

Protein Enhancement in Low-grade Maize by Fermentation with Yeast and Bacteria

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Abstract This study aimed to enhance protein content in low-grade maize to higher than 24%w/w, though fermentation with yeast (*Saccharomyces cerevisiae*, *Candida utilis*, *Candida tropicalis*) and bacteria (*Bacillus subtilis*, *Lactobacillus plantarum*, and *Streptococcus thermophilus*). Starch in low-grade maize was digested through enzyme hydrolysis into monosaccharide or glucose, which used as the main substrate in fermentation. Yeast consumed more glucose (95%) and at a faster rate than bacteria, which consumed glucose less than 20% glucose from low-grade maize hydrolysate. After the fermentation, protein contents from the cultures of yeast and bacteria significantly increased from low-grade maize substrate (8.68%w/w) to 29.05%, 31.30%, 29.85%, 28.95%, 29.85%, and 18.85%w/w when cultivated with *S. cerevisiae*, *C. utilis*, *C. tropicalis*, *B. subtilis*, and *L. plantarum*, respectively, which were significantly increased about 4 times from initial protein content in low-grade maize that higher than 24%w/w protein. But *S. thermophilus* obtained the lowest protein content as 18.85%w/w with 2 times increase. The results indicated that, the cultivation of yeast and bacteria effectively enhanced the protein cell in low-grade maize through fermentation. The reduction in carbohydrate content was inversely proportional to the increase of cell number of biomass and the amount of protein production that can be used as alternative protein sources for feeds.

Keywords: bacteria, fermentation, maize, single cell protein, yeast

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1. Introduction

In developing countries, the continual population growth at an alarming rate leads to an increase in animal and human food supply [1]. The mounting world demand for protein rich food leading to the search for the formulation of alternative protein sources to supplement the conventional protein sources, which are particularly single cell protein (SCP). SCP refers to cells or proteins derived from microorganisms such as bacteria, fungi, mold, algae, and yeasts [2,3]. The SCP can also be called biomass, bioprotein or microbial protein that contains other nutrients like carbohydrates, fats, vitamins, and minerals. Moreover, it is rich in certain essential amino acids like lysine and methionine, which are limiting in plant and animal feed [4]. This protein from microorganism can be used as additive added to the main diet feed. The SCP is produced through the process of fermentation, which uses inexpensive and agricultural wastes as substrates for conversion to

fermentable sugar [2,5].

Most of the agricultural materials with high carbohydrate content are very good substrate for microbial growth because they provide the required carbon for energy production. The microorganisms grown and increased in the cell number and harvested also serve as source of SCP [6]. Maize (*Zea mays* L.) is one of the important feed crops in the world that enrich in carbohydrate content and other basic nutrients has potential to support microbial growth. Although, it is the preferred grains for animal feed, but it is found to be low in protein content as well as protein quality, thereby limiting its nutritional value [7,8,9]. Therefore, this has necessitated a search for improving nutritive value in maize, particularly the protein quality for feed. In this study, we used low-grade maize that is inexpensive crop and low-quality protein as substrate in SCP fermentation replace a high price conventional protein feed. According to the notification of the Ministry of Agriculture and Cooperatives of Thailand in 2015 [10], the protein contents in raw material for animal feeds should in the

range of 24-60%.

Different types of microbes such as yeast and bacteria are used in this present study for producing SCP. Yeasts are probably the most widely accepted as microorganism for SCP production and can grow on agricultural material as well as from hydrocarbon. In addition, yeast biomass is regarded as an inexpensive dietary supplement as it is easily produced on industrial scale [11,12]. *Saccharomyces cerevisiae*, *Candida utilis*, and *Candida tropicalis* have been used in biomass production because of its ability to utilize a variety of carbons sources for support high microbial protein yield, which consist of amino acids for application in several industrial production both for human and animal consumption [13,14,15,16].

Bacteria are capable of growth on a wide variety of substrates, have a short generation time and are high protein content [17]. *Bacillus subtilis* is widely known for its capacity to produce and secrete large amounts of industrially relevant proteins, mostly produce various carbohydrase enzymes for polysaccharide degradation [18,19]. *Lactobacillus plantarum* commonly found in fermented food and in the gastrointestinal tract and is commonly used in the food industry as a potential starter probiotic in many types of food fermentations [20]. *Streptococcus thermophilus* is widely used for its high acidification ability in dairy products and presents many feature that make it a good production of heterologous protein [21].

This study aimed to increase the protein content in low-grade maize to higher than 24%w/w by fermentation of yeast and bacteria (*S. cerevisiae*, *C. utilis*, *C. tropicalis*, *B. subtilis*, *L. plantarum*, and *S. thermophilus*) for single cell protein production.

2. Materials and Methods

2.1. Raw Material Preparation

Low-grade maize (*Zea mays* L.), grains with high moisture content, discoloration, and incomplete or broken kernels were obtained from Phatananikom Kaset Ltd, company in Phrae province, Thailand. Maize grains were ground and sieved to give around 2 mm particle size. The samples were kept in double polypropylene plastic bags and stored at room temperature (25-30°C) until used [22].

