Effect of pH on Swelling Ratio and Fluorescence of Quantum Dots (QDs) Hydrogel ($CuInS_2/ZnS$ Hydrogel)

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ABSTRACT

Quantum dots (QDs) are semiconductor materials possessing a distinct electrical order and physical dimensions less than the excitation of the Bohr radius. The innovative combination of hydrogel and QDs has a broad range of applications in critical areas like the environment, health, and energy. QDs hydrogel has been applied as a biosensor and in the waste processing industry. The main characteristics of QDs hydrogel are fluorescence and swelling ratio. In the waste processing industry, the swollen hydrogel provides a more porous matrix that allows heavy metal to penetrate the hydrogels via passive diffusion. In the biosensor application, QDs hydrogel can be used as active sensors by changing the fluorescence properties when reacting with analytes, or by conjugating antibodies to the dot surface to act as passive label probes. This study aims to study the effect of pH on the fluorescence and swelling ratio of $CuInS_2/ZnS$ hydrogel. $CuINS_2/ZnS$ hydrogel is synthesized by sonication a mixture consisting of QDs ($CuInS_2/ZnS$), N-(3-aminopropyl) methacrylate, polyethylene glycol methacrylate, methylene bis acrylamide, and ammonium persulfate solution for 5 min with amplitude of 20 kHz. $CuInS_2/ZnS$ acts as QDs component, while N-(3-aminopropyl) methacrylate, polyethylene glycol methacrylate, methylene bis acrylamide, and ammonium persulfate act as hydrogel synthesizing components. The QDs hydrogel was then immersed in water with varying pH to observe the effect of pH on fluorescence and swelling ratio. The results show that increasing pH will reduce the swelling ratio and increase the fluorescence strength. $CuInS_2/ZnS$ hydrogel has a maximum swelling ratio at a pH of 5 and provides strong fluorescence at pH of 7, 9, and 11. The result also revealed that $CuInS_2/ZnS$ hydrogel has sufficient fluorescence and swelling ratio characteristic at the pH level of the biological fluid of the human body namely 7.35-7.45, this suggests the biological application of $CuInS_2/ZnS$ hydrogel such as for drug delivery and biosensing.

1. Introduction

Hydrogel is a macro-molecular material that has cross-linked tissue which when exposed to water forms a three-dimensional macromolecule tissue. It is capable of absorbing water exceeding its weight or volume and capable of releasing water based on external stimulation [1]. Some important applications of this hydrogel are in mechanisms for the controlled delivery of drugs, as heavy metal ion removals, as a material for lensing in tissue engineering, contact lens production, as sensors, and as super-capacitors [2], [3]. In biomedical fields, hydrogel is used to store bio-

macromolecules such as proteins and DNA [4]. The development towards the application of hydrogel is also applied as a fever-reducing plaster [5].

Meanwhile, quantum dots (QDs) are semiconductor materials possessing a distinct electrical order and physical dimensions less than the excitation of the Bohr radius. The Bohr radius is the distance in an electron–hole exciton [6]. Its value is 5.29×10^{-11} m [7]. The period of II or III–V elements from the periodic system are typically used to synthesize the QDs. QDs have potential applications in the fields of biology, nano-diagnostics, bio-imaging, drug delivery, and photodynamic therapy [8], [9]. QDs are frequently utilized in fluorescence detection because of their exceptional photoluminescence characteristics, strong water solubility, and good biocompatibility. The fluorescence quantum yield (QY) of QDs is a critical factor in determining their photoluminescence characteristics. Quantum yield is defined as the relationship between photon emission and absorption [10].

$CuInS_2$/ZnS is one of the QDs that has a 1.45 eV belt gap and has low stability, efficiency, toxicity, and spectrum in the red spectrum range to close to the visible light spectrum ranging from 600 to 1100 nm [11]–[14]. Adding Zn^{2+} or ZnS to the core of the QDs will improve the fluorescent performance of the semiconductor QDs. Deng et al. [15] indicate that an increase in the Zn in the AISe QDs system will lead to the blue spectrum at photoluminescent emission, which increases the quantum yield (QY) by 50%. Separately, Deng also observed the optical characteristics of the AISe QDs after the addition of the ZnS by hot injection process, the quantum yield of AISe QDs can reach 40% with emissions in the range of 700-820 nm. So this emission range makes QDs applicable for biological purposes [12].

