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Oil content analysis on yam bean fermented by *Aspergillus niger*

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Abstract. The richness and diversity of nutrition contained in yam bean (*Pachyrhizus erosus*) have great potential to be developed in various industries, such as food and health industries. The purpose of this study was to analyze the influence of inoculum' concentration and duration of incubation by *Aspergillus niger* on the level of oil content and glucose in fermented yam bean. Three different levels of solid state fermentation using *Aspergillus niger* molds are grouped as follows: control group (P_0), 0.2 ml/g (P_1), 0.4 ml/g (P_2), and 0.6 ml/g (P_3), and three different durations of fermentation are grouped as incubation control (K_0), 24 hours (K_1), 48 hours (K_2), and 72 hours (K_3). The oil content analysis was completed using soxhlet extractor and n-hexane solvent. The analysis results showed that the highest level of oil content of 70.15% was at the inoculum level of 0.4 ml/g with the incubation time of 96 hours.

1. Introduction

Food and health industries are developed from a variety of local plants, and one of them is yam bean (*Pachyrhizus erosus*). Various industrial products of yam bean have been widely developed in the field of food and health and are available in various forms. As food processing and health biotechnology develop, local plants gain more popularity and have become superior products.

Yam bean is classified in the group of legume plants (Leguminoceae) as a source of vegetable oil. Vegetable oil has been much used in the fields of food, health, and industry. In general, vegetable oils obtained from nuts have been extensively developed, such as those from soybean or peanut as alternatives for coconut as a source of oil. The high level of water in yam bean causes the vegetable oil extracted from yam bean has not yet been developed commercially. The extraction processing of yam bean as a source of vegetable oil requires physical, chemical, and biological processing technologies.

Several methods of extraction in producing vegetable oil from nuts are by mechanical pressing and extraction with solvent pressure [1,2]. The above conventional vegetable oil production is considered to have a low level of economic value, thus there is a need for innovation for efficiency and has a high economic value. One development of vegetable oil products is using a fermentation biotechnology process.

Fermentation is one biological conversion technique from a complex substrate to simpler components using microorganisms such as bacteria and fungi [3]. In regard to this, a fermentation technique called solid state fermentation (SSF) is introduced. SSF is a solid fermentation technology with a water-free condition, but the substrate has to have an adequate level of moisture to support the

growth and metabolism of microorganisms [4,5,6]. SSF has extensively been used in various industrial processes [7]. Its use for a fermentation medium is considered to be economical, as it yields high production volume and does not require any sophisticated machinery [8]. In the SSF fermentation method, mold can adapt much better than yeast or bacteria [9]. Filamentous fungi receive a greater attention because of their ability to produce thermostable enzymes of high scientific and commercial value, such as amylase, cellulase, protease, and lipase [2].

Amylase is an important enzyme used in the industrial process of starchy materials to hydrolyze polysaccharides, namely starch into simple sugars [10]. Cellulase can be used for bioethanol production through a hydrolysis process capable of breaking down polysaccharides into constituent sugar monomers [11]. In regard to this, Aspergillus niger has been found to have the best amylase activity [12]. and highest cellulase production [13]. through the process of solid state fermentation. Aspergillus niger is a potential filamentous mold for fermentation because it has amylolytic and cellulolytic activity and does not produce any mycotoxin.

Further, it is suggested that the enzymatic production process of vegetable oil using *Aspergillus niger* fungi can increase effectiveness and efficiency. Fermentation by *Aspergillus niger* can produce several thermostable enzymes such as amylase, cellulase, protease and lipase [2]. The use of SSF is also carried out for the production of lipase in vegetable oil waste refinement [14]. Therefore, the purpose of this study is to analyze the levels of yam bean oil fermented using *Aspergillus niger*.

2. Materials and methods

2.1. Materials

The yam bean used as the material for the research study was purchased from a traditional market, washed, and crushed by shredding it.

2.2. The making of yam bean flour and LCMS analysis

The yam bean was washed and the skin was not peeled, and directly shredded. The shredded yam bean was then squeezed and settled in order to obtain the sediment. The water is then slowly discharged and the sediment is placed at room temperature until it dries and turns into bright white color. Then, the yam bean flour is prepared for LMCS analysis under the following procedure:

2.2.1. *Extraction.* This step includes weighing yam bean flour at 100 – 250g, dissolving it in 95% methanol with the ration of 1:5, stirring it until it becomes homogenous and putting it still for 24 hours at room temperature in a closed bottle. The next step is filtering the solution using Erlenmeyer vacuum filter so that the filtrate and pulp are obtained. Then, the pulp is taken and repeating the process three times. Next, the obtained filtrate is combined and the next step is conducting evaporation process. The evaporation process is completed using an evaporator to separate the methanol solvent; so that a semi-thick extract is obtained (the solution obtained is half the volume of the previous solution). The obtained extract is then used for the next step.