2.2. Starch in Low-grade Maize Degradation by Enzyme Hydrolysis

Starch degradation included two steps of liquefaction and saccharification with α -amylase and glucoamylase enzymes, respectively. Starch in low-grade maize was firstly hydrolyzed with α -amylase from *Bacillus licheniformis* with activity of 150,000 U/ml (purchased from Union Science Co. Ltd.) then the hydrolysate continuously hydrolyzed with glucoamylase from *B. licheniformis* with activity of 260,000 U/ml (purchased from Union Science Co. Ltd.). Low-grade maize sample was gelatinized by autoclaving at 121 °C 15 psi for 15 min. After gelatinization, the sample was let cool down to the temperature in range of 70-75 °C. Then the heated sample was mixed with 1 ml of 10 mM CaCl_2 and α -amylase, 72 U/g of maize in the liquefaction. The mixture was

incubated with shaker water bath at 75 °C, 150 rpm for 3 h. Then the maize hydrolysate was continuously added with glucoamylase, 175 U/g of maize in the saccharification. The mixture was incubated in shaker water bath at 55 °C, 150 rpm for 6 h. The low-grade maize hydrolysate after enzymes hydrolysis was use as the substrate in the fermentation process.

2.3. Microorganism Preparation

Saccharomyces cerevisiae TISTR 5328, *Candida utilis* TISTR 5352, *Candida tropicalis* TISTR 5136, *Bacillus subtilis* TISTR 001, *Lactobacillus plantarum* TISTR 541 and *Streptococcus thermophilus* TISTR 894 were provided by Thailand Institute of Scientific and Technological Research. Cultures of yeast cell *S. cerevisiae* TISTR 5328, *C. utilis* TISTR 5352, and *C. tropicalis* TISTR 5136 were incubated in 250 ml Erlenmeyer flask that contained YMB medium. YMB medium was composed of 3.0 g yeast extract, 3.0 g malt extract, 5.0 g peptone, 10.0 g glucose in 1 L distilled water, and pH adjusted to 4-6. Three strains of bacterial cell were incubated in in 250 ml Erlenmeyer flask. *B. subtilis* TISTR 001 was cultured in nutrient broth (NB) that consist of 5.0 g peptone and 3.0 g beef extract in 1 L distilled water. Whereas, *L. plantarum* TISTR 541 and *S. thermophilus* TISTR 894 were cultured in MRS culture medium that composed of 10.0 g peptone, 10.0 g beef extract, 5.0 g yeast extract, 20.0 g glucose, 1.0 ml tween 80, 2.0 g K_2HPO_4 , 5.0 g sodium acetate, 2.0 g tri-ammonium citrate, 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.2 g $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ in 1 L distilled water, pH adjusted to 4-6. Erlenmeyer flask was filled by 50 ml of culture medium and sterilized by autoclaving at 121 °C, 15 psi for 15 min before used. For inoculum starter culture preparations, one single colony of yeast and bacterial cell was added into the culture media flask and then incubated at 30 and 37 °C for yeast and bacteria, respectively with continuously shaken at 100 rpm for 24 h [23].

2.4. Yeast and Bacteria in Low-grade Maize Fermentation

Low-grade maize culture medium was prepared to obtain glucose or monosaccharide that is fermentable sugar as the method of starch in low-grade maize degradation by enzymatic hydrolysis. Then 15 ml of each inoculum (*S. cerevisiae*, *C. utilis*, *C. tropicalis*, *B. subtilis*, *L. plantarum*, and *S. thermophilus*) with the cell concentration of 10^6 cfu/ml was added into semi-solid paste of low-grade maize medium flask [6]. All fermentable flasks with the except of 0 h, were placed into incubator shaker at the constantly rotate 100 rpm, incubated in 30 and 37°C for yeast and bacteria, respectively. Biomass product in a form of slurry, liquid filtrate, and solid residue was collected every 12 h for 120 h, for determine the biomass production in term of cell number counting, glucose content, and the amount of protein. The experiments comprised in three replicated experiments.

2.5. Determination of Yeast and Bacterial Cell Growth Curves

Growth of the yeast and bacterial cells were defined by

colony counting of the eighth diluted plate (from 10^{-1} to 10^{-8} serial dilutions) by pour plating method [24]. The culture medium was collected every 12 h for 120 h during incubation. One ml of culture medium was diluted about 10^{-1} times with 9 ml of sterilized distilled water and the dilutions were introduced to 3 separate plates then covered with melted culture medium agar. The culture plates were incubated at 30 °C for 3 days and colony of microbial cell was counted and recorded at the 30 to 300 colonies contained plate. The living cell numbers were plotted the common growth curve of microorganisms which were composed of lag, log, stationary, and decline phases.