The innovative combination of hydrogel and QDs has a broad range of applications in critical areas like the environment, health, and energy. QDs hydrogel has been applied as a biosensor and in the waste processing industry. Fluorescent QDs hydrogel has successfully identified several heavy metal ions such as Pb^{2+} [16], [17], Fe^{3+} [18]–[22], Cu^{2+} [19], [21], [23], Cr^{6+} [24], Ag^{+} [25] and Hg^{2+} [26]. Several types of QDs have been combined with hydrogel such as CdS, graphene-Fe3O4, ZnO, and CdSe ZnS [17], [26]. By immobilizing the QDs in a hydrogel matrix, sample conditions that produce analyte-independent effects are reduced and on-site detection of heavy metal ions is made possible [26]. However, no research has observed the potential of semiconductor $CuInS_2$/ZnS hydrogel. Therefore, this study examines the initial potential of $CuInS_2$/ZnS hydrogel in particular the influence of pH on the fluorescence of hydrogel, and studies the swelling ratio of $CuInS_2$/ZnS hydrogel to water. From this research it is expected that $CuInS_2$/ZnS hydrogel with excellent swelling and fluorescent performance will be obtained at specified pH, thus enabling its application in the biosensor and waste processing industry.

2. Research Methodology

2.1. Materials

Copper acetate (CuAc, 99.995% purity Sigma Aldrich, USA), Zinc stearate (ZS, purity 90%, Sigma Aldrich, USA), 1-dodecanethiol (DDT, 97% purity Sigma Aldrich, USA), 1-octadecene (ODE, 90% purity Sigma Aldrich, USA), Indium acetates (InAc) (99% purity Alfa-Aesar, USA), Zinc chloride (99% purity Riedel-deHaen AG Germany), Poly (ethylene glycol) methacrylate (PEGMA, Mn~360 and Mn~526), N, N-dimethyl formamide (99.8% purity, Sigma Aldrich, USA), N'-methylene bis acrylamide (98% purity Sigma Aldrich, USA), and ammonium persulfate (99% purity Sigma Aldrich, USA), N-(3-aminopropyl) metacrilamide hydrochloride (99% purity Polysciences), all organic solvents obtained from EM-Sciences, and Zinc ethylxanthate (ZE) is synthesized following a method used by Mintcheva et al [27].

2.2. Procedures

1) Preparation $CuInS_2$/ZnS QDs Solution

The preparation of $CuInS_2$/ZnS QDs followed existing literature guidelines [13]. Shortly, a mixture of 0.17517 g $In(Ac)_3$, 0.0245 g $Cu(Ac)_2$, 2.5 mL ODE, and 2.5 ml DDT was agitated at 40 ℃ for 1 h while purged in a nitrogen atmosphere. Next, the mixture underwent moderate heating, reaching 240 °C to ensure the nucleation process, which gave the solution a reddish-orange color. Then, another mixture in the form of 0.1 mL DMF, 0.031 g ZE in 1 mL toluene, and 0.504 g ZS in 3 mL ODE, was immediately added to the mixture to make the shell of core. After the addition was

complete, this mixture was cooled to 30 ℃ and centrifuged for 20 min at 6000 rpm. Next, the supernatant was thrown away. The precipitate was dispersed in 10 ml chloroform under sonication and precipitated by adding a methanol/acetone (1:1) and the $CuInS_2$/ZnS QDs formed was precipitated by centrifugation at 6000 rpm for 10 min. The Series of $CuInS_2$/ZnS QDs synthesis apparatus is shown in Fig. 1.

