PROTEIN TEST WITH CHEMICAL SOLUTION IN IDENTIFYING NUTRIENT CONTENT IN FOOD MATERIAL

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ABSTRACT

This study aims to find out the four factors that because proteins can be denatured, Knowing the solubility of proteins, as well as Knowing the bond of peptides in proteins, the presence of free and aromatic amino.

The method used in this study is qualitative research that is descriptive, with sampling techniques that use purposive sampling method. The results showed that observations of protein denaturation that aims to see the presence of deposits in milk and the results obtained that does not occur precipitation, deposition by metals as for the results obtained that is only raw egg whites that do not occur precipitation while for the other 3 samples occur precipitation.

The results of the precipitation test by alcohol obtained that tubes 1 and 2 there are no deposits while for tube 3 there are deposits, protein solubility test is obtained that the sample 1,2,4 insoluble and there are deposits while for cheese samples are only insoluble. biuret test observations obtained the occurrence of discoloration in the sample. As for the Xanthoprotein test obtained results from the sample 1,2,4 there was a change in sediment and color while for samples 3 and 5 Only changes in sediment.

Keywords: proteins, chemical solutions, nutrients

INTRODUCTION

Food is a basic human need to live life. Food consumed must have the nutrients needed by the human body for the growth, development, maintenance and repair of body tissues, which are utilized directly by the body which includes proteins, vitamins, minerals, fats and water.Protein (protos yang berarti "paling utama") adalah senyawa organik kompleks yang mempunyai bobot molekul tinggi yang merupakan polimer dari monomer-monomer asam amino yang di hubungkan satu sama lain dengan ikatan peptida [6].

Protein is a very important biomolecule. Some functions of proteins are as catalysts (enzymes), transport and storage, causes of movement, immune system support, formation and transmission of nerve impulses, growth control and differentiation, supporting structural rigidity, and others. Protein-forming monomers, amino acids, also play an important role in the metabolism of living cells. These roles are as substrates for protein synthesis, nitrogen suppliers for the synthesis of compounds containing other nitrogen, and energy sources when catabolized [11].

High protein has an assortment class in biomolecular. Starting from differences in chemical properties, such as weight, shape, size and soluble power, making it possible to carry out many biological functions. These functions include enzyme catalysts, metabolic regulation, binding and transporting small molecules, gene regulation, immunology defenses and cell structure. Cellular activity and

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such functions involve one or more proteins. The basic structure of proteins is amino acids. Where there are 20 amino acids that make up proteins, they all have a certain structural shape. This form is a carboxylic acid group (-COOH) and an amino base group (-NH 2). Therefore, what distinguishes the shape from the other is the building chain of its structure. Amino acids of proteins are connected by peptide bonds between carboxyl groups and their amino α form linear polymers [3].

Protein contains benzene-laced amino acids, if added concentrated nitric acid will settle with white deposits that can turn yellow when heated. Nitro compounds formed in alkaline atmospheres will be ionized and their color will turn older or orange. This reaction is based on the nitration test of benzene nuclei contained in protein molecules into yellow intri compounds [5].

Proteins have a variety of different biological functions, namely as enzyme catalysts, transport and storage, mechanical functions, movement, protection, and information processes [12].

Protein is derived from the Greek proteos, which means the main or the first. The word was introduced by dutch chemist Geraldus Mulder (1802-1880). He argues that protein is the most important substance in any organism. Protein molecules contain elements C, H, O, and special elements contained in proteins and are not present in carbohydrate and fat molecules are nitrogen (N) [1].

Protein is a food substance that is very important for the body, because this substance in addition to functioning as fuel in the body also serves as a building and regulatory substance. Protein is a source of amino acids containing elements C, H, O, and N that are not owned by fats or carbohydrates. Protein molecules also contain phosphorus, sulfur, and there are types of proteins that contain metal elements such as iron and copper [2].

