ANIMAL AND VEGETABLE LIPID SOLUTION TEST IN IDENTIFYING FATTY ACID HYDROLYSIS REACTIONS

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ABSTRACT

This study aims to determine the solubility of animal and vegetable lipids, and can determine the saponification reaction in fats and determine the amount of saponification and determine the unsaturation of fatty acids through the determination of the iodine value.

The research method used is a laboratory experimental method to obtain result data.

The results showed that in the fat solubility test experiment, it was seen that cooking oil could not dissolve in all solutions because distilled water was water so oil which was insoluble in water could not dissolve in the solvent. Saponification reaction test: Oil dissolves when a solvent or alcohol reagent is added. The number of saponification with the results obtained that do not match the results with the experiment is because an error occurred during the practicum so that the color that should appear, which is pink, turns cloudy.

Keywords: lipids, saponification, fatty acids.

INTRODUCTION

Lipids refer to a class of nonpolar and hydrophobic aliphatic hydrocarbon compounds. Because they are nonpolar, lipids are insoluble in polar solvents such as water, but soluble in nonpolar solvents, such as alcohol, ether or chloroform. The most important biological functions of lipids include storing energy, as structural components of cell membranes, and as signaling molecules [12].

Lipids are organic compounds obtained from the endothermic dehydrogenation process of hydrocarbon chains. Lipids are amphiphilic, meaning they are able to form structures such as vesicles, liposomes, or other membranes in a wet environment. Biological lipids are wholly or partly derived from two types of biochemical subunits or "building blocks": ketoacyl groups and isoprene groups. Using this approach, lipids can be divided into eight categories: fatty acyl, glycerolipids, glycerophospholipids, sphingolipids, sacarolipids, and polyketides (derived from condensation of ketoacyl subunits); and sterol lipids and prenol lipids (derived from condensation of isoprene subunits) [17].

Although the term lipid is sometimes used as a synonym for fat. Lipids also include molecules such as fatty acids and their derivatives (including tri-, di-, and monoglycerides and phospholipids, as well as sterol-containing metabolites, such as cholesterol. Although humans and mammals have metabolisms to break down and form lipids, some lipids do not. can be produced in this way and must be obtained through food [10].

Lipids (from the Greek lipos, fat) are constituents of plants or animals which are characterized by their solubility properties. Lipids are insoluble in water, but soluble in nonpolar solvents such as chloroform, ether, and benzene. The main

constituents of lipids are triglycerides, which are glycerol esters with three different types of fatty acids [3].

Lipids are compounds which are esters of fatty acids with glycerol which sometimes contains other groups. Lipids are insoluble in water, but soluble in organic solvents such as ether, acetone, chloroform, and benzene [13].

Lipids can generally be divided into two broad classes, namely simple lipids and complex lipids. The simplest lipids and contain the fattiest acids as their constituent units are triacylglycerols, also often called fats, neutral fats, or triglycerides. This type of lipid is an example of the most common lipid found in humans, animals, and plants. Triacylglycerol is a major component of fat storage or fat depots in plant and animal cells, but is generally absent in membranes. Triacylglycerol is a nonpolar hydrophobic molecule, because this molecule does not contain an electric charge or functional group with high polarity [6].

A lipid is defined as an organic compound that occurs in nature and is insoluble in water, but soluble in a non-polar organic solvent such as a hydrocarbon or diethyl ether. Lipids do not have the same molecular formula, but are made up of several different groups. Based on their similar chemical structure, lipids are divided into several groups, namely fatty acids, fats and phospholipids. Fats are chemically defined as esters of fatty acids and glycerol. The general formulas of fats, namely R1, R2, and R3 are hydrocarbine chains with the number of carbon atoms from 3 to 23, but the most common are 15 and 17 [14].

Fats and oils are triglycerides or triacylglycerols, both terms mean “triester (of) glycerol”. The difference between a fat and an oil is arbitrary: at room temperature fats are solid and oils are liquid. Most of the glycerides in animals are in the form of fat, while the glycerides in plants tend to be in the form of oil [2].