2.2.2. *Dilution of extract.* The obtained extract is dissolved with methanol until the concentration reaches under 100ppm. In order to dissolve this, the tube is put on a vortex to obtain a homogenous solution. To separate the solids, centrifugation is completed at 8,000 rpm for 10 minutes. Then, the supernatant is obtained and used for the next step.

2.2.3. *Protein precipitation.* The step is completed by taking about 2 ml supernatant extract, putting it into a centrifuge tube, adding 3 ml acetonitrile acidified with 0.2% formic acid. Then the centrifugation process is completed at 8,000 rpm for 30 seconds. The supernatant is then taken.

2.2.4. *Purification with Solid Phase Extract (SPE).* This step is started with putting in Sep-Pak C18 Cartridge (1 cc, 1 mg) which has previously been conditioned with 1ml liquid of 80% acetonitrile and

20% water. Accommodating 0.5 ml of the solution that comes out and adding 1 ml of the sample into the Sep-Pak column, and accommodating another 0.5 ml of the solution that comes out and adding 0.5 ml of 80:20 acetonitrile-water solution to the Sep-Pak column. Then, the next step is accommodating again another 0.5 ml 80:20 acetonitrile-water solution to the Sep-Pak column. Then, the next step includes accommodating again 0.5 ml of the solution that comes out and adding 0.25 ml of 200 mM ammonium formate in a 50:50 acetonitrile-water solution to the Sep-Pak column, taking 0.5 ml of the solution that comes out and adding 0.2 ml of a 25:75 acetonitrile – buffer solution (25 mM ammonium formate, pH 4.5). Then the solution is ready to be used for injection on the LCMS. The solution is filtered with a cellulose acetate of 0.45 μm filter membrane and continued with degassing.

2.2.5. *Solution preparation.* The soluble bottle is cleaned until it is dry and free from any contaminants. All solutions are filtered with 0.45 μm cellulose acetate filter membrane. After filtering, all the solution undergoes a degassing process to remove any gas contained in the solution. The next step is operating LCMS analysis. The sample is injected and analyzed using LCMS method according to the procedure outlined by Shimadzu-Japan. The LCMS procedure is then completed in compliance with the procedure proposed in [15,16].

2.3. Making Aspergillus niger inoculum

The making process is initiated by inserting 1 ose *Aspergillus niger* into 500 ml physiological water, adding 10 g glucose, 1.5 g KH2PO4, 0.75g MgSO4 aseptically, then mixed using a shaker with an orbital rotator at 180 rpm for 20 minutes. The inoculum is then incubated for 24 hours at room temperature and used for yam bean fermentation [17].

2.4. Yam bean fermentation

50 g of grated yam bean is put in sterile infusion bottles and added 10 ml physiological water then mixed by being shaken. The substrate is then put into an autoclave at 121ºC for 20 minutes. The substrate that has been sterilized is inoculated with 10ml, 20ml, and 30ml inoculum aseptically and shaken using orbital rotator at 180 rpm for 30 minutes [17]. The fermentation media is then incubated for 0 hour, 24 hours, 48 hours, and 72 hours.

2.5. Soxhlet extraction

A sample of ± 10 g is weighed and put into a thimble of which size is adjusted with the size of the soxhlet extractor used. A 250 ml boiling flask is then installed along with the condenser. The solvent used is n-hexan as many as 1.5 to 2 times to be put into the reaction tube. The soxhlet unit is installed and equipped with a reverse cooler. Heating is then done at the boiling temperature of the solvent. Then, the circulation occurs until the solvent is clear. The extraction is completed for 3 hours. The sample is dried in a vacuum oven at 100ºC for 15 minutes. Determination of the oil content is determined by weighing the sample in the thimble after extraction and after being dried in the oven so that the constant weight is obtained. The oil content is determined using the following formula:

$$\text{oil content} : \frac{b-c}{a} \times 100\%$$

a = sample weight
b = sample weight and filter paper before extraction
c = sample weight and filter paper after extraction

3. Results and discussion

3.1. LCMS Analysis of yam bean flour

The analysis results of yam bean flour complex compounds are depicted in Figure 1.

