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Optimization of Nitrogen and Mineral Sources in Liquid Media for Amylase Production Thermophilic *Aspergillus sp*. LBKURCC304

Mhd. Muslim Syaifullah*, Silvera Devi, Saryono, Itnawita, Mukhlis
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Riau
Bina Widya Campus, Jl. HR Soebrantas Km 12,5, Pekanbaru, Indonesia

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Abstract. Amylase has a wide range of applications and is utilized in several industries, including textiles, pulp and paper, feed, detergents, and food. Consequently, the continuous process involves isolating and optimizing the production of amylase from different microbes. The production of amylase from *Aspergillus sp*. LBKURCC304 was optimized by using different nitrogen sources (soy flour, tempeh flour, or catfish flour) and minerals ($FeSO_4.7H_2O$, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, $MnSO_4.H_2O$, $BaCl_2.2H_2O$) in liquid media. The incubation was carried out at a temperature of 50ºC for a duration of 11 days with an agitation speed of 150 rpm. The amylase activity was assessed using the Nelson-Semogyi method, the protein content was measured using the Lowry method, and the specific activity was derived by dividing the amylase activity by the protein content. The research data underwent statistical analysis using ANOVA and the multiple-distance Duncan technique, with a significance threshold set at 5%. The analysis revealed that the medium containing tempeh as a nitrogen source and $MgSO_4.7H_2O$ at a concentration of 0.05% exhibited the maximum amylase activity, with a value of 0.0084±0.0014 U/ml. The protein content measured 0.5111±0.0073 mg/ml, whereas the specific activity was 0.0164±0.00 U/mg.

*Corresponding author.
E-mail address: m.m.syaifullah@lecturer.unri.ac.id

1. Introduction

Thermophilic enzymes are enzymes derived from microorganisms that thrive in elevated temperatures, with a maximum growth temperature of 50ºC (Mohammed et al., 2017). An example of a thermophilic enzyme is amylase, which is commonly employed in various industries including biotechnology, textiles, pulp and paper, feed, detergent, and food (Sindhu et al., 2017; Tiwari et al., 2015). Amylase has the capability to convert starch into sugar (Leveque et al., 2000). In 2018, Saryono and colleagues successfully extracted three thermophilic fungi from the Bukik Gadang hot springs in Solok Regency. One of these fungi was identified as *Aspergillus sp*. LBKURCC304, which has the ability to produce amylase. The amylase produced by this fungus forms a clear zone on solid media with a ratio of 1.90.

In 2020, Widylia conducted a quantitative analysis to determine the amylase activity produced by *Aspergillus sp*. LBKURCC304. The experiment was carried out in Unal liquid production media, using starch as the carbon source and yeast extract as the nitrogen source. The culture was incubated in a rotary shaker at a speed of 150 rpm and a temperature of 50ºC for a duration of 11 days. The resulting amylase activity was measured to be 0.0302 ± 0.0041 U/ml. Lorena (2021) conducted amylase

production optimization by utilizing the optimal conditions determined in Widylia's research. This involved testing various carbon sources (carbohydrates) while employing yeast extract as a nitrogen source and $MgSO_4.7H_2O$ as a mineral supply. The sago carbon source yielded the highest amylase crude extract activity, measuring 0.0391 ± 0.0017 U/ml, with a specific activity of 0.0874 ± 0.0049. This is a 29.47% increase in amylase activity compared to the findings of Widylia's research.

Amplification of amylase production from *Aspergillus sp*. LBKURCC304 can still be accomplished by altering the composition of the media. This is because, as stated by Kizhakedathil (2020), factors related to the components of the media and the surrounding environment have a significant impact on enzyme production. These factors include the carbon source, nitrogen source, pH level, metal ions, and temperature. The sources of nitrogen for microbes include peptone, yeast extract, soybean flour, urea, and fish meal (Nofiani, 2008). In addition, microorganisms require minerals and metal ions as cofactors for their normal growth (Suhartono, 1989). The research conducted by Kizhakedathil (2020) focused on the isolate Pseudomonas Balearica VITPS19, which was obtained from agricultural land in Kolathur, India. The isolate was incubated in malt extract or corn extract media, supplemented with Fe2+, Ca2+, Mn2+, and Ba2+ cations. The results showed that the isolate was capable of producing specific amylase activity in each production medium, with values of 980 U/mg, 1150 U/g, 980 U/mg, and 1020 U/mg, respectively.

