will produce glycine and cysteine (Mark *et al.,* 1996). When the fermentation process was completed, the molds cell and the remaining substrate that was not separated from a mass called Microbial Biomass Product (MBP). The protein will accumulate in BMP thus increasing the protein content in fermented substrate (Muhiddin, 2001).

**Table 4.** The Increase of fat content of fermented coconut dregs at different fermentation days

| Length of Fermentation (days) | Decrease of fat content (%) |
|---|---|
| 2 | 69.27 |
| 3 | 57.95 |
| 4 | 67.34 |
| 5 | 60.11 |

**Tabel 5.** The carotenoids content of fermented coconut dregs at different fermentation days

| Length of fermentation (day) | Average of carotenoids content (%) |
|---|---|
| Unfermented coconut dregs (0 day) | Not detected |
| 2 days fermented coconut dregs | 0.33 |
| 3 days fermented coconut dregs | 0.83 |
| 4 days fermented coconut dregs | 1.65 |
| 5 days fermented coconut dregs | 1.95 |

**Table 6.** The Increase of carotenoids content of fermented coconut dregs at different fermentation days

| Length of Fermentation (days) | Decrease of Carotenoids content (%) |
|---|---|
| 2 | - |
| 3 | 151 |
| 4 | 98.8 |
| 5 | 18.18 |

The increase of protein content in fermented products can also occur due to a decrease in carbon content in the substrate related to catabolism of fat, carbohydrates and cellulose on the substrates through the respiration process that produces water and CO2 molecules (Cui *et al.,* 2012). This decrease in carbon content will increase the percentage of protein-forming nitrogen in the remaining substrate.

The increase of mold population on coconut dregs should be in line with an increase of protein content because the molds are mostly composed of protein (Wizna, 2009), but the results appears that the protein content was decrease after second days of fermentation. This pattern occurs because the amino acids and peptides formed in the early stages of fermentation are further catabolized as an energy source.

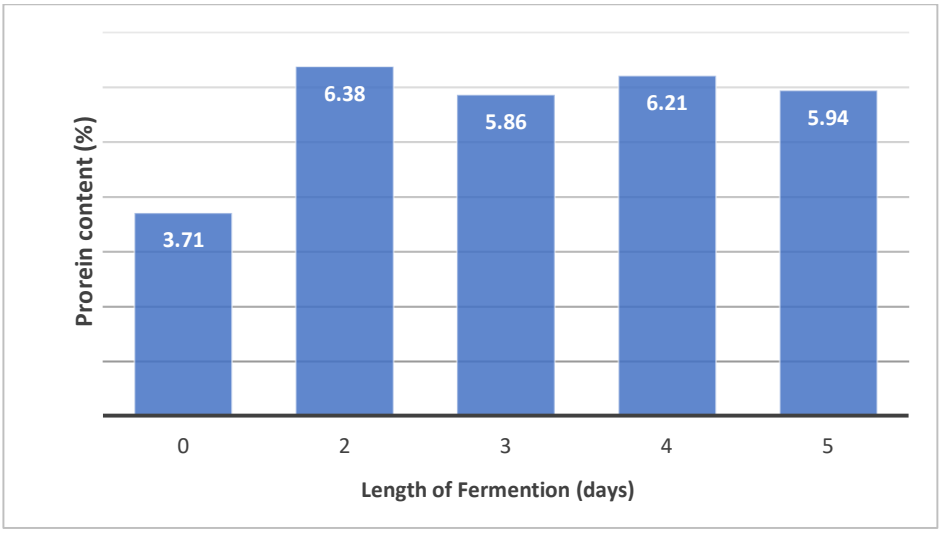


**Figure 2.** Changes in protein content during fermentation process

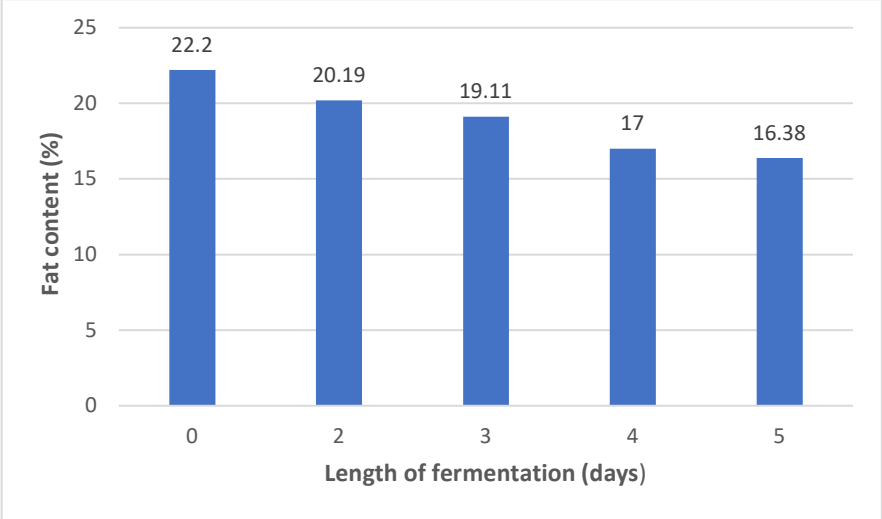


**Figure 3.** Changes in fat content during fermentation process

Contrary to the protein content, a total fat content of fermented coconut dregs constantly decreases during fermentation process starting from 39.92% on the second day to 31, 25% on the fifth day of fermentation. The decrease of fat content describe a high lipolytic activity of *Neurospora sp* which hydrolyze fat or triglycerides into free fatty acids. The lipolytic activity of Neurospora sp has been confirmed in previous research which found the extracellular triacylglycerol lipase from the conidia of *Neurospora crassa* in fermented palm kernel cake (Pratama *et. al.,* 2017).

The carotenoids content of coconut dregs tend to increase during the fermentation process. The interesting fact that the carotenoids was not detected in non-fermented coconut dregs indicate the capacity of *Neurospora sp* to convert certain compounds in unpigmented substrate into pigmented carotenoids compounds. The biosynthetic pathway of carotenoids commonly has same steps with the biosynthetic pathway of other isoprenoids compounds. Since the demand for natural pigment especially in modern food and cosmetics industry increasing rapidly, the capability of *Neurospora sp* to produce carotenoids was potential to be further developed.

## CONCLUSION

The nutrition value of coconut dregs confirmed to increase through fermentation process using *Neurospora sp* derived from traditional oncom cake. The increase of protein and carotenoids content, and the decrease in fat content can be significantly detected with the highest increase of protein content by 9.27%, decrease of fat content was 26.21% and carotenoid production was 1.95 (µmol/L). These

results indicate that of fermentation of coconut dregs with *Neurospora sp* was potential be further developed to produce food ingredients with good nutrition value. Further research should be conducted to discover the biochemical composition of fermented coconut dregs and its effect on health.

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