Fats are classified according to the saturation of the bonds in their fatty acids. The classification is saturated and unsaturated fatty acids. Fats that contain saturated fatty acids, namely fatty acids that do not have double bonds. In animal fats such as pork fat and beef fat, the saturated fatty acid content is more dominant. Unsaturated fatty acids are fatty acids that have double bonds. This type of fatty acid can be identified by an addition reaction, where the double bond will be broken to form a saturated fatty acid [14].

Saponification is a process that reacts a fat or a glyceride with a base [1]. [15]. The nutritional content of virgin coconut oil has quite a lot of benefits. The components of virgin coconut oil consist of saturated fatty acids (90%). The content of saturated fatty acids is fatty acid solution. From the saturated fatty acid content, the uses of virgin coconut oil can be used as cosmetic raw materials such as lotion, lip moisturizer, hair conditioner, and bath soap.

Lipids (Greek lipos fat) are a large group of natural compounds that are insoluble in water, but soluble in nonpolar organic solvents such as n-hexane, chloroform and diethyl ether. It is this property that distinguishes lipids from carbohydrates, proteins, nucleic acids and most other biological molecules. The molecular structure of lipids is very diverse, so we must review many of the functional groups we have studied previously. Compounds belonging to the lipid group are triglycerides, waxes, phospholipids, glycolipids, steroids, terpenes, prostaglandins [9].

Lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds that are related more by their physical properties than by their chemical properties. This compound is an important food

constituent not only because of its high energy value, but also because of the fat-soluble vitamins and essential fatty acids contained in natural dietary fats. Fat is stored in adipose tissue, where it also functions as an electrical insulator, and allows the propagation of depolarizing waves along myelinated nerves. The combination of lipids and proteins (lipoproteins) is an important cellular constituent that is present in both the cell membrane and in the mitochondria, and also functions as a transport vehicle for lipids in the blood. Knowledge of lipid biochemistry is required to understand many important biomedical areas,

DAmong the many types of oil, coconut oil is the most commonly used. Crude coconut oil contains non-oil components such as phosphatides, gums, sterols (0.06%-0.8%), tocopherols (0.003%), and pineapple fatty acids less than 5%. The color of the oil is caused by the presence of natural color pigments, carotene, which is an unsaturated hydrocarbon (Ketaren, 1986). The most important property of coconut oil is that it does not melt step by step like other fats, but immediately turns into a liquid because the melting points of the constituent fatty acids are close together [7].

Margarine is a food product that has a semi-solid texture with physical and chemical characteristics that are very dependent on the constituent components. Margarine is an emulsion system consisting of 20% water in 80% oil. The raw material content of margarine has high brittleness and resistance to development [18].

GliSerol is a major component of all fats and oils, in the form of esters called glycerides. Glycerol was found to have a wide variety of uses in the manufacture of various domestic, industrial, and pharmaceutical products. Glycerol (CH2OH.CHOH.CH2OH or propane-1, 2, 3-triol) has a pure form, which is clear, odorless, and viscous. Glycerol is soluble in water and alcohol, slightly soluble in many common solvents such as ether and dioxane, and insoluble in hydrocarbons. At low temperatures, glycerol sometimes forms crystals which tend to melt at 17.9oC. Liquid glycerol boils at 290oC under normal atmospheric counterparts [16].

testThe lipid tests in this study included the unsaturation test and the acrolein test. The degree of unsaturation is expressed by the iodine number. The amount of salt that can be absorbed by 100 grams of fat for the saturation reaction. The greater the iodine number, the higher the unsaturation [14]. The unsaturation test is used to determine whether the fatty acids tested are saturated or unsaturated fatty acids. Hubl's iodine is used as an indicator of change. A positive reaction is indicated by the appearance of a pink color, then the color returns to its original color (clear). The color that returns to the original color indicates that there are many double bonds in the fatty acid hydrocarbon chain. The pink color lost during the reaction indicates that the unsaturated fatty acids have reduced Hubl's Iod reagent. Meanwhile, the acrolein test is a test on glycerol in its free form or in fats and oils when it is dehydrated to form axyl aldehyde or also called acrolein. A positive reaction occurs when the heated and dehydrated fat smells like burning fat with white smoke.

The grouping is divided into two based on the identity and location of the three constituent fatty acid components. Compounds with the same fatty acid content in all three positions of the hydroxyl group are called simple triglycerides. Triglycerides containing two or more different fatty acids are called mixed triglycerides.