In Kizhakedathil's (2020) research, the production of the amylase enzyme from the thermophilic fungus *Aspergillus sp*. LBKURCC304 in liquid production media was optimized. This was achieved by varying the nitrogen source (soybean flour, tempeh flour, and catfish meal) and the types of minerals ($FeSO_4.7H_2O$, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, $MnSO_4.H_2O$, and $BaCl_2.2H_2O$). The production process was conducted at 50ºC for 11 days with a speed of 150 rpm. The selection of the nitrogen source for tempeh was made based on its availability and cost-effectiveness, whereas catfish meal is a traditional and locally sourced ingredient from the Pekanbaru region. The amylase enzyme's activity was assessed using the Nelson-Semogyi method, while the protein content was evaluated using the Lowry method. The specific activity of the enzyme was then computed by comparing its activity to the protein content.

The Nelson-Somogy method is used to measure the activity of the amylase enzyme by measuring the concentration of reducing sugar produced from the hydrolysis of starch. The concentration of reducing sugar is directly proportional to the enzyme activity. The copper arsenomolybdate reagent is employed in this technique to quantify the amounts of reducing sugars. The absorbance can be used to measure the sugar concentration in the sample by analyzing the color generated. The conventional approach involves using glucose as the standard solution, specifically by establishing a linear relationship between the concentration of glucose and its absorbance (Mahardikaningrum and Yuanita, 2012). The Lowry method was employed to do protein content analysis, utilizing Folin-Ciocalteau reagent to identify phenol groups and quantifying light absorption through blue complex bonds (Boyer, 1993). The Lowry technique operates based on the interaction between protein and phosphotungstic-phosphomolybdic acid in an alkaline environment, resulting in the formation of a blue hue that corresponds to the protein content. Within this environment, Cu^{2+} ions undergo complexation with peptide bonds, resulting in the reduction of Cu^{2+} to Cu^{+}. The protein will undergo a chemical reaction with the Folin Ciocalteau reagent, resulting in the formation of a colorful complex molecule. This reaction occurs between the copper base and the protein sample being analyzed (Bintang, 2010). The enzyme's specific activity is determined by correlating its activity to the protein content.

2. The Methods

2.1. Equipments and Materials

Equipments: The equipment used includes an autoclave (All America model 1925/KY-23D), a rotary shaker (Lab TechScientific International), a water bath (Grant Instrument Type SUB 28), a vortex mixer (H-VM-300), a pH meter (Hanna Instrument H18014), an incubator (Herraeus Instrument D6450), a vacuum rotary evaporator (Heidolph WB 2000), a Laminar Air Flow system, Whatman filter paper (no

1), a UV-Vis spectrophotometer (Thermoscientific Genesis 10S), analytical scales, and other standard laboratory equipment as required by the procedures.

Material and Reagents: Potato Dextrose Agar (PDA) (Merck Cat. No.1.10130.0500), sterile distilled water, 70% antiseptic alcohol (Brataco), $FeSO_4.7H_2O$, $CaCl_2$. $2H_2O$, $MnSO_4.H_2O$, $MgSO_4.7H_2O$, $BaCl_2.2H_2O$, NaH_2PO_4, Na_2HPO_4, NaOH, HCl, Bufer Fosfat pH 7 0.05 M, $NaKC_4H_4O_6.4H_2O$, Na_2CO_3, $NaHCO_3$, Na_2SO_4, $CuSO_4.5H_2O$, H_2SO_4, sago carbohydrate/flour, Nelson-Somogyi reagent, and Ammonium Molybdate $(NH_4)_2MoO_4$, $Na_2HAsO_4.7H_2O$, arsenomolybdate reagent, Glucose, Bovine Serum Albumin, Folin-Ciocalteu reagent, soybean flour as a nitrogen source, tempeh flour, and catfish meal.