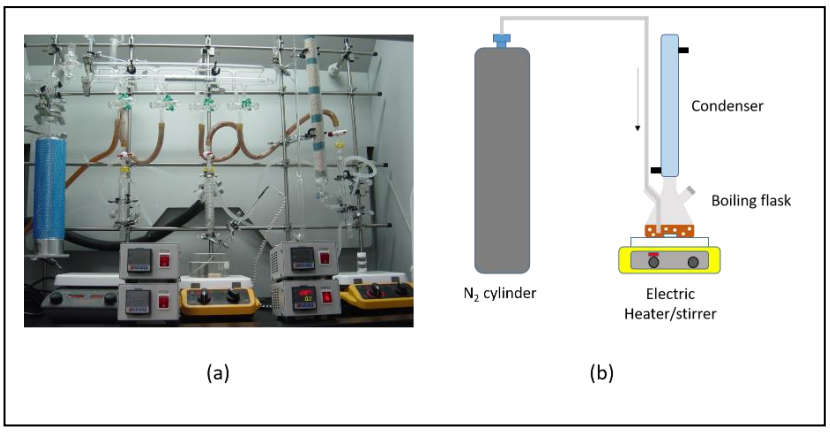

Fig. 1.$CuInS_2$/ZnS (QDs) synthesis apparatus, a) the photograph of the core synthesis apparatus, b) the series of the core-shell synthesis apparatus

2) Synthesis $CuInS_2$/ZnS Hydrogel

$CuInS_2$/ZnS hydrogel was synthesized by mixing and sonicating a solution of 40 mg N-(3-aminopropyl) methacrylate/100 µL water, 10 mL polyethylene glycol methacrylate, 3 mg methylene bis acrylamide/100 µL water, 3 mg ammonium persulfate/100 µL water and 0.5 ml $CuInS_2$/ZnS (QDs). After 5 min of sonication at an amplitude of 20 kHz, the solution became a hydrogel. This immobilized $CuInS_2$/ZnS hydrogel system exhibits unique luminescence characteristics for semiconductor QDs nanocrystals. To determine the effect of pH on the $CuInS_2$/ZnS hydrogel, the hydrogel was immersed in a solution with pH variations of 1, 3, 5, 7, 9, 11, and 13 for one and five days, with NaOH and HCl as pH control levels. Fig. 2 shows the sonication process of $CuInS_2$/ZnS hydrogel synthesis using Ultrasonic Homogenizer Mixer Sonicator 125 W. Next, the $CuInS_2$/ZnS hydrogel was irradiated using a UV lamp to observe fluorescence characteristics.

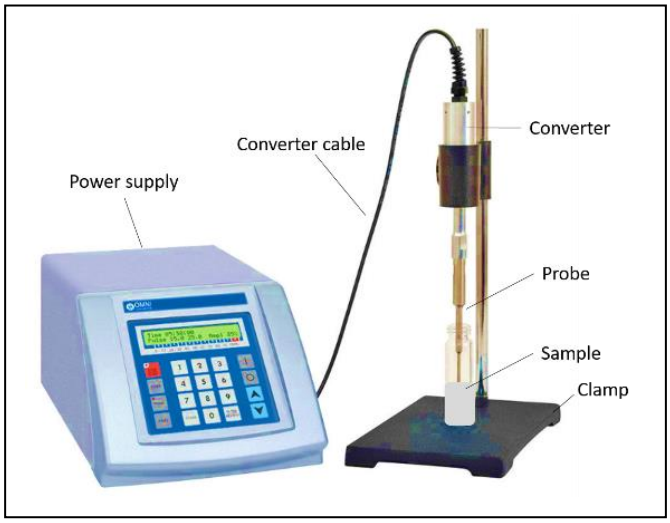

Fig. 2. Sonication process of $CuInS_2$/ZnS hydrogel synthesis

3) Swelling Ratio Calculation

The swelling behavior of hydrogel is their most interesting feature. Hydrogels begin to absorb fluid when they come into contact with solutions, which causes them to swell. The following equation applies to hydrogel's swelling ratio (*Sr*):

$$Sr = \frac{Ws - Wi}{Wi} \times 100 \quad (1)$$

Where *Wi* is the hydrogel's initial weight before submersion in water, and *Ws* is the weight of the swollen hydrogel [28].