2.6. Determination of Glucose Content

Glucose content was analyzed by dinitrosalicylic acid (DNS) method using glucose as a standard [25]. The reaction was carried out by mixing 0.5 ml of liquid part culture medium with 0.5 ml of dinitrosalicylic acid solution (DNS). The mixture was boiled for 15 min and immediately cooled down to room temperature. The absorbance was read by spectrophotometer at 540 nm and the reducing sugar concentration was calculated by using standard curve constructed with known concentration of glucose.

2.7. Determination of Protein Content

Protein content was determined by Bradford method and Kjeldahl method. After fermentation, biomass samples were collected and centrifuged at 3000 rpm for 10 min which separated liquid and solid part. The solid residue was dried at 50°C for 48 h kept in sealed plastic bag at room temperature until used in the protein analysis.

2.7.1. Bradford Method

Protein was extracted from dried biomass by soaking with 0.1 M pH 7.6 phosphate buffer for 12 h and the supernatant was collected for protein analysis by Bradford assay which use bovine serum albumin (BSA) as a standard. The reaction was carried out by mixing 0.1 ml of supernatant with 3 ml of Coomassie Brilliant Blue G-250 reagent. The mixture was left at room temperature during 2 to 60 min and mixed well by vortex. The absorbance was recorded by spectrophotometer at 595 nm and the protein concentration was calculated by using standard curve of BSA [26].

2.7.2. Kjeldahl Method

Accurately weighed of 0.10xx g dried biomass sample was mixed with 3.5 g K_2SO_4 and 0.4 g $CuSO_4$ as catalyst to digest by adding 15 ml sulfuric acid in Kjeldahl flask at high temperature about 350-380 °C for 30 min until a clear solution was obtained. All nitrogen bonds in the sample were broken by digestion procedure and convert all organically bonded nitrogen into ammonium ions (NH_4^+). After digestion was completed, the sample was allowed to cool to room temperature, then diluted with water and transferred to the distillation unit. The digested solution was added with 40% NaOH in the digestion step that the ammonium ions (NH_4^+) were converted into ammonia (NH_3) and captured by 2% boric acid solution. Then the solution was directly titrated with 0.01 M HCl and a mixture of indicators. The titration was completed when

the green solution became a slightly violet color. The volume and concentration of HCl needed were used to calculate the number of mol of nitrogen atoms in the sample and then the percentage of protein in sample also calculated [27].

2.7. Statistical Analysis

Results were analyzed and data recorded as mean $\pm$ SD in three replications (n=3). The statistical treatment of obtained data and mathematical calculations of models generated were performed by employing the IBM SPSS statistics (Version 22). The analysis of variance indicated significant F-values comparisons were all accomplished with a confidence level of 95%.

3. Results and Discussions

3.1. Determination of Glucose Content

The initial glucose content of low-grade maize grain was 10.28 ± 0.43 g/l but the glucose content increased after starch in maize grain was hydrolyzed through two separate steps of enzymes hydrolysis, liquefaction and saccharification by α -amylase and glucoamylase, that provided glucose contents made up 85.20 ± 1.08 and 112.50 ± 0.98 g/l, respectively. Maize starch was hydrolyzed into simple fermentable sugars, which was fermented using the strains of yeast (*S. cerevisiae*, *C. utilis*, *C. tropicalis*) and bacteria (*B. subtilis*, *L. plantarum*, *S. thermophilus*) to acquire the single cell protein in the fermentation.

After inoculation with yeast, glucose content in the low-grade maize culture media rapidly decreased during the cultivation time from 0 to 120 h. The glucose content in the culture media of *S. cerevisiae* decreased faster than *C. tropicalis* and *C. utilis* as shown in Figures 1-3. The lowest amount of glucose in culture of *S. cerevisiae*, *C. tropicalis*, and *C. utilis* were presented at 36, 84, and 84 h, respectively. Glucose content in the culture of yeast was significantly decreased from about 100 g/l (at 0 h) to nearly 0 g/l. From the results, yeast strains could consume glucose from low-grade maize hydrolysate more than 95%. The glucose content in low-grade maize culture media decreased after fermentation for 84 h as presented in Figure 7. The amounts of glucose remained in the culture of three strains yeast, *S. cerevisiae*, *C. utilis*, and *C. tropicalis* were 1.49 ± 0.02 , 2.70 ± 0.06 , and 4.09 ± 0.04 g/l, respectively in which the culture of *S. cerevisiae* remained the lowest glucose content. Glucose is differently degraded in varies yeast types: *S. cerevisiae* is glucose sensitive yeast in the presence of surplus glucose for use in fermentation but *C. utilis* and *C. tropicalis* are the intermediate types [28,29]. So, in this study *Saccharomyces* yeasts showed the higher fermentative power compared to the most non-*Saccharomyces* yeasts. However, many microorganisms usually prefer to grow in the culture media that contain glucose as carbon and energy source [30].