Microorganism: Isolated *Aspergillus sp*. culture LBKURCC304 from the Laboratory of Enzyme, Fermentation and Bio-molecular at FMIPA UNRI.

2.2. Quantification of moisture content for every nitrogen source

The nitrogen source employed is flour derived from soybeans, tempeh, and catfish. It is subjected to an oven at a precise temperature of 100ºC to ascertain the water content using the Gravimetric method (SNI 01-2891-1992).

2.3. Revitalizing *Aspergillus sp*. isolates LBKURCC304 on slanted agar media

The use of PDA media is an effective method for revitalizing LBKURCC304 fungal stocks. A total of 3.9 grams of PDA was combined with 100 ml of DM aqueous. The resulting solution was then heated and agitated until complete dissolution occurred. Subsequently, it underwent sterilization in an autoclave for a duration of 15 minutes, at a temperature of 121°C and a pressure of 15 lb. Approximately ±5 ml of PDA media is added to each test tube, which is then inverted and left at room temperature. If no contamination is observed, the media is deemed suitable for usage. The *Aspergillus sp*. LBKURCC304 isolate was aseptically obtained from the stock using a loop needle and then inoculated onto the surface of the PDA agar slant medium. The media was thereafter incubated at a temperature of 50ºC for a period of 7 days.

2.4. Cultivating an inoculum of *Aspergillus sp* LBKURCC304 using solid media

After renewal, 3 ml of sterile distilled water was added to *Aspergillus sp.* LBKURCC304. Subsequently, the surface of the media was gently crushed with a loop needle to liberate the spores. A volume of approximately 250 µl of the fungal suspension was extracted and subsequently introduced into a petri dish containing potato dextrose agar (PDA). The suspension was then equally distributed throughout the surface of the agar using a sterile spreader. Following a time period of approximately 1 hour, the container is inverted, securely covered, and subjected to incubation at a temperature of 50ºC for a duration of 7 days.

2.5. Production of amylase enzyme by *Aspergillus sp* LBKURCC304 in liquid media

The nitrogen sources present in soybean flour, tempeh flour, and catfish flour were individually subjected to a temperature of 100ºC in an oven. Subsequently, the water content was evaluated using the Gravimetric method (SNI 01-2891-1992). The amylase enzyme production medium is prepared by dissolving the chemical components listed in Table 1. The media solution undergoes sterilization through autoclaving, which involves subjecting it to a temperature of 121°C and a pressure of 15 lb for a duration of 15 minutes.

Table 1. Composition for 100 ml of liquid starch production media with variations in nitrogen and mineral sources (Unal, 2015).

Variable	Composition	Amount

Carbon sources	Sago flour	0,5 g
Nitrogen sources	Flour of soy bean, tempeh, and catfish	0,2 g
	KH_2PO_4	0,1 g
Mineral sources	$MgSO_4.7H_2O$) ($FeSO_4$. $7H_2O$), ($CaCl_2$. $2H_2O$), $MnSO_4.H_2O$ and $BaCl_2.2H_2O$)	0,05 g
Solvent	Phosphate buffer 0,05M pH 7	100 ml

Amylase production with variations in nitrogen sources, mineral sources and mineral concentrations using the half factorial method in the following stages:

First stage: 100 ml of production media containing three distinct nitrogen sources (soybean flour, tempeh flour, and catfish meal) were individually placed into Erlemeyer flasks. Subsequently, a sterile cork borer was used to extract a 1 cm inoculum of *Aspergillus sp.* LBKURCC304 from 2 plugs, which was then introduced into the production medium. Subsequently, the sample was subjected to incubation on a rotary shaker, operating at a velocity of 150 revolutions per minute, while maintaining a temperature of 50ºC, for a duration of 11 days. The amylase extract obtained was fractionated using Whatman paper no 1. Subsequently, the amylase crude extract was assessed for both its amylase activity and protein content. The maximum amylase crude extract activity was observed in these 3 production media.

