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Analysis of Primary Metabolite Compound Content in Tempe Durian Seeds (Durio zibethinus)

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Abstract: Durian seed tempe is tempe made from durian seed waste through a fermentation process by the same microorganisms used to make soybean tempe, namely Rhizopus sp. This research aims to determine the content of primary metabolite compounds in durian seed tempe. The process of determining the fat content in durian seed tempe uses the soxhletation method with n-hexane solvent. The protein content is determined using the Kjedhal method, which uses 3 processes, namely digestion, distillation, and titration. Meanwhile, carbohydrate levels are determined using the anthrone method. Based on the results obtained in this research, namely: the results of the analysis of the fat content contained in durian seed tempe were 0.006 grams. The protein content in durian seed tempe is 0.216, and the carbohydrate content in durian seed tempe is 2.421 grams.

Keywords: Carbohydrates; Durian seeds tempe; Fat; Protein

Introduction

Indonesia has very diverse and abundant natural resources, which can be used as a food source, as a source of nutrition, protein, carbohydrates, and fats that come from plants (Kumoro et al., 2020). Primary metabolites and secondary metabolites are the two categories of metabolites found in plants. While secondary metabolites are created in some levels by plants as a sort of self-defense against environmental perturbations, primary metabolites are important for plant development (Robe et al., 2021).

Chemicals generated by living things that are necessary for cell creation and metabolism are known as primary metabolite chemicals. Plants include a variety of primary metabolite chemicals, such as lipids, proteins, carbs, vitamins, fiber, energy, potassium, calcium, copper, and phosphorus. Additionally, food that has been prepared from natural or vegetable (plant) food contains primary metabolite chemicals (Perchuk et al., 2020).

Natural food ingredients are food ingredients that come from biological sources. Food sources can come from animals (animal food sources) and plants (vegetable food sources). One of the plant-based foods that comes from grains and is very popular is tempe (Teoh et al., 2024).

The largest tempe producer in the world and the largest market for soybean tempe in Asia is Indonesia. Tempe has been consumed for generations by children and the elderly (Rizzo, 2024) . Tempe is a very popular food product in Indonesia (Kadar et al., 2020). Soybean tempe is processed through a fermentation process over a certain period using the fungus Rhizopus oligosporus. In general, tempe has a characteristic white color due to the growth of fungal mycelia that connect the soybean seeds, resulting in a compact tempe texture. However, recently the price of soybeans has become more expensive, which is the cause of the increase in tempe prices. This has become less affordable for underprivileged communities, therefore breakthroughs are needed as a substitute for soybeans, including processing durian seeds into a food source.

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According to Khaksar et al., (2024) durian seeds have potential as a source of nutrition, namely containing protein, carbohydrates, fat, calcium, and phosphorus. The nutrients contained in 100 grams of durian seeds include 28.3 grams of carbohydrates, 67 grams of minerals, 520 KJ of energy or the equivalent of 124.8 calories, 2.5 grams of fat, 2.5 grams of protein, and 1 .4 gr. Meanwhile, 100 grams of soybeans have 331 calories, 34.9 grams of protein, 34.8 grams of carbohydrates, 227 mg of calcium, 585 mg of phosphorus, 8 mg of iron, and 1.1 mg of vitamin B1 (Kumoro et al., 2020). The relatively complete nutritional content of durian seeds makes it possible to use them as a substrate for the production of durian seed tempe.

The durian seeds used in this research were local durian originating from Binangga Village, Parigi Tengah District, Parigi Moutong Regency. Because the seeds of this local durian are large and suitable to be used as a basic ingredient for making food, especially tempe. From research by Yarlina et al., (2023), Based on the literature analysis and the nutritional value of durian seeds as a food source, researchers examined the major metabolite content of durian seed tempe. Samples of durian seeds from Binangga Village in Parigi Moutong Regency's Central Parigi District.

