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Nutritional comparison of *Spirulina* sp powder by solid-state fermentation using *Aspergillus* sp (FNCL 6088) and *Lactobacillus plantarum* (FNCL 0127)

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Abstract. The *Spirulina* sp powder contains high levels of protein and Solid-State Fermentation (SSF) improved protein level. The aims of the study was to find the proximate contents in *Spirulina* sp's powder fermentation. The experiments were conducted by SSF of *Spirulina* sp's powder using fungi *Aspergillus* sp (FNCL 6088) and lactic acid bacteria *Lactobacillus plantarum* (FNCL 0127). SSF was carried out for 10 days at 35% moisture level. The protein contents of *Spirulina* sp's powder fermented by *L. plantarum* were consistently lower ($p < 0.05$) about 43.28% than compare with the other one about 46.12% (SSF by *Aspergillus* sp) until the end of fermentation. The *Spirulina* sp fermented products contained the highest level of protein after 6 days.

Keywords : fermentation, powder, protein, proximate, Spirulina sp

1. Introduction

Dried *Spirulina* powder was containing essential and non-essential amino acids [1]. The amino acid compounds on *Spirulina* are also one potential source of umami such as glutamates and aspartate. The combination of free glutamates and aspartate are high in amount, those commonly found in fermented and aged foods [2]. Solid state fermentation (SSF), an alternative for submerged fermentation for enzyme production, was found to be more favorable, which can be performed under limited financial and labor requirement [3]. SSF have potentially been used for secondary metabolites, for improve the important component in food products. The utilization of *Spirulina* for umami flavor has been made possible by the process of solid-state fermentation (SSF) which involves fermentation in limited moisture levels [4,5]. The aims of this study was to compared the appropriate microorganism (*Aspergillus* sp FNCL 6088 and *Lactobacillus plantarum* FNCL 0127*)* promoting high yield of protein.

2. Material and Methods

2.1 Cultivation for Spore Production and Inoculum Preparation
Selected fungi and LAB strains : *Aspergillus* sp (FNCL 6088) and *L.plantarum* (FNCL 0127) and re-cultured in Potato Dextrose Broth (PDB) (Oxoid) at 30 $\pm$ 1 **℃** in medium bottles. The spores was harvested after 4-5 days of cultivation with sterile distilled water containing 0.1% (w/v) peptone water.

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The spore suspension at 1 x 10^8 spore /ml and volume of 20% (v/w) was mixed with the prior autoclaved growth medium. This medium was used as inoculum for SSF process.

2.2 Substrate Preparation

Solid-state fermentation (SSF) was carried out in 0.5 L conical flasks containing 50 g substrate with the moisture content adjusted to 35% (autoclaved at 121 ℃ at 15 psi pressure for 1 h) for a period of 10 days. An inoculum size of 2 x 10^7 spores 50 g^{-1} substrate was used for each flask and incubated at 30 $\pm$ 2 **℃**. Sampling was done at every 48 h interval starting from days 0 to 10 for both experiments [4] . The substrate was mixed for 5 min prior to sampling process. The sample was used to determine the nutrition composition.