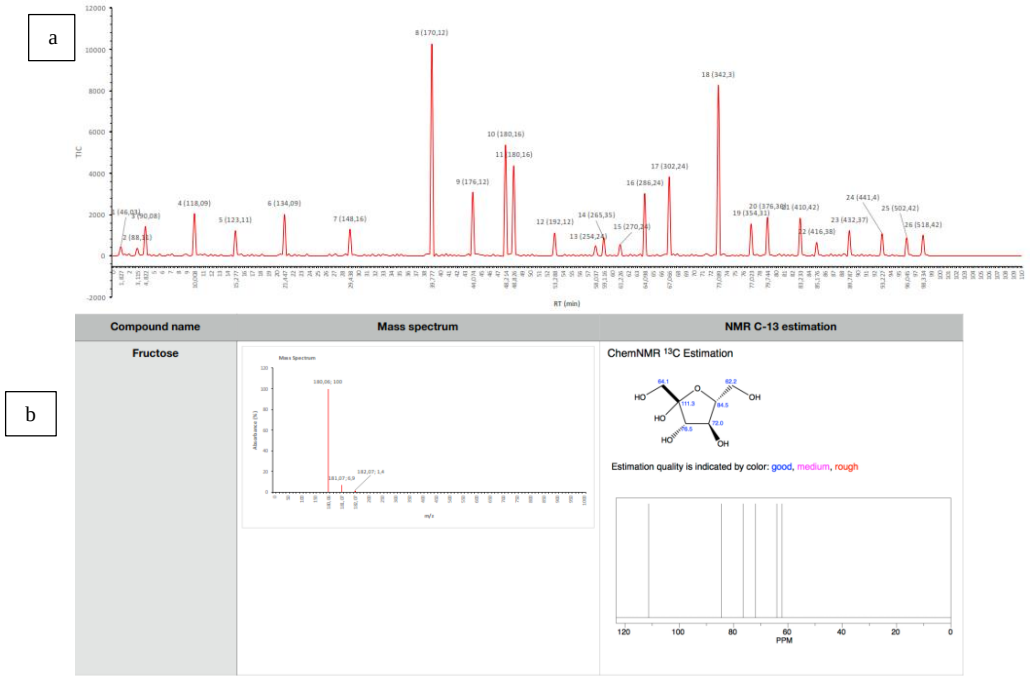

Figure 1. LCMS Analysis of yam bean flour

a) Description: apparatus model: Shimadzu LCMS-8040 LCMS; column: Shimadzu, FC-ODS Shim pack (2mm, 90/100 at 24 min); flow rate: 0.5 ml/min; sampling cone 23.0 V; solvent: CH3CN (0.1% TFA) / H2O (0.1% TFA); MS focused ion mode: ion type [M] +; collision energy: 5.0 V; desolvation gas flow: 600 L/hr; desolvation temperature: 350ºC; ionization: ESI; scanning: 0.6 sec / scan (mz: 10-1000); source temperature: 100ºC; run time: 110 minutes; LCMS method.

b) One example of the NMR spectrum analysis
The complex compounds in the yam bean flour in Figure 1 can be described as in Table 1.

Table 1. Results of LCMS analysis of yam tuber starch

No	Composition (%)	Molecular weight (g/mol)	Name	No	Composition (%)	Molecular weight (g/mol)	Name
1.	0,721	46,03	Formic acid	14.	1,461	265,35	Thiamine
2.	0,613	88,11	Butanoic acid	15.	0,914	270,24	Genistein
3.	2,372	90,08	Lactic acid	16.	5,032	286,24	Kaempherol
4.	3,401	118.09	Succinic acid	17.	6,374	302,24	Quercetine
5.	2,033	123,11	Niacin	18.	13,758	342,30	Maltose
6.	3,339	134,09	Malic acid	19.	2,572	354,31	Chlorogenic acid
7.	2,140	148.16	Cinnamic acid	20.	3,107	376,36	Riboflavine
8.	17,092	170,12	Gallic acid	21.	3,035	410,42	Rotenolone
9	5,132	176,12	Ascorbic acid	22.	1,077	416,38	Daidzin

10.	8,937	180,16	Glucosa	23.	2,039	432,37	Genistin
11.	7,257	180,16	Fructose	24.	1,794	441,4	Folic acid
12.	1,840	192,12	Citric acid	25.	1,461	502,42	Malonyl daidzin
13.	0,810	254,24	Daidzein	26.	1,671	518,42	Malonyl genistin

3.2. Inoculum concentration analysis and incubation duration on oil content

The results of the control treatment (P_0K_0) showed average oil content of 24.45%, while the inoculum concentration treatment was 0.4 ml / g and the treatment time was 72 hours (P_2K_3) the average oil content was 70.15%. The influence of inoculum concentration and incubation time on yam beam fermentation oil content is described in Figure 2.