3. Results and Discussion

The manufacture of $CuInS_2$/ZnS hydrogel is carried out in two stages. The first stage is the $CuInS_2$/ZnS (QDs) solution synthesis stage, and the second stage is the $CuInS_2$/ZnS hydrogel synthesis. The first stage of synthesis was carried out using Copper acetate (CuAc), Indium acetates (InAc), Zinc stearate (ZS), 1-dodecanethiol (DDT), 1-octadecene (ODE). Copper acetate (CuAc) is a source of Cu, Indium acetates (InAc) is a source of In, while 1-dodecanethiol (DDT) is a solvent and source of S. Cu, In, and S (CIS) are the core of QDs. The CIS QDs satisfy the needs of down-conversion materials with their adjustable emission wavelengths, increased stoke shifts, low toxicity, and affordable price [29]. ZnS (which is made from zinc stearate (ZS) and Zinc ethyl xanthate (ZE)) coating of the CIS core structure is required to enhance its stability and fluorescence characteristics [29], [30]. DMF acts as an organic solvent. Meanwhile, a polyvalent metal salt solution (ZnCl2) is used to form $-(COO)_{ns,}$ which is then used to produce a second cross-linking effect on the gel [31]. The polyvalent salts act as coordination centers in hydrogels that can enhance the mechanical characteristics of hydrogels [32].

The main materials in the second stage are polyethylene N-(3-aminopropyl) methacrylate (AMP), glycol methacrylate (PEGMA), methylene bis acrylamide (MAA), and ammonium persulfate (AP). These monomers are used in the radical copolymerization process that created the QDs hydrogel, which is also characterized by ionic coordination, micro crystallinity, and hydrophobic association [32]. AMP is the main material for hydrogel fabrication. To improve the hydrogel's hydrophilicity and water retention, MAA monomer was added. PEGMA monomers were added to enhance their mechanical performance through the synergistic strengthening impact of ionic coordination (IC) and hydrophobic association (HA) [32] and also act as coating material that can reduce nanoparticle aggregation [33]. AP monomer acts as an initiator agent. The addition of chloroform and acetone aims to purify the QDs [29]. Heating at a temperature of 240 ℃ is carried out to ensure that the nucleation process of the core and the growth of the shell occurs completely [29].

3.1. Effect of pH on Swelling Ratio

The swelling properties of the hydrogel are their most intriguing characteristic. The swelling mechanism is a critical component in the biological uses of hydrogel. Since biological fluid makes up the majority of the human body, the hydrogel's capacity to expand and release the drug is a crucial characteristic for drug delivery applications [2]. Estimating the swelling ratio can be done using several hydrogel characteristics. A hydrogel's type of porosity is one of its key parameters. Hydrogel pores facilitate cellular ingrowth, vascularization, and nutrition transfer. When pore size is increased, angiogenesis in the hydrogel is more efficient, resulting in faster swelling kinetics and better absorbent qualities than in non-porous hydrogel [34].

Table 1 shows that increasing the pH from 1 to 5 increases the weight of the hydrogel, for pH above 5 the weight of hydrogel tends to decrease. The reason for the decreasing weight of hydrogel is that hydrogels are three-dimensional macromolecule networks. The hydrogel contains N-(3-aminopropyl) methacrylate, which is aminoalkyl methacrylate derivative monomers with double bonds and tertiary amino or quaternary ammonium (cationic) groups in the molecule. The polymer's tertiary amine side chains become protonated at a specific temperature when the pH drops into the acidic zone, raising the network's charge density. The network's internal osmotic pressure rises sharply in tandem with the concurrent rise in mobile counterion content, causing swelling transitions that have been observed. On the other hand, if the hydrogels were submerged in the alkaline solution, the internal osmotic pressure decreased, causing the hydrogels to shrink [35][36]. This result is by the study conducted by Ali et al. [36], which examines swelling behavior, mechanical properties, and network parameters of pH- and temperature-sensitive hydrogels of poly((2-dimethyl amino) ethyl methacrylate-co-butyl methacrylate). This study shows a decrease in the swelling ratio as the pH increases from 3 to 9. This result is different from the study conducted by Xiongfei et al.