The initial glucose content of bacteria, *L. plantarum* and *S. thermophilus* culture (124.92 ± 3.12 g/l) were higher than yeast culture because of the amount of glucose in MRS broth for *L. plantarum* and *S. thermophilus*

inoculum higher than YM broth for yeast inoculum. Nevertheless, there was no glucose content in NB broth for *B. subtilis* inoculum, therefore the initial glucose of *B. subtilis* culture showed the lowest content (90.12 ± 1.09 g/l). From Figures 4-6, remaining glucose contents in bacterial culture were higher than yeast culture, which means bacterial cell consumed glucose less than 20%. *L. plantarum* used glucose more than *S. thermophilus* and *B. subtilis*. After fermentation for 84 h, the amounts of glucose remained in the culture of *B. subtilis*, *L. plantarum*, and *S. thermophilus* were 77.24 ± 1.01 , 89.68 ± 1.53 , and 103.81 ± 1.71 g/l, respectively (Figure 7). Many strains of bacteria like bacilli and lactic acid bacteria are able to use glucose as sources for carbon and energy to synthesis a variety of organic acid. However, *B. subtilis* and lactic acid bacteria secrete amylolytic enzymes to hydrolyze chains of α -1, 4 and α -1, 6 glycosidic linkages into simple sugar [31,32]. In this study, starch from low-grade maize was degraded to glucose by secreted enzymes from bacterial strain, then glucose was consumed for bacterial cell production. Therefore, the large amounts of glucose were remained in bacterial culture during fermentation.

3.2. Determination of Yeast and Bacterial Cell Growth

All microbial strains in this study were able to generate and reproduce cell in low-grade maize culture, which showed the growth behavior in four main phases as follow: lag, log (exponential), stationary, and death phase as shown in Figure 1-6. Lag phase duration of *S. cerevisiae* and *C. utilis* shown the shortest time at 0-12 h follow by *C. tropicalis*, *B. subtilis* and *L. plantarum*, which occurred at 0-24 h. While *S. thermophilus* shown the longest time at 0-36 h. After that, the cells of bacterial strains were generated in exponential phase for 36 h in which *B. subtilis* and *L. plantarum* entered to exponential phase earlier than *S. thermophilus*; the exponential phase of *B. subtilis* and *L. plantarum* revealed during 24-60 h but *S. thermophilus* revealed during 36-72 h. Whereas, the generation times of three yeast strains in exponential phase was different with *S. cerevisiae*, *C. utilis*, and *C. tropicalis* being generate for 24, 36, and 48 h, respectively. Moreover, *S. cerevisiae* and *C. utilis* entered to exponential phase earlier than *C. tropicalis* and three bacterial strains. At the end of this phase, cell numbers reached the highest peak, and number of cell produced can be ranked from high to low as, *C. utilis*, *L. plantarum*, *S. thermophilus*, *S. cerevisiae*, *C. tropicalis*, and *B. subtilis*. In the culture of *C. utilis* (Figure 2), the highest cell number was observed at 60 h with 8.84 log cfu/ml that showed the highest cell number with the high growth rate. The culture of *B. subtilis* showed the lowest cell number at 72 h with 6.84 log cfu/ml as presented in Figure 4.

The stationary phase entry time of most yeast occurred earlier than bacteria after exponential phase of 36-48 h, which was related to the glucose content in low-grade maize. In the case of yeast cells after exponential phase, remained glucose contents were close to 0 g/l. Yeast could consumed glucose more than bacteria, therefore yeast utilized glucose to generate the cells with faster growth rate and also entered death phase faster. *S. cerevisiae* and

C. utilis entered death phase at 84 and 96 h, respectively but all bacteria enter death phase at 108 h. Both cell numbers of yeast and bacteria were related to the glucose content from low-grade maize. The glucose utilization by yeast resulted in rapidly reduced amount of glucose during the exponential phase due to active metabolism and cell generation of the yeast cells. After exponential phase, the available glucose content in yeast culture remained nearly 0 g/l but glucose content was higher in the bacterial cell. Yeast could consume more glucose than bacteria, thus yeast utilized glucose for generate the cells with fast growth rate, also fast entered to the stationary phase. *S. cerevisiae* showed the fastest that entered to the stationary phase because it could consume glucose well and glucose content sharply decreased in the shortest time. Cells enter to the stationary phase, meaning that the cells are no growing and cell number constantly survive in the culture due to the lack of carbon or nutrient source. Therefore, cells stay in a resting state for increased ability to survive the extended periods of starvation [33,34,35]. The remained glucose contents in bacterial culture were more so, bacterial cells entered stationary phase but not due to starvation of carbon source from glucose but maybe of other nutrients. In fact that, excess glucose in media could also inhibit bacterial cell formation and changed in the environment typically caused by high accumulation of cell density [35].