Second stage: four Erlemeyers will be supplied and filled with the identical media composition as the one holding the crude amylase extract with the highest activity in the first stage. Ferrous Sulphate Heptahydrate ($FeSO_4.7H_2O$), Calcium Chloride Dihydrate ($CaCl_2.2H_2O$), Manganese Sulfate ($MnSO_4.H_2O$), and Barium Chloride Dihydrate ($BaCl_2.2H_2O$) are added to each medium. The manufacturing of amylase was conducted using the same methodology as in the first stage. The crude extract of amylase had the highest activity among the 5 production media.

*Third stage***:** three erlenmeyer flask are used with the same media composition as the second stage, which yields the maximum amylase activity. The mineral concentration is then altered, specifically at 0.025%, 0.05%, and 0.075%. The manufacture of amylase is carried out in the same manner as in the first stage. Subsequently, the amylase extract obtained was assessed for its amylase activity and protein content.

2.6. Determination of amylase enzyme activity

The amylase enzyme's activity can be quantified by measuring the reducing sugar generated during the hydrolysis of starch by amylase, employing the Nelson-Somogyi method. Prepare three test tubes, one for the experimental test, one for the control, and one for the blank. Treat all three tubes concurrently. Inject 0.5 ml of a 2% starch substrate into the test tube, while leaving the control and blank tubes devoid of any substance. Subsequently, place these three tubes into a water bath and incubate them at a temperature of 50ºC for a duration of 5 minutes. Subsequently, 0.5 ml of the unrefined amylase enzyme extract was introduced into both the test and control tubes, followed by another incubation period of 30 minutes.

Subsequently, the test tubes, control tubes, and blanks were extracted from the water bath. Then, 1 ml of Nelson-Somogyi reagent was added to each test tube. In the case of the control tubes, 0.5 ml of 2% starch substrate was added, whereas for the blanks, 1 ml of 0.05 M phosphate buffer pH 7 was added. The resulting solutions were thoroughly mixed using a Vortex mixer to achieve homogeneity. The test, control, and blank tubes were immersed in a boiling water bath for a duration of 20 minutes, while the opening of each tube was sealed with a marble. Subsequently, the three tubes were cooled to the ambient temperature, and 1 ml of arsenomolybdate reagent was introduced into each tube to generate a pigmented compound. Additionally, 6 ml of DM aqueous solution was added and mixed vigorously, then allowed to stand undisturbed for a duration of 30 minutes. The solution's absorbance was quantified at a wavelength of 540 nm using a UV-Vis spectrophotometer (repeated 5 times).

The unit value of amylase enzyme activity is determined by measuring the amount of enzyme required to generate 1 μmol of reducing sugar per minute per ml of crude extract, as calculated using the below formula:

$$\text{Enzyme activity} = \frac{\text{Test reductor sugar} - \text{control reductor sugar}}{\text{enzyme crude extract volume incubation time}} \quad (1)$$

2.7. Protein content and specific enzyme activity determination

The mother liquor was prepared by dissolving 0.01 gram of glucose in a tiny quantity of 0.05 M pH 7 phosphate buffer in a 10 ml volumetric flask, followed by the addition of phosphate buffer to reach the desired volume. The intermediate solution was prepared by taking 1 ml of a concentrated glucose solution and diluting it in a 10 ml volumetric flask until it reached the mark. 10 reaction tubes were filled with 0.1 to 1 ml of glucose solution for protein standards. The solution in each tube was then diluted with DM water until the total volume reached 1 ml. Tubes 11 to 14 are filled with different substances. Tube 11 contains 1 ml of deionized water, whereas tubes 12 to 14 contain 1 ml of enzyme crude extract.