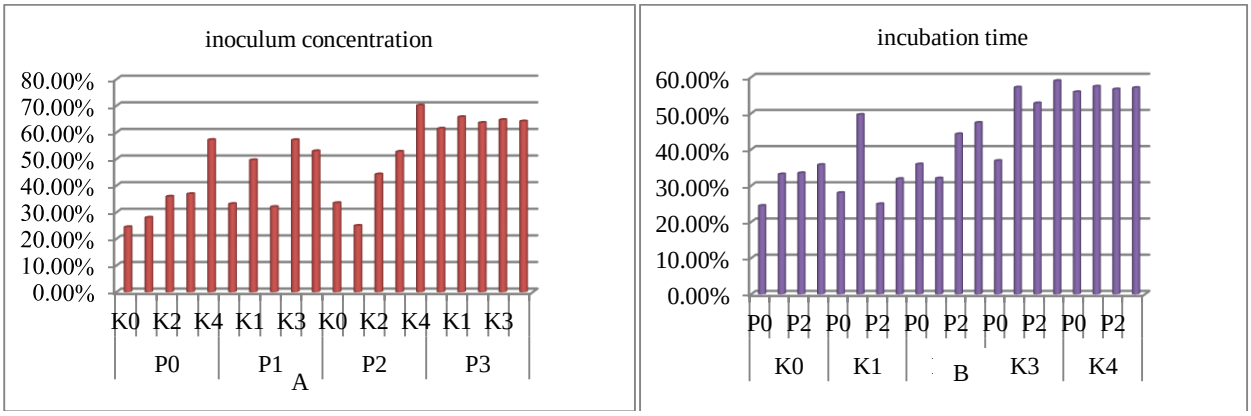

Figure 2. The influence of inoculum concentration and incubation duration on the oil content of fermented yam bean. Inoculum concentration with *Aspergillus niger* control (P_0), 0.2 ml/g (P_1), 0.4 ml/g (P_2) and 0.6 ml/g (P_3); control incubation duration: 0 (K_0), 24 hours (K_1), 48 hours (K_2), and 72 hours (K_3)

Pachyrhizus erosus which is often called yam bean is a plant species that can grow in tropics. Yam bean is originated from Greece, spreading to Central America and Mexico [18,19] and is widely cultivated in Mexico, Africa, the Philippines, and Indonesia [20,21]. Its nutritional values and complex compounds of yam bean make it an important food group so that its conservation and processing are widely developed using food biotechnology and nanotechnology [22]. Yam bean consists of starch/flour of about 56-58% of the dry weight of the yam bean [23].

The results of HPLC analysis of yam bean flour show that there are complex compounds in it (Table 1). The diversity of yam bean complex compounds can be developed into various food, health and industrial products. One of the biotechnology developments of yam bean is through the fermentation technique using mold called *Aspergillus niger*. *Aspergillus niger* can break down complex fibres into much simpler fibers, also inorganic nitrogen sources can also be converted into protein cells and produce hydrolysis enzymes [24].

Fermentation is a conversion technique from a complex substrate into its simpler components by microorganisms, namely bacteria and fungi [3]. Factors that influence fermentation are the inoculum dose and fermentation time, [25,26] this is related to the size of the microbial population that

determines the speed of microbial development in producing enzymes to break down the substrate into simpler components. Increased inoculum concentration affects the performance of *Aspergillus niger* lipase enzyme in hydrolyzing fat. Increasing cell weight and increasing number of cells influence the growth and propagation of these microbes [27]. The volume of inoculum added will affect the metabolic output, namely the lipase enzyme which catalyzes the formation of unsaturated fatty acids and lipids [28]. Several studies have shown that fermentation of solid substrates using *Aspergillus niger* fungi can reduce crude fibre content, increase levels of protein, lipids and digestibility in vitro [29,30].