The microbial initiation cells of three strains yeast and bacteria in the individual low-grade maize were 6 log cfu/ml then the cell number of microbial increased after fermentation for 84 h. The cell numbers in the yeast cultures were greater than bacteria cultures. The cell numbers of three strains of yeast, *C. utilis*, *C. tropicalis*, and *S. cerevisiae* were 8.68, 8.02, and 7.98 log cfu/ml, respectively while the cell numbers of three strains bacteria, *L. plantarum*, *B. subtilis*, and *S. thermophilus* were 7.79, 7.69, and 6.80 log cfu/ml, respectively (Figure 8). In comparison, yeast is able to grow better in the high starch substrates as the carbon source than bacteria, and this means that yeast can intensively degrade the starch as a nutritional source of its growth. Yeast will secrete extracellular enzymes both α -amylase and glucoamylase into the culture medium that is effective in improving fermentation process from starchy materials [36,37]. Moreover, *B. subtilis* and *L. plantarum* were also consumed starch from low-grade maize to produce their cell by increasing 28.17 and 29.83%, respectively. Le *et al.* [38] demonstrated that *B. subtilis* and *L. plantarum* are grown in the culture of jackfruit seed starch in which *B. subtilis* reach its maximum growth rate at 0.44 h⁻¹ in the 14.9 mg/ml substrate, while *L. plantarum* reach highest growth rate at 0.16 h⁻¹ in the required 3.84 mg/ml substrate. *B. subtilis* and *L. plantarum* are among the lactic acid bacteria that may synthesize lactic acid from starch via extracellular amylase activity during fermentation. Starchy substrates are hydrolysed to saccharify starch, which are utilized by lactic acid bacteria to produce lactic acid. Therefore, these bacterial strains are able to employ the starch materials for cell growth with high productivity and substrate conversion yield. Even though lactic acid bacteria have the ability to degrade polysaccharides for produce monosaccharides or lactic acid. However, lactic acid bacteria made a greater contribution to fermented dairy products and they have a

weak ability to hydrolyze sugars in grains [32]. Thus, some strains of lactic acid bacteria have a less employ polysaccharide from grains that cause to less in cell production as *S. thermophilus* in this study.

3.3. Determination of Protein Content

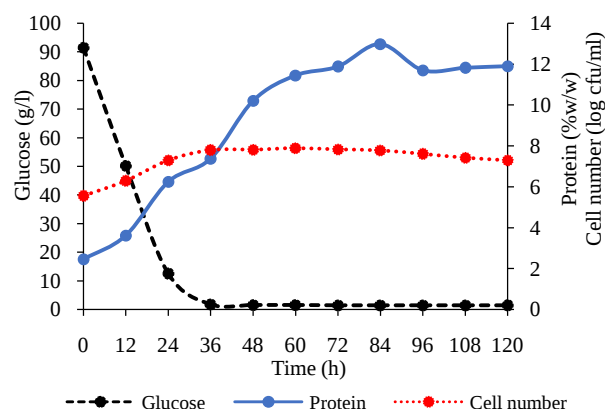
Protein contents in this experiment were analyzed by Bradford method, which focused on soluble protein and basic amino acid. The initial protein of low-grade maize was 1.25 %w/w and protein significantly increased to the maximum content after 72-84 h of fermentation with yeast and bacteria. The protein content showed relationship with cell number and glucose content as presented in Figure 1-6. In addition, the protein content increased during fermentation time due to microbial cells generated in the preference nutrients and suitable environment. The increase of microbial cells resulted in the rise of amount of protein to the highest content with the lowest glucose content in low-grade maize culture. The protein in *C. utilis* culture presented the highest content (15.00 %w/w) that was higher than the highest protein content of *L. plantarum*, *C. tropicalis*, and *S. cerevisiae* as 13.41, 13.00, and 12.98 %w/w, respectively at 84 h of fermentation. Moreover, most strains of yeast in this study produced protein content higher than bacteria especially *B. subtilis* and *S. thermophilus* that provided protein contents 10.66 and 8.12 %w/w, respectively.

Because the microorganisms interact differently to each substrate, production of protein via fermentation also varies with the suitable substrate used [39]. Especially, yeast protein biomass has a higher protein-to-carbohydrates ratio as the result of protein content after fermentation with *S. cerevisiae*, *C. utilis*, and *C. tropicalis*. As shown in the report of Tian *et al.* [40], *S. cerevisiae* can efficiently convert glucose, sucrose, and maltose. *C. utilis* can metabolize a variety of carbon sources and survive in various complex matrices. *C. tropicalis* has strong ability to degrade starch and hydrocarbon in organic matters.

The strains of yeast and bacteria that could be attribute to the activities and increase in the number of microorganisms during fermentation. Some of producing microbe such as filamentous cell, may be multicellular as provide to protein or its multiplication in form of SCP [41,42]. Moreover, the protein contents of starch materials after fermentation presumably increase due to extracellular protein secretion by microorganisms in metabolization. This corresponds to the research of Oboh and Akindahunsi [43], in which protein level in cassava product increased due to possible secretion of some extracellular enzymes (proteins); amylase and cellulase into the substrates which break starch or polysaccharides into simple sugars that are easily metabolized by yeast and bacteria as a source of carbon [44].