Based on the source protein is classified as two, namely animal protein and vegetable protein. Animal protein is a protein in foodstuffs derived from animals. Examples of proteins from meat, milk protein, and so on. Vegetable protein is a protein derived from plant food. Examples of proteins from corn, from wheat, and so on [10].

Protein means "first or foremost" is the most abundant macromolecule in cells and makes up more than half the dry weight of almost any organism. Amino acids, protein structure units, and simple peptides, which consist of several amino acids combined by peptide bonds. Protein structure consisting of polypeptides that have a very long chain, composed of many units of amino acids [7].

Nitrogen is the main element of protein as much as 16% of the protein weight. Protein molecules also contain phosphorus, sulfur, and there are types of proteins that contain metal elements such as copper and iron [8].

An amino acid is usually classified as a molecule that has both carboxyl and α-amino α and chemically a distinctive side chain (R group) attached to the carbon-α [8].

Protein quality can be defined as the efficiency of protein use by the body.2 The quality of protein is determined by the type and proportion of amino acids it contains. In principle a protein that can provide essential amino acids in a comparison that equals human needs, has a high quality. In contrast, proteins that lack one or more essential amino acids have low quality [8].

Protein classification based on its bilogy function consists of: enzymes, building proteins, contractile proteins, transporting proteins, hormone proteins, toxic proteins, protective proteins, and

reserve proteins [8]. Protein classification is found in the form of fibers (fibrous), globular, and conjugated. Fiber-shaped proteins consist of several spiral-shaped peptide chains that are intertwined with each other so that they resemble rigid stems. The protein characteristic of the fiber form is that it has low soluble power, high mechanical strength, and is resistant to digestive enzymes [8].

Conjugated proteins are simple proteins that are bound to non-amino acidic ingredients. This non-amino acid group is called a prosthetic group. Nucleoprotein, lipoprotein, phosfoprotein, metaloprotein, hemoprotein, and flavoprotein are included in conjugated proteins [8].

Proteins are of giant biomolecules, in addition to polysaccharides, lipids, and polynucleotides, which are the main constituents of living things. In addition, proteins are one of the most studied molecules in biochemistry. The protein was discovered by Jons Jakob Berzelius in 1838. Natural protein biosynthesis equals genetic expression [9].

The genetic code carried by DNA is transcribed into RNA, which acts as a mold for ribosoma translations. Until this stage, proteins are still "raw", composed only of proteinogenic amino acids. Through the posttranslation mechanism, proteins are formed that have full biological function. Protein has a special function that can not be replaced by other nutrients, namely building and maintaining the cells clan tissues of the body [9].

In physic, protein plays an important role, chemical processes in the body can take place well due to the presence of enzymes that are inflated as biocatalysts. In addition, hemoglobin in red blood grains or erythrocytes that serve as a transport of oxygen from the lungs to all parts of the body is one type of protein. Similarly, substances that play a role in fighting disease bacteria or called antigens, as well as a protein [9].

Protein is needed for growth, development, muscle formation, formation of red blood cells, the body's defense against diseases, enzymes and hormones, and the synthesis of other body tissues. Proteins are dicema into amino acids, which are then formed body proteins inside muscles and other tissues. Protein can serve as a source of energy when the kaibohydrate consumed is insufficient such as during strict dieting or during intensive physical exercise. Preferably, approximately 15% of the total calories consumed come from protein [9].

Biuret is a compound with two peptide bonds formed at the heating of two urea molecules. Cu2 ions from biuret reagents in alkaline atmospheres will react with polypeptides or peptide bonds that make up proteins forming complex compounds in purple or violet . this reaction is positive to two or more peptide bonds, but negative for free amino acids or dipeptides [6].

Determination of protein levels in milk has been done by Kamizake, et al., (2003) by method of Kjeldahl and Spectrophotometry using Biuret, Lowry, and Bradford reagents. Based on the study, the spectrophotometry method provides the results of determining protein levels that are not much different from using the Kjeldahl method. Bradford's reagent spectrophotometry has the best sensitivity compared to Biuret and Lowry reagents. Compared to the Kjeldahl method, determining protein levels in the sample in spectrophotometry needs to be done fat separation first and also requires BSA (Bovine Serum Albumine) or Casein as the default comparison [5].