Next, add 5 ml of Lowry's reagent to each tube, mix thoroughly, and allow them to incubate at room temperature for approximately 10 minutes. Subsequently, 0.5 ml of Folin Ciocalteau reagent was introduced to each sample and mixed vigorously until a uniform mixture was achieved. The samples were then allowed to rest undisturbed for a duration of 30 minutes at ambient temperature. The UV-Vis spectrophotometer was used to measure the absorbance of this colored solution at a specific wavelength of 700 nm. The enzyme's precise activity value is calculated using the following equation:

$$\text{Specific activity} = \frac{\text{Enzime activity}}{\text{Total protein content}} \quad (2)$$

2.8. Data analysis

The research findings are presented through graphical representations and tabular formats, which also include statistical analyses of the amylase enzyme. The data analysis employed Analysis of Variance (ANOVA) followed by the Duncan multiple range test at a significance level of 5%.

3. Result and Discussion

3.1. Water contents of the nitrogen sources

The water content obtained from various nitrogen sources using the gravimetric approach is presented in Table 2. Soybean flour, tempeh flour, and catfish flour are employed as nitrogenous substrates in the production media for amylase by *Aspergillus sp*. LBKURCC304. Initially, the water content is determined to ensure that the weight of the nitrogen source remains consistent, specifically at 0.2%. Cahyani (2014) asserts that nitrogen sources play a crucial role in the formation of cellular macromolecules, particularly in the production of proteins and nucleic acids. Common nitrogen sources utilized in microorganism growth media comprise peptone, yeast extract, soybean flour, urea, and fish meal (Nofiani, 2012).

Tabel 2. Water content in nitrogen source

No.	Sources	Water content (%)
1.	Soybean flour	8.50
2.	Tempeh flour	6.00
3.	Catfish flour	6.00

The utilization of liquid media for the generation of amylase from *Aspergillus sp*. LBKURCC304 in this study is based on the research conducted by Unal (2015). The liquid media consisted of a starch carbon source, yeast extract nitrogen source, and the minerals KH_2PO_4 and $MgSO_4.7H_2O$. The unaltered media was subsequently adjusted according to the findings of prior research. Specifically,

sago was chosen as the carbon source based on Lorena's (2021) research results. Additionally, the nitrogen source yeast extract was substituted with soybean flour, tempeh flour, and catfish flour. Furthermore, the mineral $MgSO_4.7H_2O$ was replaced with $FeSO_4.7H_2O$, $CaCl_2.2H_2O$, $MnSO_4.H_2O$, and $BaCl_2.2H_2O$. The ideal temperature for incubating *Aspergillus sp*. LBKURCC30 is 50ºC, as determined by Saryono et al. (2018). The production time for this strain is 11 days, and the recommended agitation speed is 150 rpm, according to the research conducted by Widylia (2020). A single unit of amylase activity corresponds to the capacity of the amylase enzyme to break down starch into reducing sugars at a rate of 1 µmol per minute per ml of enzyme (µmol/minute/ml).

3.2. Enzymatic activity in different nitrogen sources

Table 3 displays the quantitative data on amylase activity, protein content, and specific activity of the crude amylase extract from *Aspergillus sp.* LBKURCC304, considering various nitrogen source factors. The statistical analysis of the measurement data in Table 3, using the Duncan multiple distance method, revealed that the nitrogen source of tempeh flour had the highest amylase activity and specific activity. Furthermore, the amylase activity of tempeh flour was significantly different ($p \geq 0.05$) from that of soybeans and catfish. However, the amylase activity of catfish was not significantly different from that of soybeans.

Table 3. Crude extract from *Aspergillus sp* LBKURCC304: amylase, protein, and specific activity.

Sources	Enzyme activity (U/ml)	Protein content (mg/ml)	Specific activity (U/mg)
Soybean flour	0,0011±0,0004[a]	0,3672±0,0028[a]	0,0068±0,0076[ab]
Tempeh flour	**0,0084±0,0014[b]**	0,5111±0,0073[b]	0,0164±0,0027[b]
Catfish flour	0,0021±0,0004[a]	0,8778±0,0015[c]	0,0023±0,0004[a]

Note: The superscript in the same column indicates no significant difference at 5% ($p \geq 0.05$) using the Duncan multiple range test.