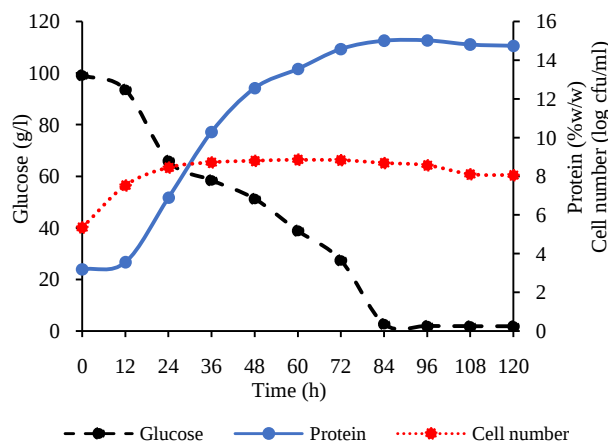
Protein contents from Kjeldahl analysis is presented in Figure 9. The protein contents were observed in the culture after fermentation for 84 h of *S. cerevisiae*, *C. utilis*, *C. tropicalis*, *B. subtilis*, and *L. plantarum* as 29.05, 31.30, 29.85, 28.95, and 29.85% w/w, respectively, which increased from the initial protein of

low-grade maize (8.68% w/w) and protein increased to higher than 24% w/w. These results indicated that the protein percentage increased at the end of substrate fermentation by yeast and bacteria. Generally, protein of final substrate after fermentation originates from two main sources: material and yeast or bacterial cell. During starch fermentation, the growths of yeast or bacteria produces cell mass that mainly consists of proteins [45] because proteins are the basic structural and functional components of every cell [11]. Carbohydrate is useable as substrate for microbial growth, which breakdowns complex substrates into simple compound and allows the obtainment of a substrate enrich in protein [5]. From the results, the lowest protein content (18.85%w/w) was obtained in the culture of *S. thermophilus* in low-grade maize. This is because lactic acid bacteria like *S. thermophilus* normally favour to utilize lactose in milk product as carbon source more than glucose from starch materials. However, Alloysius *et al.* [46] reported that the protein content of maize flour increased from 9.44% to 12.97% after fermentation with lactic acid bacteria for 48 h. It's also due to the synthesis of protein as probiotic enzymes by fermenting substrates with organisms that could resulted in increased production of amino acid [47].



Note: Mean (n=3 samples in each fermentation time)

Figure 1. Cell number, glucose and protein content from *S. cerevisiae*



Note: Mean (n=3 samples in each fermentation time)