RESEARCH METHODS

Methods Used

In this study used a type of research that is descriptive. In this study using

qualitative laboratory examination with Xanthoprotein test method, Biuret Test, Metal Deposition Test, Protein denaturation test, protein solubility test, Precipitation Test on alcohol in food and beverage ingredients. Qualitative research was conducted to determine whether or not there is a protein content in the sample.

In this study sampling techniques used purposive sampling method. Purposive sampling method is a method of deliberate sampling for a specific purpose based on research purposes.

In this practicum the tools used are drip pipettes, test tubes, test tube racks, tube clamps, water handlers, beakers, and measuring cups. As for the materials used are HCl 0.1 M, NaOH 0.1 M, buffer acetate 1M (pH 4.7),, solution Pb-acetate 5% (pH 4.7), ammonium sulfate, Breast milk, , HgCl2 solution 2%, AgNO3 solution 5%, ethanol 95%, NaOH 40%, concentrated HNO3, casein, aquadest, chloroform, millon reagent, NH4OH, NaOH o,1 N, NaOH 10%, CuSO4 1%, K-oxalate, PP indicator, Farmaldehyde, and sample

Working Procedure

1. Xanthoprotein Test
   a. Prepare 4 clean and dry test tubes and label each test tube,
   b. Pour samples on each measuring glass as much as 2 ml,
   c. Add on each tube 1 ml HNO3 concentrated. Note the presence of white deposits formed,
   d. Heat 1 minute until the precipitate dissolves again and the solution turns yellow,
   e. Observe and record changes that occur. The reaction is positive, if at the border number (interface), between the protein and NaOH formed orange color.

2. Biure Test
   a. Prepare 4 pieces of tubes that have been given first and then label each tube make sure the appliance is dry,
   b. Filling each tube with albumin and casein solution and cheese as much as 2 ml,
   c. Add to the tube setiaap 2 ml NaOH 10% and CuSO4 1% as much as 10 drops,Mixing well,
   d. Observe and record changes that occur.
3. Metal Precipitation Test
   a. Prepare 4 pieces of tubes that have been given first and then label each tube make sure the appliance is dry,
   b. Insert each tube with a solution of albumin as much as 1 ml,
   c. Dripping sequentially with 5 drops of 5% Pb-acetate solution, HgCl2 solution 2% and AgNo3 solution 5%,
   d. Observing changes that occur.
4. Protein denaturation test
   a. Prepare tools and materials and label each tool to use,
   b. Prepare 3 test tubes and racks of test tubes,
   c. Pour 2 ml of milk on each test tube,
   d. Added HCl 0.1 M on the first tube,
   e. Added 1 ml naoh 0.1 M on the second tube,
   f. Add acetate buffer on third tube,
   g. Heat the sample in boiling water for 15 minutes,
   h. See what changes are happening,
   i. Cool the sample at room temperature or room temperature,
   j. Added 10 ml acetate buffer on tube containing milk + HCl and milk + NaOH,
   k. Observe the changes that occur.
5. Protein solubility test
   a. Prepare 20 pieces of tubes that have been given first and then label the numbers (1,2,3,4,5) on each tube make sure the appliance is dry,
   b. Inserting 1 ml aquadest in consecutive tubes 1, HCl 10% as

much as 1 ml on tubes that have label number 2, and on labeled number 3 filled with NaOH 0.25% as much as 1 ml, on tubes labeled number 4 added with alcohol 96% as much as 1 ml as well, and tubes labeled number 5 added with chloroform as much as 1 ml ,

c. And on the number tubes on each tube containing samples of chemicals added with raw egg albumin, whole milk, dissolved cheese and cooked egg albumin, each is added as much as 2 ml to each test tube that has been labeled on each type,
d. Then all the tubes containing the sample are shaken or shaken slowly in order to see the changes that occur,
e. Observe changes to the sample.