Method

Tools and materials

The tools used in this research arestove, knife, scale, 10 mL measuring flask, oven, analytical balance, socket set complete with aluminum foil, pan, spatula, petri dish, polypropylene plastic, 50 mL measuring cup, UV-Vis spectrophotometer, distillation tool, stand and clamps, burette, Erlenmeyer, 50 mL and 100 mL beakers, funnel, test tube and test tube rack, hot plate, funnel, 50 mL and 100 mL volumetric flask, 5 mL volume pipette. The ingredients used are durian seeds, distilled water, tempe yeast, methylene red, 30% NaOH, concentrated H_2SO_4, 1 N NaOH, 0.1 N HCl, glucose solution, concentrated HNO3, 5% phenol, filter paper, N-hexane, kjedhal tablets, anthrone, glucose, concentrated HCl, Na_2CO_3.

Ways of working

Making durian seed tempe

Durian seeds are washed with clean water then drained, then dried in the sun for 4 days until dry. Weigh 1 kg of dried durian seeds, then boil them until cooked, then remove the skin, then wash them until the mucus is gone. After that, slice it into small pieces the size of soybeans. The next step is to steam it again and then cool it, by letting it air in front of a fan until it is cold. Next, the fermentation process is carried out, namely using 1 g of yeast. Then wrapped in banana leaves and then aged (fermented) for 2 days. The process of making tempe follows the procedure of (Gamay et al., 2024), using modified durian seeds.

Fat Content Analysis

The initial step to determine the weight of an empty flask is by first heating the flask in the oven in the oven for 10 minutes, then cooling it in a desiccator for 15 minutes, then weighing it until constant. The next step is determining total fat using the Soxhlet method. Weighed 10 grams of durian seed tempe sample and put it in a filter paper sleeve, then put it in a Soxhlet column. Put the n-hexane solvent into the flask. The next step is to assemble the soxhletation tool. Open the water inlet valve and turn on the heating mantle. The solvent in the flask, boils and evaporates. The solvent vapor is condensed by the return cooler and this hot solvent vapor descends. The vapor droplets will hit the sample, and the solvent will dissolve the fat. When it reaches the surface of the siphon tube, all the liquid will fall back down to the gray round bottom through the siphon capillary tube, resulting in circulation and so on. Fat extraction in this study lasted 9 cycles (the solvent was clear). The extracts obtained were collected. Next, evaporate using an desiccator, until the solvent has evaporated completely. The extracted fat will remain in the pumpkin. Then the pumpkin was heated for 5 minutes in the oven, then cooled for 15 minutes in a desiccator. The flask containing the extracted fat was weighed until constant weight.

Protein Content Analysis

1. In the digestion stage, 10 grams of durian seed tempe samples were then put into a 100 mL kjedhal flask. Then add 1 kjedhal tablet and 10 mL concentrated H_2SO_4. Next, destroy (heat) all the ingredients in a digestion flask until it becomes a clear solution.
2. The next stage is the distillation stage. The results of the digestion stage are diluted with 100 mL distilled water, then stirred until homogeneous. Next, 10 mL was taken and put into a distillation flask. Then 10 mL of 30% NaOH solution was added through the walls of the flask until two layers were formed. Then the flask is connected to the condenser. The steam from the boiling liquid will flow through the condenser and be collected in an Erlenmeyer filled with 10 mL of 0.1 N HCl solution and dripped with methyl red indicator.
3. After the distillation process, the distillate is titrated using 0.1 N NaOH solution. The titration is stopped after the distillate changes color to pink.
4. Make a blank solution, which is done in the same way, but replace the sample with distilled water.