Figure 2. Cell number, glucose and protein content from *C. utilis*

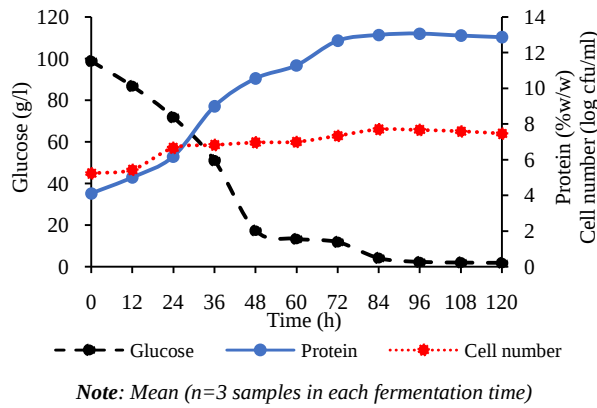


Figure 3. Cell number, glucose and protein content from *C. tropicalis*

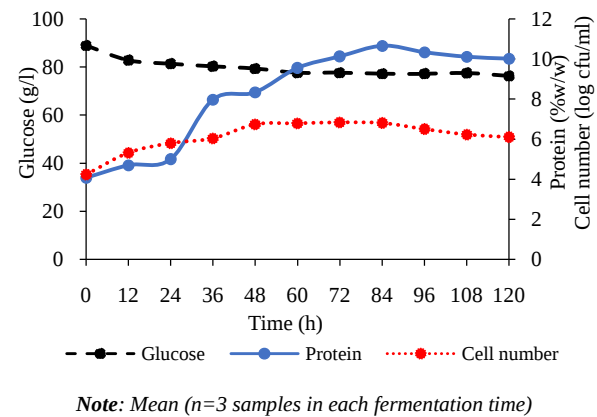


Figure 4. Cell number, glucose and protein content from *B. subtilis*

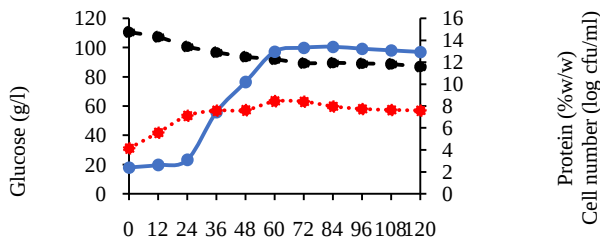


Figure 5. Cell number, glucose and protein content from *L. plantarum*

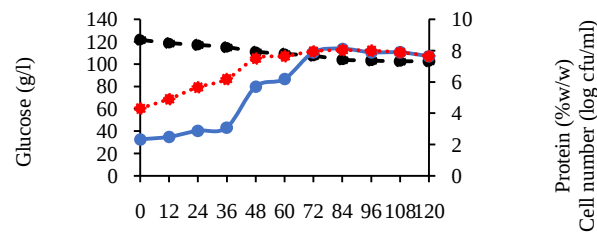
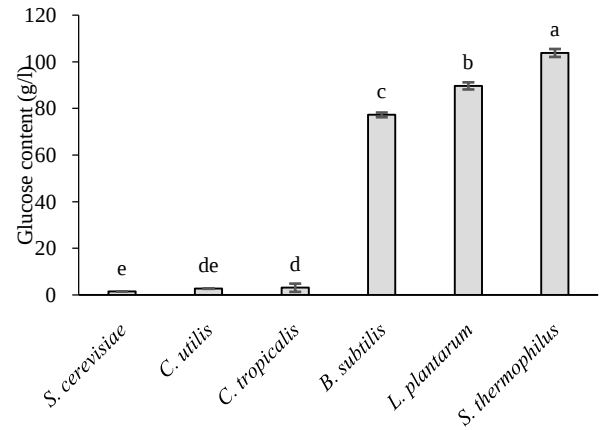
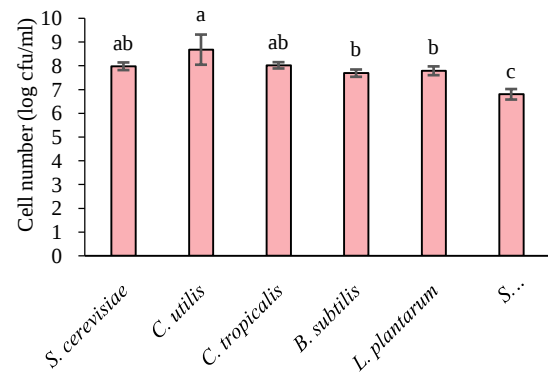


Figure 6. Cell number, glucose and protein content from *S. thermophilus*



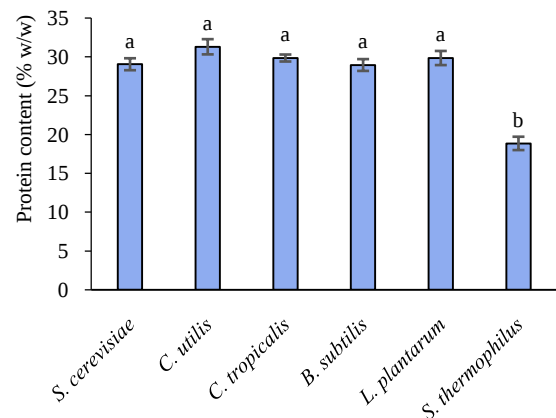
Note: Mean $\pm$ SD (n=3 samples in each fermentation time)
abc letters show the significantly different glucose content ($p < 0.05$) with the different strain of microorganisms.

Figure 7. Glucose content after fermentation by yeast and bacteria for 84 h.



Note: Mean $\pm$ SD (n=3 samples in each fermentation time)
abc letters show the significantly different cell number ($p < 0.05$) with the different strain of microorganisms.

Figure 8. Cell number after fermentation by yeast and bacteria for 84 h



Note: Mean $\pm$ SD (n=3 samples in each fermentation time)
abc letters show the significantly different protein content ($p < 0.05$) with the different strain of microorganisms.

Figure 9. Protein content after fermentation by of yeast and bacteria for 84 h

Table 1. Chemical compositions of monoculture of yeast and bacteria in low-grade maize culture for 84 h

Biomass	Chemical composition (%w/w dry basis)					
	Moisture	Fat	Protein	Ash	Crude fiber	Carbohydrate
Low-grade maize	4.43±0.10 ^{*c**}	5.33±0.10 ^e	8.68±0.87 ^c	1.83±0.13 ^d	2.82±0.14 ^a	76.91
<i>S. cerevisiae</i>	5.08±0.22 ^b	10.55±0.24 ^a	29.05±0.77 ^a	2.08±0.09 ^{bc}	2.52±0.05 ^b	50.72
<i>C. utilis</i>	5.44±0.07 ^a	9.55±0.09 ^c	31.30±0.98 ^a	2.67±0.10 ^a	2.08±0.11 ^c	48.96
<i>C. tropicalis</i>	4.89±0.06 ^b	10.03±0.23 ^b	29.85±0.45 ^a	2.28±0.09 ^b	2.89±0.12 ^a	50.06
<i>B. subtilis</i>	5.54±0.09 ^a	8.95±0.12 ^d	28.95±0.76 ^a	2.09±0.06 ^{bc}	2.12±0.04 ^c	52.35
<i>L. plantarum</i>	5.12±0.06 ^b	9.14±0.08 ^d	29.85±0.91 ^a	1.98±0.10 ^{cd}	2.78±0.03 ^a	51.13
<i>S. thermophilus</i>	5.63±0.10 ^a	9.67±0.12 ^c	18.85±0.86 ^b	2.51±0.07 ^a	2.55±0.09 ^b	60.79

Note: *Results were expressed as Mean ± SD (n=3).