Tests related to Lipid, In lipids There are various kinds of tests related to lipids

which include qualitative and quantitative analysis. Qualitative lipids such as lipid solubility test, arcolein test, lipid saturation test, rancidity test, Salkowski test for cholesterol. While the quantitative test is the Lieberman Buchard test and the iodine number test [15].

Lipid solubility test consists of analysis of the solubility of lipids and lipid derivatives against various solvents. In this test, the solubility of lipids is determined by the nature of the polarity of the solvent. When a lipid is dissolved in a polar solvent, the result is that the lipid will not dissolve. This is because lipids have nonpolar properties so they will only dissolve in solvents that are both nonpolar [9].

Another lipid qualitative test is the acrolein test. In this test, there is dehydration of glycerol in the free form or in fat/oil to produce aldehyde acrylate or acrolein. The acrolein test is used to test for the presence of glycerin or fat. When fat is heated after adding a dehydrating agent (KHSO4) which will attract water, then the glycerol portion will be dehydrated into an unsaturated aldehyde form known as acrolein (CH2=CHCHO) which has an odor like burning fat and is characterized by white smoke [4].

The lipid unsaturation test was used to determine whether the fatty acids tested were saturated or unsaturated by using Hubl's Iod reagent. Hubl's iodine is used as an indicator of change. Fatty acids tested plus the same amount of chloroform. The tube is shaken until the material is dissolved. After that, drop by drop the Hubl Iod reagent was put into the tube while being shaken and the color change that occurred to the mixture was observed. Saturated fatty acids can be distinguished from unsaturated fatty acids by looking at their structure. Unsaturated fatty acids have double bonds in their hydrocarbon groups. The positive reaction of unsaturation of fatty acids is indicated by the appearance of a red color of fatty acids, then the color returns to the initial clear yellow color.

Triglycerides containing fatty acids that have double bonds can be added by the halogen group. In the unsaturation test, Huble's iodine reagent will oxidize fatty acids that have double bonds in their molecules to become single bonds. The pink color lost during the reaction indicates that the unsaturated fatty acids have reduced the huble's iodine reagent [6].

Another lipid qualitative test is the rancidity test. In this test, it is identified which lipids are rancid and which are not rancid caused by lipid oxidation. The oil to be tested is mixed with HCl. Next, a filter paper is dipped in the phloroglucinol solution. This phloroglucinol functions as a spot marker. After that, the paper is hung in an erlenmeyer containing the tested oil. CaCO3 powder was put into an erlenmeyer and immediately closed. The added HCl will donate hydrogen ions which can break down fat elements to form free radicals and hydrogen free radicals. Both forms of these radicals are very reactive and in the final stage of oxidation will produce peroxide [3].

The Salkowski test is a qualitative test performed to identify the presence of cholesterol. Cholesterol is dissolved in anhydrous chloroform and then sulfuric acid is added to the same volume. Sulfuric acid functions as a lipid ester bond breaker. If cholesterol is present in the sample, then the cholesterol layer at the top becomes red and sulfuric acid appears to turn yellow with a green fluorescent color [11].

The Lieberman Buchard test is a quantitative test for cholesterol. The principle of this test is to identify the presence of cholesterol by adding sulfuric acid to the mixture. A total of 10 drops of acetic acid were dissolved in a solution of cholesterol and chloroform (from the

Salkowski experiment). After that, concentrated sulfuric acid is added. The tube is shaken gently and left for a few minutes. The mechanism that occurs in this test is when sulfuric acid is added to a mixture containing cholesterol, water molecules move from the C3 group of cholesterol, cholesterol is then oxidized to form 3,5-cholestadiene. This product is converted to a polymer containing a chromophore which produces a green color. This green color indicates a positive result.

In lipids there is an iodine number test. Animal fats are generally solid at room temperature, while plant fats are liquid. Fats that have a high melting point contain saturated fatty acids, while liquid or alkaline fats are called oils containing unsaturated fatty acids. Animal and plant fats have different fatty acid compositions. To determine the degree of unsaturation of the fatty acids contained in it, it is measured by the iodine number. Iodine can react with double bonds in fatty acids. Each iodine molecule undergoes an addition reaction to a double bond. Therefore, the more double bonds, the more iodine that can react [17].