6. Alcohol Deposition Test
   a. Prepare 4 pieces of tubes that have been given first and then label each tube make sure the appliance is dry,
   b. Insert each of 3 test tubes as much as 3 ml of milk,
   c. Added 1 ml HCl 0.1 M and ethanol 6 ml on test tube 1,
   d. Add 1 ml naoh 0.1 M, and ethanol in test tube 2 as much as 6 ml,
   e. Insert 1 ml of acetate buffer solution and 6 ml of ethane in the test tube to 3,
   f. Observe the changes that occur.

RESEARCH RESULTS

The results we get from the protein practicum are:

Tabel 1: Observations of protein denaturation

No.	Sampel	Result			
		Added HCl 0,1 M	Added NaOH 0,1 M	Added buffer asetat	Once simmered and added buffer asetat
1.	milk	Deposition occurs	No Deposition occurs	No deposition occurs	No deposition occurs

Source: Primary data, 2019

Tabel 2: Precipitation result by metal

No.	Sampel	Result			Description
		$HgCl_2$	pb-asetat	$AgNO_3$	
1.	Raw egg whites	No sediment	No sediment	No sediment	No change
2.	Raw egg yolks	There are sediment	There are sediment	There are sediment	Changes have occurred
3.	Cooked egg whites	There are sediment	There are sediment	There are sediment	There is a change
4.	Cooked egg yolks	There are sediment	There are sediment	There are sediment	There is a change

Source: Primary data, 2019

Tabel 3: Results of precipitation test by alcohol

No.	Sample	Observations
1.	Tube 1 HCl+ethanol	No sediment
2.	Tube 2 NaOH+ethanol	No sediment
3.	Tube 3 acetic buffer solution+ethanol	There are sediment

Source: Primary data, 2019

Tabel 4: Protein solubility test results

No	Sample	Result				
		Aquades	HCl	NaOH	alkohol	klorofo rm
1.	Raw egg albumin	soluble	Insoluble	soluble	soluble	Insoluble and there are sediment
2.	Whole milk	soluble	Insoluble	soluble	soluble	Insoluble and there are sediment
3.	cheese	soluble	Insoluble and there are sediment	soluble	soluble	Insoluble
4.	Albumin egg white cook	Soluble	Insoluble	Soluble	Soluble	Insoluble and there are sediment

Source: Primary data, 2019

Tabel. 5: Biuret Test Observation Results

No	Sample	Observations
1.	Raw egg whites	Light green
2.	Cooked egg whites	Solid light blue
3.	cheese	Clear light blue
4.	Cooked egg yolks	Yellowish green

Source: Primary data, 2019

Tabel. 6: Xanthoprotein Results

NO.	Sample	Result		information
		HNO_3	NaOH	
1.	Sweetened condensed milk	There are sediment and yellowing	Positively contains protein and turns orange	Changes in precipitate and color
2.	Bearbrand milk (whole milk)	There are sediment and yellowing	Positively contains protein and turns orange	Changes in precipitate and color
3.	Cheese	There are sediment and yellowing	Negatively protects protein and does not change color	Only changes in sediment
4.	Egg white (Albumin)	There are sediment and yellowing	There are sediment and turn orange	Changes in precipitate and color
5.	Egg yolk	There are sediment	There are sediment and not discolored	Only changes in sediment

Source: Primary data, 2019

DISCUSSION

Protein (protos which means "most important") is a complex organic compound that has a high molecular weight which is a polymer of amino acid monomers that are connected to each other with peptide bonds [5].

Biuret is a compound with two peptide bonds formed at the heating of two urea molecules. Cu2 ions from biuret reagents in alkaline atmospheres will react with polypeptides or peptide bonds that make up proteins forming complex compounds in purple or violet . this reaction is positive to two or more peptide bonds, but negative for free amino acids or dipeptides [5].