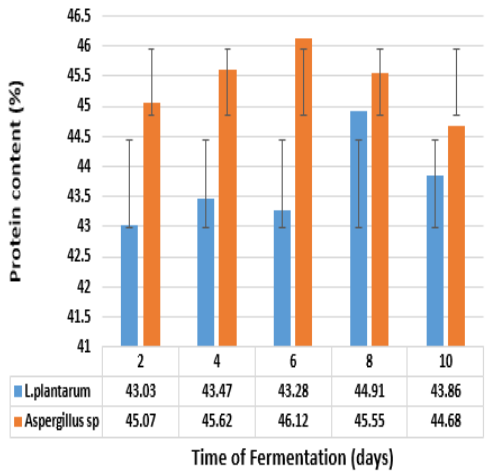
2.3. Fatty acid profile

To analyze the fatty acid composition, fat was extracted according to the method of Folch et al. [6], and 2 mL 0.5N NaOH/methanol was added to 20 mg of fat, which was later saponified for 10 minutes at 105 ^{0}C. It was examined after applying 2 mL boron trifluoride/methanol, and methylated. Then, 2-3 mL hexane (HPLC grade) and 2 mL saturated NaCl solution were added. The supernatant of the mixture used the separated funnel was analyzed by gas chromatography (Hewlett Packard 6890 series; Palo Alto, CA, USA). The column was set up with an HP-FFAP capillary column (25 m x 0.32 mm internal diameter, 0.5 µm film thickness); initial oven temperature of 130 ℃ (1 minute), increased at 2.5 ℃/min to a final temperature of 230 ℃ (10 minutes); injector temperature 230 ℃, detector temperature 250 ℃; helium carrier gas with a spilt ratio of 20 : 1, and flow rate of 1 mL/min.

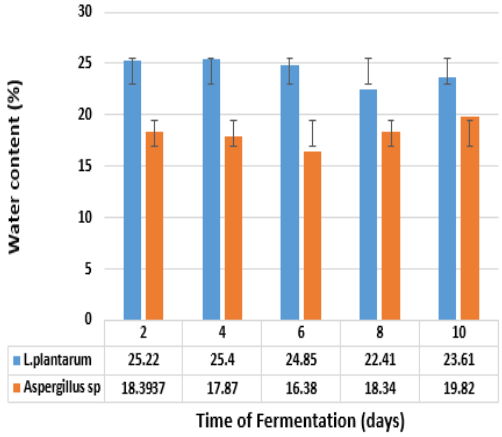
3. Result and Discussion

3.1. The proximate contents of Spirulina sp *with Solid State Fermentation*

Proximate contents of *Spirulina* sp with SSF treatment using isolates culture of *Aspergillus* sp FNCL 6088 and *Lactobacillus plantarum FNCL* 0127 shown in Figure 1.



(a)



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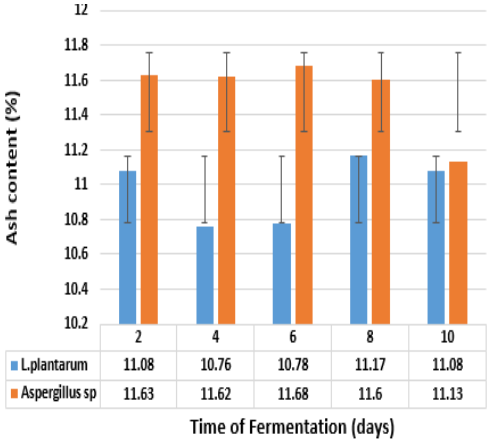
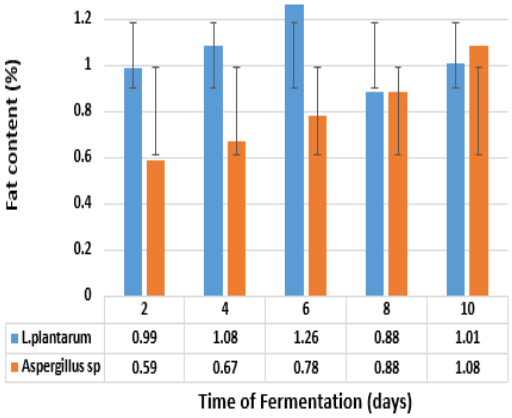
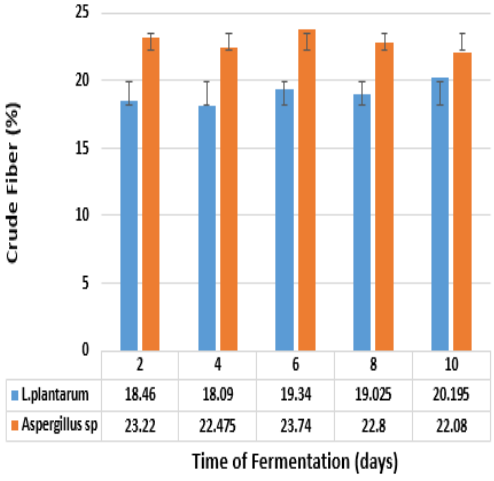