Increasing incubation time can lead to increased performance of *Aspergillus niger* for growth and fermentation [31,32] so that the longer the incubation time, the higher the chance of *Aspergillus niger* to degrade fermented yam bean. K_1 and K_2 show the results of this increase to be not quite significant because the microbes in the substrate experience lag phase. The lag phase is a phase of microbial adaptation to the new environment. During the lag phase, the cell division takes place slowly. The incubation time that produced the highest oil content was in K_3 because *Aspergillus niger* experienced a logarithmic (log) or exponential growth phase. This is in line with the statement proposed by [33] that *Aspergillus niger* in the exponential phase produces the highest lipase enzyme. [34] states that in the log phase, microbes produce enzymes to synthesize substrates and under optimal conditions microbial cell populations will multiply. The next phase is the stationary phase, the phase where microbes do not develop. This phase of the cell becomes small because the cell continues to divide even though the availability of nutrients in the medium is greatly reduced and ends in the death phase.

4. Conclusion

Yam bean contains complex compounds and fermentation of Aspergillus niger can increase oil content. Further phytochemical testing on yam tuber oil is needed

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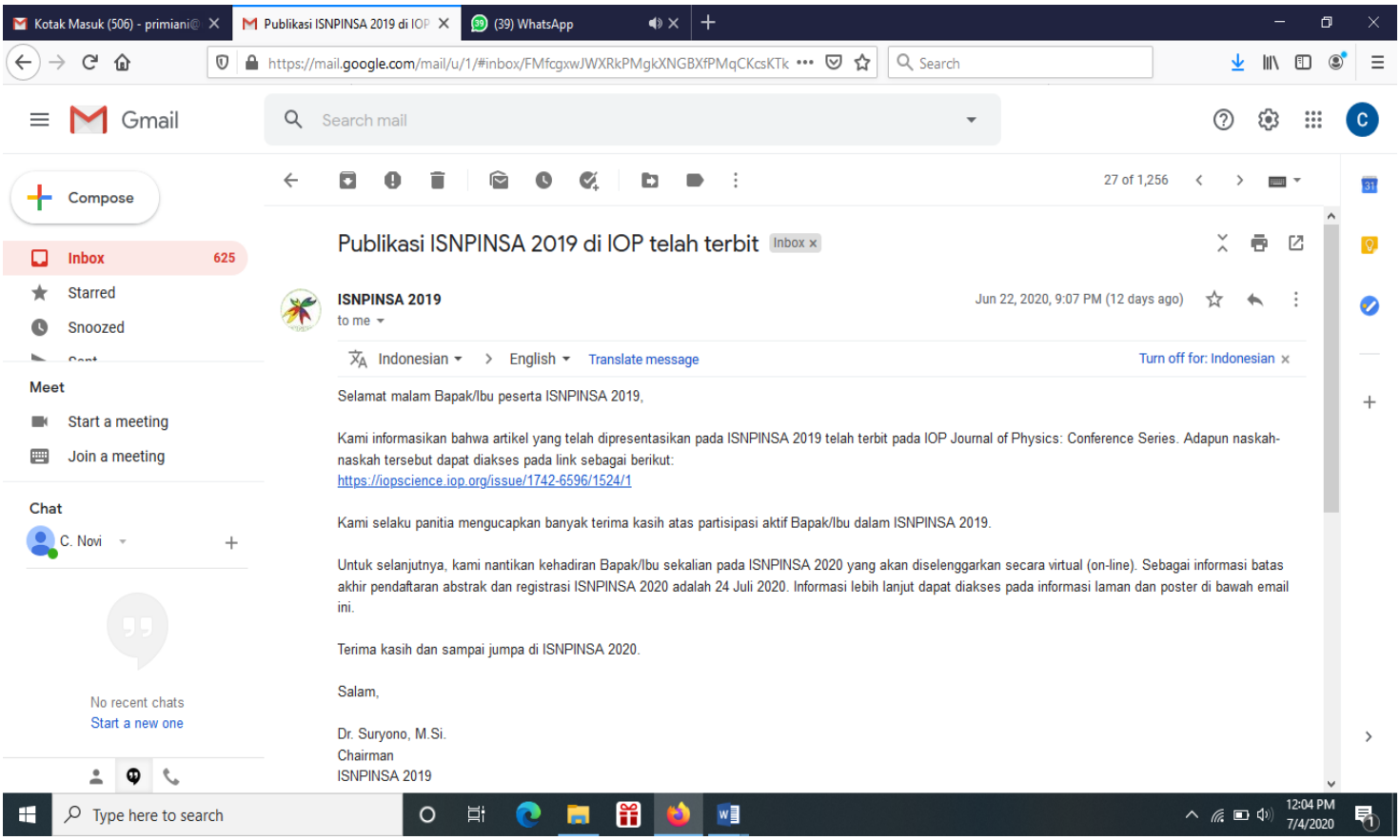
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