Carbohydrate Content Analysis

1. Preparation of standard glucose solutions with respective concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm. Making a standard solution with a concentration of 2 ppm is done by taking 0.1 mL of the stock solution using a measuring pipette and putting it into a 50 mL measuring flask, then adding distilled water to the mark. Next, use the same method to make concentrations of 4 ppm, 6 ppm, 8 ppm, and 10 ppm by taking respectively 0.2 mL, 0.3 mL, 0.4 mL, and 0.5 mL by adding distilled water. use the same volume, namely 50 mL.
2. Determination of total carbohydrates using the Anthrone method by weighing 10 grams of sample and placing it in a beaker. Then 3 mL of concentrated HCl and 20 mL of distilled water were added. Next, the beaker is covered using aluminum foil. Then it was destroyed using a hot plate for 3 hours for the hydrolysis process. After the digestion process is complete, it is cooled and then neutralized with Na_2CO_3 until no foam appears. After no foam appears, the hydrolysis liquid is mixed with distilled water and filtered. Next, 1 mL of the filtrate was taken, put into a test tube and 3 mL of anthrone reagent was added, then heated for 10 minutes. Next, cool it, then measure its absorption at a wavelength of 630 nn using a UV-Vis spectrophotometer. Based on the measurement results, a graph of the concentration of the adsorbent was created.

Result and Discussion

Results

Fat Content Analysis

The results of the analysis of the fat content of durian seed tempe were carried out using the Soxhlet method, to extract free fat compounds with hexane solvent. Based on the research results, the fat content was found to be 0.006 grams of fat in 10 grams of sample or around 0.06%.

Protein Content Analysis

The results of protein analysis from durian seed tempe using the Kjedhal method obtained an average of 0.216 grams in 10 grams or around 2.16%

Carbohydrate Content Analysis

The results of the analysis of carbohydrate content obtained from durian seed tempe using the anthrone method are presented in Table 1. In this table are the results of measurements of standard glucose concentrations of 2, 4, 6, 8, and 10 ppm, using a UV-Vis spectrophotometer instrument with a wavelength of 630 nm.

Table 1. Results of absorbance measurements of standard glucose solutions

Concentration	Adsorbance 630 nm
2	1.15
4	1.185
6	1.238
8	1.288
10	1.347

Measurement of the absorbance of a standard glucose solution with varying concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm obtained a curve as in Figure 1.

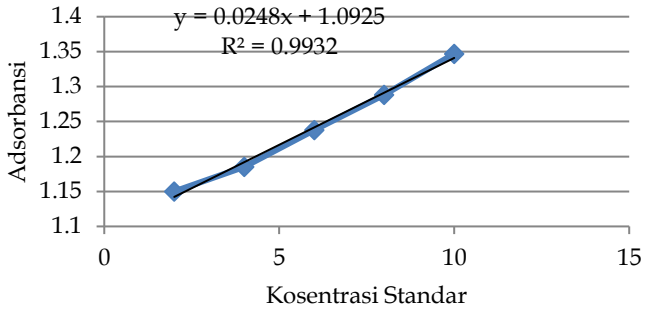

Figure 1. Glucose standard calibration curve

The figure shows that the absorbance value in the standard glucose solution has increased starting from the lowest concentration, namely 2 ppm, to the highest concentration, namely 10 ppm. At a concentration of 2 ppm, it has an absorbance of 1.15 nm, at a concentration of 4 ppm it has an absorbance of 1.185 nm, at a concentration of 6 ppm it has an absorbance of 1.238 nm, at a concentration of 8 ppm it has an absorbance of 1.288 nm, and at a concentration of 10 ppm, it has an absorbance of 1.347 nm. Then the value of the linear regression equation is obtained, namely y = 0.0248x + 1.0925 and the value of R2 = 0.9932. The R2 value lies in the interval 0.9 < R2 < 1 so this value meets the requirements to be used as a calibration curve. This shows that there is a linear correlation between absorbance and concentration so glucose levels can be determined based on the absorbance value.

The percentage of carbohydrate content in durian seed tempe showed different results from the three sample repetitions. The results obtained in repetition 1 were 2.266 grams, repetition 2 was 2.604 grams, and repetition 3 was 2.395 grams so the average carbohydrate was 2.421 grams in the 10-gram samples used.

Discussion

Sample Preparation (Tempe Making)

The process for making durian seed tempe is almost the same as the process for making soybeans. Making durian seed tempe can be seen in Figure 2.