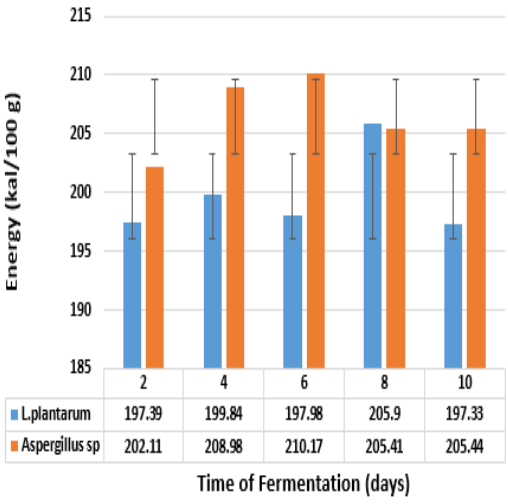
(c)



(d)



(e)



(f)

Figure 1. The proximates composition of Dried Powder SSF *Spirulina* sp : (a) protein , (b) water, (c) ash, (d) fat, (e) crude fiber, and (f) energy

The highest protein of 46.12% *Spirulina* sp SSF with *Aspergillus* sp was obtained after 6 days of fermentation process (Figure 1a). Figure 1 (a) showed that the protein content of *Spirulina* sp treated with bacteria increased in significant level. Figure 1 (d) showed that treatment of SSF of *Spirulina* using *Aspergillus* sp generates that fat content more predictable and tends to increase (p<0.05) during the SSF as compared to the use of *L. plantarum*. Crude fiber content of *Spirulina* sp on Figure 1 (e), tends to fluctuate. For *Aspergillus* sp optimum value is achieved at day 6 while for *L.plantarum* on the 8th day (Figure 1f). SSF treatment with *Aspergillus* sp tends to produce more higher fat and energy value compared to *L. plantarum* since the occurrence of assimilation between media that *Spirulina* sp growth with microbes, especially *Aspergillus* sp. These microorganism suspected converted fat of *Spirulina* sp into protein. The previous study conducted by [7,8] states that the loss of nutrients of fat in palm oil during SSF is because of the conversion of fats in palm oil into biomass protein.

3.2. Fatty acid composition

The fatty acid composition of *Spirulina* sp treated with SSF are shown in Figure 2.

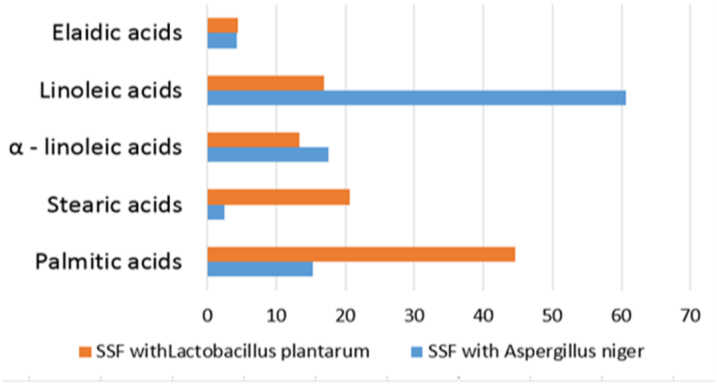


Figure 2. Fatty acids composition of SSF *Spirulina* sp

The *Spirulina* sp SSF with *Aspergillus niger* had the highest unsaturated fatty acids (linoleic acids) concentration at 60.63%, which was significantly higher (p < 0.05) than that of SSF with *L. plantarum* (16.93%). On the other hand, the saturated fatty acids composition of *Spirulina* sp powder treated with *L. plantarum* showed highest proportion (p < 0.05). This result can be seen as an effect of solid state fermentation. Significant improvement in fatty acid content was detected with reduction of amount of substrate in the fermentation process, because the substrate was used by microbial for growth to produce secondary metabolites. This study was similar with the research studied by Lee et. al [3] stated that time of process in fermentation makes the depth substrate was relatively less than begin.

4. Conclusion

The overall study showed that the 6th days until 10 days of SSF were able to increase the protein content of *Spirulina* sp. *Aspergillus* sp isolate was more potential to improve the *Spirulina* protein value compared to *L. plantarum*. The condition of SSF was suitable for growth of *Aspergillus* sp compared with *L.plantarum.*

5. Acknowledgment

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