Saponification is a process of alkaline hydrolysis of fats and oils, and the siponification reaction is not an equilibrium reaction. The initial product of saponification is a carboxylate because the mixture is basic. After the mixture is acidified, the carboxylate turns into a carboxylic acid.

RESEARCH METHODS

In this study used a descriptive type of research. In this study, qualitative laboratory examinations were used with the method of testing the content of globulin, biuret and hemoglobin in the blood. Qualitative research was conducted to determine the presence or absence of globulin, biuret and hemoglobin in the blood of adolescent women.

Material Description

The materials used in the blood lab include the following:

1. 90% ethanol, also known as ethyl alcohol, pure alcohol, absolute alcohol, or simply alcohol, is a kind of volatile, flammable, colorless liquid, and is the most commonly used alcohol in everyday life,
2. PP indicator, is a dye that acts as a pH indicator. Phenolphthalein is often used as an indicator in acid-base titrations. For this application, it changes color from colorless in acidic solution to pink in alkaline solution,
3. KOH 0.1 N, is a strong base made of the alkali metal potassium with atomic number 19 on the periodic table. Here are some useful ways to make most potassium salts,
4. Glacial acetic acid, is a colorless hygroscopic liquid, and has a freezing point of 16.7°C. Vinegar contains 3–9% by volume of acetic acid, making acetic acid the main component of vinegar besides water,
5. Chloroform, is the common name for trichloromethane (CHCl3). Chloroform is known because it is often used as an anesthetic, but its use has been banned because it has been proven to damage the liver and kidneys. Chloroform is mostly used as a nonpolar solvent in the laboratory,
6. Aquadest, is distilled/distilled water equal to pure water or H2O, because H2O almost does not contain minerals. While mineral water is a universal solvent,
7. Sodium thiosulfate, is a chemical compound and medicine. This compound is an inorganic compound which is usually available as the pentahydrate, Na2S2O3·5H2O. The solid is an eflorescent (loses water easily) crystalline substance that is well soluble in water.

8. Starch indicator (starch/starch. Another starch element, namely amylopectin, forms a reddish complex (violet) with iodine, which is difficult to remove because of the long chain.
9. Oil, is a general term for all organic liquids that are insoluble/miscible in water (hydrophobic) but soluble in organic solvents. Oil is a compound of triglycerides or triasglycerol, which means "triester of glycerol".
10. Fat, refers to a large group of natural molecules consisting of the elements carbon, hydrogen, and oxygen including fatty acids, waxes, sterols, fat-soluble vitamins (eg A, D, E, and K).), monoglycerides, diglycerides, phospholipids, glycolipids, terpenoids.

Tools and materials

The tools used in this practicum are; stand, clamp, burette, erlenmeyer, measuring cup, test tube, test tube rack, dropper, glass beaker, water bath, stirring rod.

The ingredients used are cooking oil, butter, gasoline, water, ether, 96% alcohol, hubl iodine reagent, 1 N NaOH, 1 N HCl solution, 90% ethanol, PP indicator, 0.1 N KOH.

Work procedures

1. Saponification Number
   a. Prepare tools and materials;
   b. Weigh 0.3 grams of fat, put it in an Erlenmeyer;
   c. Adding 25 ml of 90% ethanol and adding 5-10 drops of pp indicator;
   d. Then titrate with 0.1 N KHO until the solution turns pink;
   e. Record the volume of KOH used;
   f. Calculating the saponification number
2. Saponification Reaction
   a. Prepare tools and materials;
   b. Measuring 5 gr each sample;
   c. Enter cooking oil with a temperature of 70ºC;
   d. Adding NaOH little by little;
   e. Observing the changes that occur
3. Fat Solubility Test
   a. Prepare 4 ml test tube and test tube rack and label it;
   b. Melt the butter;
   c. Adding 1 ml of butter into each test tube that has been prepared;
   d. Add 1 ml of oil into each test tube that has been prepared;
   e. Mix with the following ingredients:
      Tube I : add 1 ml of water,
      Tube II : plus 1 ml of 96% alcohol,
      Tube III : plus 1 ml of ether,
      Tube IV : added 1 ml NaOH 1 N Stir until homogeneous.
   f. Let stand a few minutes and observe and note the changes that occur.

g. RESEARCH RESULT

The results of this study are an overview of the experimental animal and vegetable lipid solubility tests in identifying the hydrolysis reactions of fatty acids and bases, for students who take even semester biochemistry practicum courses.