Figure 2. Making Durian Seed Tempe

The tempe produced is white and harder than soybean tempe, this is because the texture of durian seeds is harder than soybeans. It has a distinctive aroma of durian seeds, however, the color of durian seed tempe is the same as tempe in general.

Fat Content Analysis

The results of research that has been carried out are in determining the fat in durian seed tempe using the Soxhlet method and using a non-polar solvent, namely N-hexane. This is because fat is a non-polar compound so it can be dissolved with non-polar solvents too. Solvent n-hexane is used to extract free fat compounds in solid form in samples. Method.

Chemically, fat is triglyceride which is part of the lipid group. Triglycerides are the result of the condensation of one glycerol molecule with three fatty acid molecules as in Figure 3. There are two types of fat related to the fatty acids that form it, namely fat in liquid form (oil) which contains many unsaturated fatty acids, such as oleic acid ($C_{17}H_{33}COOH$), linoleic acid ($C_{17}H_{35}COOH$), and linolenic acid ($C_{15}H_{29}COOH$). Meanwhile, solid fats are saturated fatty acids. Saturated fatty acids have a higher boiling point than unsaturated fatty acids. The decrease in fat content of boiled durian seeds was caused by longer boiling. The longer the boiling process at a high enough temperature will cause the fat to oxidize, especially the unsaturated fatty acids. So the more fat or fatty acids that are oxidized, the lower the fat content obtained during the analysis process. The presence of water in boiling durian seeds affects the extraction process, the process of solvent entering the fat cells which causes the fat extraction process to be less efficient (Adimas & Abera, 2023).

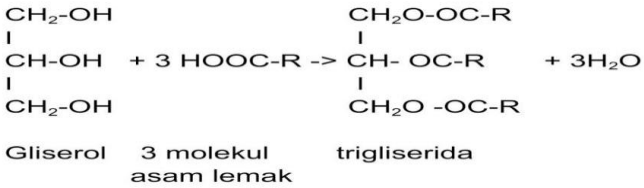

Figure 3. The structure results from the condensation of one glycerol molecule with three fatty acid molecules.

Protein Content Analysis

The results of research that has been carried out in determining protein in durian seed tempe using the Kjedhal method. This method is used to see the quantitative value of nitrogen determination. This method is used to indirectly analyze crude protein levels in a food ingredient. Analysis of protein levels using the Kjedhal method is divided into 3 processes, namely, digestion, distillation, and titration. In the destruction stage, the sample is heated in concentrated sulfuric acid so that the protein is destroyed into its elements. Adding a catalyst will increase the boiling point so that the process runs quickly. The N element in protein is used to determine the protein content in a material. In the distillation stage, the ammonium sulfate formed will be broken down into ammonium (NH4) by adding base and a heating process. This stage ends when all the ammonium is completely distilled, which is indicated by the distillate no longer reacting. The next process is the titration stage which is marked by a change in the color of the solution to pink.

The results of the protein test carried out on extraction samples with 10 grams of each sample, obtained results on durian seed tempe samples 2 times in a row arerepetition 1 obtained 0.007 grams and repetition 2 was 0.425 grams so that the average protein content was 0.216 grams from 1 gram of the average total weight of durian seed tempe after freeing the fat and water content. These results show that the protein content of durian seed tempe is quite high when compared with research (Melianti et al, 2019) which states that durian seed tempe has a protein content of 0.05 grams.

The protein content before fermentation is almost the same when compared to after the fermentation

process, where the level before fermentation is 2.1 grams in 100 grams of durian seeds (Shi et al., 2021).Meanwhile, after it becomes tempe, the content obtained is 0.216 grams in 10 grams. This is due to fermentation using the fungus Rhizopus oligosporus which is proteolytic and this is important in breaking down proteins (Rizal et al., 2020). The longer the fermentation process takes, the longer the opportunity for the fungus to degrade protein, so that more and more protein is degraded, as a result, the protein in tempe decreases, this is indicated by the appearance of a rather pleasant aroma from tempe. As a result of the hydrolysis process, peptide bonds in proteins are broken. Then this opinion is supported by research by (García Arteaga et al., 2021) which states that protein will be graded or hydrolyzed during the fermentation process, as illustrated in Figure 4.