Protein contains benzene-laced amino acids, if added concentrated nitric acid will settle with white deposits that can turn yellow when heated. Nitro compounds formed in alkaline atmospheres will be ionized and their color will turn older or orange. This reaction is based on the nitration test of benzene nuclei contained in protein molecules into yellow intri compounds [5].

And in this experiment on protein analysis practice conducted several trials in which:

1. Xanthoprotein test, Prepare 4 clean and dry test tubes and label each test tube, Pour samples on each measuring cup as much as 2 ml, Adding to each tube 1 ml HNO_3 concentrated.

Figure 1: xanthoprotein test samples

Note the presence of white deposits formed, Heat 1 minute until the precipitate dissolves again and the solution turns yellow, Observe and note the changes that occur. The reaction is positive, if at the border number (interface), between the protein and NaOH formed orange color. So the result that we get is a sample of sweetened condensed milk produces a color that is orange which means positively contains protein, in the whole milk sample also undergoes discoloration and the resulting color is also orange, but in the cheese sample does not experience a discoloration that means the cheese sample used negative or does not contain protein, and in albumin also undergoes a discoloration as well as the 2 samples above that are orange , and in the yolk also does not experience discoloration only changes in the resulting deposits.

2. Biuret Test, Prepare 4 pieces of tubes that have been given first and then label each tube make sure the appliance is dry, Fill each tube with albumin and casein solution and cheese as much as 2 ml, Add to the tube setiaap 2 ml NaOH 10% and CuSO4 1% as much as 10 drops, Mix well, Observe and record the changes that occur.

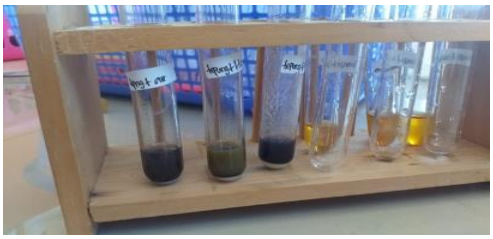


Figure 2: Biuret Test

So the results that we get is in the raw egg albumin sample produces a color that is light green, and in the sample of ripe egg whites produce a deep light blue color, in the cheese sample produces a fairly clear or clear light blue color, and in the yolk sample produces a yellowish color.

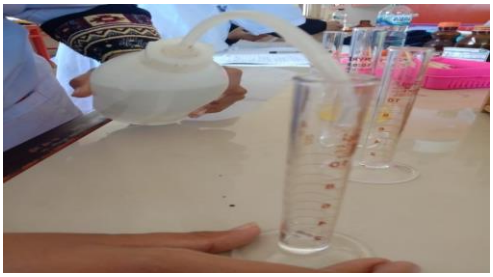
3. Metal Deposition Test, Prepare 4 pieces of tubes that have been given first and then label each tube make sure the appliance is dry, Insert each tube with albumin solution as much as 1 ml, Drip in sequence with 5 drops of Pb-acetate solution 5%, HgCl2 solution 2% and AgNo3 solution 5%, Observing the changes that occur.

Figure 3. Uji pengendapan oleh logam

So the result that we get is that the raw egg whites added with the three solutions also do not experience precipitation at all, in the raw egg yolk samples added the three chemical solutions undergo changes in which in the sample occurs deposition, and in the mature egg white samples added with 3 chemical solutions also produce deposits in the sample used , as well as in the yolk that has matured also produces the same result that is the occurrence of deposits in the sample.