From experiments using three reagents, the following results were obtained:

Table 1. Observation results of fat solubility test

Sample	Solvent			
	Water/Aquades	Ether	Alcohol	NaOH
Butter	Does not dissolve	Does not dissolve	Experiencing solubility	Experiencing solubility
Oil	Does not dissolve	Does not dissolve	Does not dissolve	Does not dissolve

Source: Microbiology Practicum Report.

Table 2. Observation results of saponification reaction test

Reagent	Information
Coconut oil is added with NaOH, HCl and alcohol	Dissolving when added with alcohol

Source: Microbiology Practicum Report.

Table 3. Test observation results saponification reaction

Sample		Result	Information
Titrate	titrant		
Fat, ethanol and pp. indicator	KOH	What is used is 9 ml, and it changes color from blue to a cloudy color	There is a color change

Source: Microbiology Practicum Report.

DISCUSSION

1. Fat solubility test, this experiment was conducted to determine the solubility of lipids in several solvents. In this experiment, the sample used was coconut oil and tested its solubility by adding distilled water, alcohol, NaOH and ether. And from the experimental results, it can be seen that cooking oil cannot be dissolved in all solutions because distilled water is water so oil that is insoluble in water cannot dissolve in the solvent. Even though alcohol is also included in organic solvents, in our experiments, cooking oil cannot dissolve in alcohol. This is because the solvent has been contaminated with other chemicals or the solvent has been around for a long time so its activity to dissolve fat has also been low or even lost, as well as other solvents.
2. While in butter, from the experimental results, it can be seen that alcohol and NaOH have dissolved this is because butter is non-polar, that is, it is insoluble in water, the same as the two organic solvents, so that it makes butter soluble in non-polar solvents (water insoluble), while But in aquadest and ether, butter is not soluble, this is because aquadest is water so oil which is insoluble in water cannot be dissolved in that solvent, as well as ether is also included in organic solvents, but in the experiments we did butter cannot dissolve in ether. . This is because the solvent has been contaminated with other chemicals.
3. Saponification reaction test, in this experiment using NaOH solution as one of the reagents where the results of observations of oil are added with 1 N NaOH little by little to produce a lot of foam (soap) and after the oil that forms the soap is added 1 N 3 drops of HCl, the result is that the foam begins to decrease then the sample is added with 90% alcohol little by little, the results you get the foam is meaningless, the oil dissolves when you add a solvent or alcohol reagent.
4. Saponification number, at this number of saponification what we do is weigh 0.3 g of fat and put it into Erlenmeyer, then we add 25 ml of 90% ethanol and also add 10 drops of pp indicator, then we titrate with 0.1 N KOH until the solution turns red young man, here we use 2 samples namely titrate and titrant, titrate samples in tube 1 are fat, ethanol and pp indicator. then the titrant is KOH with the result that tube 1 changes color from blue to a cloudy color, the volume of KOH used is 1 ml. and tube 2 the titrant sample is ethanol and the pp indicator and the titrant sample is KHO by getting the results of a color change from blue to a cloudy color, and the volume of KHO used is 5 ml,

CONCLUSION

Lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds that are related more by their physical properties than by their chemical properties. This

compound is an important food constituent not only because of its high energy value, but also because of the fat-soluble vitamins and essential fatty acids contained in natural dietary fats.

Suggestion

It is expected that the practitioner can be more orderly and skilled in conducting experiments and for the lecturer assistants are expected to be able to supervise and guide the practitioner so that he is not wrong in conducting the experiment. And practitioners are expected to always use PPE (Personal Protective Equipment) when doing practicum in order to avoid accidents if they occur.

Practical Obstacles

An error occurred in this practicum on the saponification number test where the result that should be obtained, namely the pink color, turned out to be a cloudy color, this was caused by an error during the practicum.

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