**Values in the same column with different superscript letters differ significantly ($p < 0.05$)

3.3. Chemical Compositions

The chemical compositions of low-grade maize after 84 h fermentation in the culture of yeast and bacteria is presented in Table 1. The moisture contents after monoculture fermentation significantly increased from unfermented low-grade maize (4.43 %w/w), which moisture contents of fermented low-grade maize increased in range of 4.89 to 5.63%w/w. These results are similar to findings of Alloysius *et al.* [46] who reported that the moisture content increase from 9.66 to 10.82% after fermentation with lactic acid bacteria in the culture of maize flour due to the addition of water to the substrate prior to fermentation.

The fat contents increased from 5.33%w/w in unfermented low-grade maize to a range of 8.95–10.55%w/w after fermentation by yeast and bacteria. This is similar to results reported from Aruna *et al.* [48] in which the fat content of yam peels fermented with *S. cerevisiae*, increased from 1.12% to 1.52% after 72 h fermentation. The significant increase of fat contents after fermentation that may be attributed to bio-conversion carbohydrate to fat, and microorganism can build up fat during fermentation.

Ash content significantly increased from 1.83%w/w of unfermented low-grade maize through the fermentation. The highest ash content presented after inoculated with *C. utilis* and *S. thermophilus* that showed 2.67% and 2.51%w/w, respectively. Alloysius *et al.* [46] who investigated that ash content of maize flour increased from 1.88% to 3.14% after 12 h of fermentation with lactic acid bacteria. Moreover, ash content increment leads to increase the level of mineral composition in the substrates.

Crude fiber contents of low-grade maize after fermentation with *S. cerevisiae*, *C. utilis*, *B. subtilis*, and *S. thermophilus* were significantly decreased but not in the culture of *C. tropicalis* and *L. plantarum*. Alloysius *et al.* [46] found that crude fiber content decreased from 3.62% in unfermented maize flour to 0.93% in fermented sample after 48 h fermentation by lactic acid bacteria. The general decrease in fiber composition could be due to the ability of the fermenting organisms to metabolize the fiber by secreting enzymes to breakdown the fiber and microorganisms utilize the fiber as carbon source during fermentation.

Low-grade maize enriched carbohydrate about 76.91%w/w when low-grade maize samples were used as carbon source in fermentation by yeast and bacteria, carbohydrate contents significantly declined. Carbohydrate after fermentation by *C. utilis* remained the lowest content

48.96%w/w whereas, *S. thermophilus* culture remained the highest carbohydrate content of 60.79%w/w. While the amount of carbohydrate decreased, the protein content increased after fermentation. The protein contents from this experiment were indicated as total protein analysed by using Kjeldahl method. After fermentation, protein contents from the culture of *S. cerevisiae*, *C. utilis*, *C. tropicalis*, *B. subtilis*, *L. plantarum*, and *S. thermophilus* were observed to be 29.05, 31.30, 29.85, 28.95, 29.85, and 18.85% w/w, respectively. This indicated significantly increased from protein content of unfermented low-grade maize (8.68% w/w). Materials rich in carbohydrate content that could support microbial growth and use as substrate for protein cell production [49]. Therefore, low carbohydrate values recorded after fermentation may be due to carbon source from carbohydrate utilization by the fermenting organisms [50]. The reduction in carbohydrate content is inversely proportional to the increase in cell number of biomass and the amount of protein production [51]. Moreover, it could also be due to the ability of *S. cerevisiae* to hydrolyze carbohydrate into sugars that serve as carbon source in microbial biomass synthesis that are high in protein during fermentation process as reported by Akintomide and Antai [52].

4. Conclusions

The strains of yeast in this study (*S. cerevisiae*, *C. utilis*, and *C. tropicalis*) utilize glucose from low-grade maize as a carbon source more than bacteria (*B. subtilis*, *L. plantarum*, and *S. thermophilus*). The organisms converted fermentable sugar into formation of single cell proteins. This led to four-times increase of protein in low-grade maize after fermentation by culture of yeast and bacteria, when cultivated with *S. cerevisiae*, *C. utilis*, *C. tropicalis*, *B. subtilis*, and *L. plantarum* that were 29.05, 31.30, 29.85, 28.95, and 29.85%w/w, respectively. Only *S. thermophilus* showed the protein content of 18.85%w/w after the fermentation process, which is 2 times increase. Therefore, materials rich in carbohydrate content such as low-grade maize can support microbial growth and be used as substrate for protein cell production.

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