The findings presented in Table 3 indicates that the maximum amylase activity shown by *Aspergillus sp.* LBKURCC304 was measured to be 0.0084±0.0014 U/ml, when utilizing a sago carbon source and tempeh nitrogen source. The level of amylase activity is really modest, measuring around ±36.54%. A study conducted by Lorena in 2021, utilizing sago as the carbon source and yeast extract as the nitrogen source, yielded an amylase activity of 0.0391 ± 0.0017 U/ml. Although tempeh flour can serve as a nitrogen source, its impact on enhancing amylase activity in the synthesis of amylase enzymes from *Aspergillus sp.* LBKURCC304 is rather negligible when compared to yeast extract.

For the synthesis of amylase from *Aspergillus sp.* LBKURCC304, the nitrogen source yeast extract outperforms the nitrogen source tempeh. As stated by Wardani et al (2017), yeast extract is included as a nutrient in the production medium due to its composition of carbohydrates, amino acids, peptides, and vitamins that are highly advantageous for microbial development. Within tempeh, the protein molecules that need to undergo hydrolysis before to synthesizing amino acids are comparatively larger than the amino acids themselves, allowing for prolonged absorption by cells. The observed disparity is believed to have an impact on the growth rate of *Aspergillus sp.* LBKURCC304, thereby influencing both the enzyme concentration and the magnitude of amylase activity generated. Tempeh was selected as a nitrogen source in this study because to its intricate chemical makeup and its affordability and accessibility, making it a preferred food source among the residents of Pekanbaru. An essential criterion for fermentation technology is that the materials employed must be economical and readily available.

As a result of a fermentation process by *Rhyzopus oligosporous*, the protein in tempeh is partially hydrolyzed into amino acids, making it more readily utilized as a nitrogen source by *Aspergillus sp*. LBKURCC304 compared to soybeans. Consequently, the amylase activity of tempeh is higher than that of soybean nitrogen source, at 26.91%. An interesting inference is that tempeh contains a somewhat elevated concentration of the amino acids Arginine (0.36%) and Tyrosine (0.72%) in comparison to

other amino acids. According to Kumar and Nussinov (2001), arginine and tyrosine serve as precursors for the production of amylase enzyme protein. This is because these two amino acids constitute the dominant active core of the amylase enzyme, thereby enhancing its activity. An analysis of the correlation between the percentage of tempeh mass and the amylase activity produced is important to determine if tempeh may serve as a nitrogen source for the development of amylase from *Aspergillus sp*. LBKURCC304 fungus and to create amylase with higher activity.

3.3. Amylase activity, protein content, and specific activity with different mineral sources

The measurements of amylase activity, protein content, and specific activity of the crude amylase extract from *Aspergillus sp* LBKURCC304 with different mineral sources are presented in Table 4. After undergoing statistical analysis using the Duncan multiple distance method, the measurement data in Table 3 revealed that the mineral source $MgSO_4.7H_2O$ exhibited the highest amylase activity and specific activity. The activity of this material exhibits a significant difference ($p \geq 0.05$) compared to other minerals.

Tabel 4. Amylase activity, protein content, and specific activity of *Aspergillus sp* LBKURCC304 crude extract with different minerals.

Sources	Enzyme activity (U/ml)	Protein content (mg/ml)	Specific activity (U/mg)
$MgSO_4.7H_2O$	**0,0084±0,0014^b**	**0,5111±0,0073^c**	**0,0164±0,0027^b**
$MnSO_4.H_2O$	0,0024±0,0000^a	0,3980±0,0001^a	0,0061±0,0000^a
$CaCl_2.2H_2O$	0,0025±0,0004^a	0,4529±0,0014^b	0,0056±0,0009^a
$FeSO_4.7H_2O$	0,0021±0,0000^a	0,4371±0,0272^b	0,0049±0,0002^a
$BaCl_2.2H_2O$	0,0031±0,0000^a	0,4900±0,0211^c	0,0063±0,0002^a

Note: The superscript in the same column indicates no significant difference at 5% ($p \geq 0.05$) using the Duncan multiple range test.