4. Protein denaturation test, In the 4th test the practice will Prepare tools and materials and label each tool to be used, Prepare 3 test tubes and test tube racks, Pour 2 ml of milk on each test tube, Add HCl 0.1 M on the first tube, Add 1 ml NaOH 0.1 M on the second tube, Add acetate buffer on the third tube. Heat the sample on boiling water for 15 minutes, See the changes that occur, Cool the sample at room temperature or room temperature, Add 10 ml acetate buffer on the tube containing milk + HCl and milk + NaOH, Observe the changes that occur. So the result that we get is and the result obtained is on the first tube containing milk samples + HCl 0.1 M undergoing precipitation, and in the second tube milk sample + NaOH 0.1 M no deposition occurs at all, and the last in the third tube of milk samples + acetate buffer also does not produce changes such as the occurrence of sediment.

5. Protein solubility test, Prepare 20 pieces of tubes that have been given first and then label the numbers (1,2,3,4,5) on each tube make sure the appliance is dry, Enter 1 ml aquadest on consecutive tubes 1, HCl 10% as much as 1 ml on tubes that have a number label 2, and on the labeled number 3 filled with NaOH 0.25% as much as 1 ml , on the tube labeled number 4 added with alcohol 96% as much as 1 ml as well, as well as tubes labeled number 5 added with chloroform as much as 1 ml,



Figur 4: uji kelarutan protein

And on the number tubes on each tube containing samples of chemicals added with raw egg albumin, whole milk, dissolved cheese and cooked egg albumin, each is added as much as 2 ml to each test tube that has been labeled on each type,Then all the tubes containing the sample shaken or shaken slowly in order to see the changes that occur, Observe the changes in the sample. And the results obtained are in the sample of raw egg albumin on the first tube albumin can be dissolved, and on the second tube of raw albumin is not dissolved, on The third tube of raw albumin can be dissolved, the tube to the four raw albumin can dissolve as well and the fifth tube of raw albumin is insoluble and produces deposits. Then the result of the whole milk sample, on the tube one milk sample can be dissolved, the second tube of the sample can not be dissolved, the tube the three milk samples were dissolved, the tubes of four milk samples were also dissolved, and on the fifth tube also the milk samples were not dissolved and precipitation occurred. After that, the practice of observing the results obtained in the third sample is in the dissolved cheese which in the first tube of cheese can be dissolved, but in the second tube the cheese sample is not dissolved and undergoes hardening back on the cheese sampe, then on the third tube and the four cheese samples can be dissolved, and on the fifth tube the cheese sample does not experience solubility. And the latter practically observes the changes that occur in mature albumin samples wherein in the first tube albumin undergoes solubility while the second tube of mature albumin does not experience solubility, and in the third and fourth tubes albumin undergoes solubility, only in the fifth tube mature albumin does not experience solubility and there are sediment.

6. Precipitation Test on alcohol, Prepare 4 pieces of tubes that have been given first and then label each tube make sure the appliance is dry, Insert 3 test tubes as much as 3 ml milk, Add 1 ml HCl 0.1 M and ethanol 6 ml on the test tube 1, Adding 1 ml of NaOH 0.1 M, and ethanol to the 2nd test tube as much as 6 ml, Inserting 1 ml of a 6 ml acetate buffer solution and 6 ml of ethane in the 3rd test tube, observe the changes that occur. And the result obtained in the first tube is that the sample has no sediment or no change, and the second tube of the sample also has no change or absence of deposits, then in the third tube the sample has been precipitation or has been subjected to changes in the sample added with acetic buffer solution and ethanol.

CONCLUSION

The conclusion of this experiment is that there are some foodstuffs that we often find are food ingredients that contain many nutrients including protein, perotein itself can be tested on each foodstuff by using some chemical solutions that are specific to be able to analyze the positive and negative ingredients contain protein nutrients. Protein itself is a nutrient that is also needed in the body after carbohydrates. Prtein is a source of development for the human body.

In this practice, it is recommended to wear personal protective equipment and also practice is expected to be able to understand the practical process or work procedures performed at the time of the experiment.

The obstacles obtained are in this protein experiment there are some

experiments that can not be done by practice because of the absence of materials provided in the laboratory so that the practice is only able to conduct 6 experiments on this biochemical practicum.

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