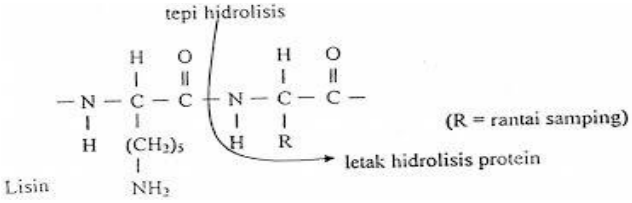

Figure 4. Protein hydrolysis during the fermentation process

The protein content in durian seed tempe is smaller when compared to soybean tempe, this is because in making durian seed tempe the boiling process occurs twice which results in the protein content in the durian seeds being reduced. The differences in protein levels obtained in each repetition are influenced by several factors, including the high water content in durian seed tempe, temperature, and also time. This is proven by research by (Zhang et al., 2020) that the factors that influence the high and low percentage of protein content are temperature, time, and the optimum ratio between water and collagen as well as the number of processing processes carried out.

Carbohydrate Content Analysis

The results of research that has been carried out in determining carbohydrates in durian seed tempe using the methodanthrone. In this study, the aim was to determine the results of the carbohydrate content in durian seed tempe. First, a glucose standard calibration curve was created.

The durian seed tempe samples were subjected to three consecutive repetitions of the carbohydrate content test, with an average value of 2.421 grams for repetition 1 (2.266 grams), repetition 2 (2.604 grams), and repetition 3 (2.395 grams). The test was conducted on the extraction samples, with 10 grams of each sample.

When starch is hydrolyzed with HCl, the starch molecules are broken down into smaller sugar molecules. With alpha-glycosidic linkages, starch is a homopolymer of glucose. Heating and an acidic hydrolysis treatment will break down the alpha-glycosidic linkages in starch molecules. The starch molecules will disintegrate into simpler or smaller molecules upon breaking this link.

According to (Shiferaw Terefe & Augustin, 2020) changes in nutritional value often occur in carbohydrates, this is due to the handling, preservation, and storage processes during the manufacture of tempe. The longer the boiling process, the more the carbohydrate content in a sample increases because the starch granules swell which can increase the total value of carbohydrates, in the process the saccharides break down into smaller pieces to produce simple starches. When the starch is heated, it will cause the granules to expand so that they will break and disintegrate. This event is illustrated in Figure 5.

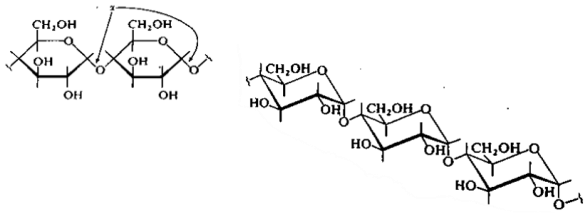

Figure 5. Starch hydrolysis

Conclusion

The study of the fat content of durian seed tempe yielded a value of 0.006 grams, according to the research results collected. Among 100 grams of durian seed samples collected in Binangga Village, Central Parigi District, Parigi Moutong Regency, the protein content of the durian seed tempe is 0.216, and the carbohydrate content is 2.421 grams.

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Author Contributions

Siti Nuryanti, Safna Nurfadila, Sitti Rahmawati, Anang Wahid M. Diah: conceptualization, analysis, methodology, discussion, conclusion, visualization, investigation, writing–original draft, and proofreading; Dewi Satria Ahmar: review and editing, Sri Mulyani Sabang: funding acquisition and resources.

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Conflicts of Interest

The Authors declare no conflict of interest regarding the publication of this paper.

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