The data shown in Table 4 demonstrates the variation in amylase activity of *Aspergillus sp*. LBKURCC304 when cultivated on liquid media containing different mineral sources. Additionally, the levels of amylase activity and protein production also vary. The amylase crude extract exhibited the greatest activity of 0.0084 ± 0.0014 U/ml when the mineral $MgSO_4.7H_2O$ was added to the liquid media. In addition to its role as a cofactor, the mineral $MgSO_4.7H_2O$ is believed to function as an activator in the amylase enzyme derived from *Aspergillus sp*. LBKURCC304. This hypothesis is supported by Amin's (2020) research findings, which indicate that the metal ions Mg and Ca, when added to the corn starch substrate, are more effective as cofactors for the α-amylase enzyme. This is because Mg and Ca can replace other metals that are less efficient in the active site of the α-amylase enzyme. Kizhakedathil (2021) also documented that the Ca metal ion functions as a catalyst for the amylase enzyme derived from the bacteria Pseudomonas Balearica VITPS19. These findings demonstrate that the identical enzyme derived from several microorganisms might exhibit either identical or distinct activators.

3.4. Effects of mineral concentrations

The measurements of amylase activity, protein content, and specific activity of amylase crude extract from *Aspergillus sp*. LBKURCC304 with different mineral sources are presented in Table 5. After undergoing statistical analysis using the Duncan multiple distance method, the measurement data in Table 4 revealed that the mineral source $MgSO_4.7H_2O$ with a concentration of 0.050% exhibited the highest amylase activity and specific activity. The activity exhibited a statistically significant difference ($p \geq 0.05$) compared to other mineral concentrations.

Tabel 5. Analysis of amylase activity, protein content, and specific activity of a crude extract from *Aspergillus sp*. LBKURCC304 with varying concentrations of MgSO4.7H2O

$MgSO_4.7H_2O$ concentration	Enzyme activity (U/ml)	Protein content (mg/ml)	Specific activity (U/mg)
0.025%	0.0049±0.0026[a]	0.4912±0.0164[ab]	0.0099±0.0010[a]
0.050%	**0.0084±0.0014[b]**	**0.5111±0.007[b]**	**0.0164±0.0027[b]**
0.075%	0.0031±0.0003[a]	0.4346±0.0444[a]	0.0071±0.0116[a]

Note: The superscript in the same column indicates no significant difference at 5% ($p \geq 0.05$) using the Duncan multiple range test.

The data presented in Table 5 indicates that the highest activity of the amylase crude extract is observed in liquid medium containing 0.05% $MgSO_4.7H_2O$, specifically at a level of 0.0084 ± 0.0014 U/ml. However, when the concentration is increased to 0.75%, the amylase activity reduces by almost 53%. The value is 0.0031±0.0003 U/ml. The drop in amylase activity is attributed to the mineral $MgSO_4.7H_2O$, which is known to operate as an activator. The concentration of this mineral was increased by 50%, causing an excess concentration that leads to an inhibitory process in amylase, resulting in a decrease in its activity. This assertion is reinforced by the declaration made by Dixon and Webb (1979) that an overabundance of activator can lead to competitive inhibition. The mineral supply $MgSO_4.7H_2O$, present at a concentration of 0.05%, resulted in the highest protein content and specific activity. The protein content was measured at 0.5111 ± 0.007 mg/ml, while the specific activity was measured at 0.0164 ± 0.0027 U/mg. Specific activity is a quantitative metric that shows the level of purity of an enzyme. As the enzyme activity increases and the protein level decreases, the specific activity also increases. This is a result of the elevated enzymatic activity of the amylase crude extract and the relatively low concentration of proteins.

Conclusion

At a level of 0.0084±0.0014 U/ml, the crude extract of *Aspergillus sp* LBKURCC304 had the highest possible amylase activity. This activity was accomplished by using tempeh flour as the nitrogen source and $MgSO_4.7H_2O$ at a concentration of 0.05% as the mineral supply. Both of these were incorporated into the tempeh fermentation process, exhibiting a specific activity of 0.0164±0.0027 U/mg and a total protein content of 0.5111±0.0007 g/ml.

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