[32] and Sudarsan et al. [37], which uses sodium alginate and acrylic acid as the main materials for making hydrogel. The use of sodium alginate will cause an increase in the swelling ratio along with increasing pH. This hydrogel contains abundant –COO– (from sodium alginate) and –COOH (from Acrylic acid) groups. In alkaline environments, the -COOH groups can be reversibly converted to negative -COO– groups, while in acidic media, the -COO– groups can be reversibly converted back to -COOH groups. The hydrogel's –COOH groups decreased and its negative –COO– groups increased upon immersion in the alkaline solution. This resulted in a weakening of the hydrogen bonds between the –COOH and –OH/–NH2 groups as well as an increase in the repulsive force between the polymer chains due to an electrostatic repulsive interaction between the negative –COO– groups. The swelling ratio increased as a result of the increased repulsive force between the polymer chains, which created more space in the hydrogel network and allowed more water to enter the hydrogels [32]. So, the pH value has a significant impact on how quickly the hydrogel network relaxes. As a result, the pH value and the hydrogel's resistance are related to the pace at which free ions migrate throughout the network [32]. Fig. 3 shows the hydrogel swelling data and its interpolation curve at varying pH. The maximum swelling occurs at a pH of 5. Fig. 3 shows that increasing pH can prevent hydrogel formation. The interpolation curve shows that the optimum swelling ratio exists at the pH level of the biological fluid of the human body namely 7.35-7.45 [38], this suggests the application of QDs hydrogel for drug delivery. In the waste processing industry, the swollen hydrogel provides a more porous matrix than the shrunken hydrogel. Via passive diffusion, ions, and small molecules can enter the hydrogels through their porous matrix. In the matrix, functional molecules that are the same size as or greater than the pore size can be physically immobilized. On-site detection of the heavy metal ions is made possible by immobilizing the QDs in a hydrogel matrix [26], [39].

Table 1. Effect of pH on the weight of $CuInS_2$/ZnS hydrogel

	pH						
	1	***3***	***5***	***7***	***9***	***11***	***13***
(Botol + gel= P) (mg)	14.8	14.97	14.86	14.86	15.09	14.79	14.88
Water, 200 ml	16.76	16.94	16.83	16.83	17.08	16.78	16.87
(P + water= Q) (mg)	15.118	15.22	15.22	15.109	15.448	15.107	15.097
Q-P (mg)	0.318	0.25	0.36	0.249	0.358	0.317	0.217

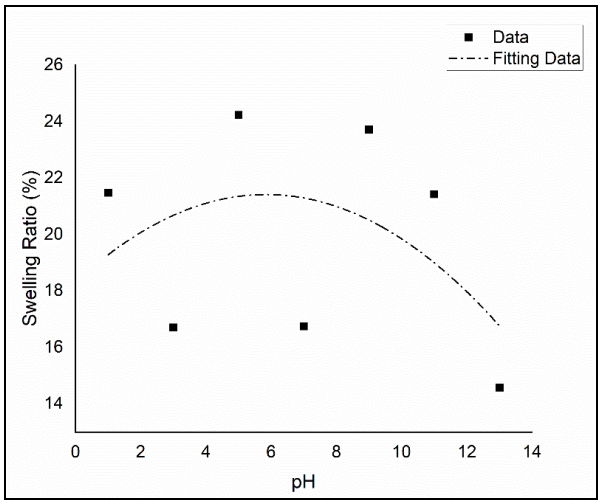

Fig. 3. Swelling ratio of $CuInS_2$/ZnS hydrogel at different pH levels

3.2. Effect of pH on Fluorescence Properties

Hydrogel fluorescent characteristics are attractive because of their enormous potential for biological imaging [40], biosensing [41], [42], and biological monitoring [43]. QDs are regarded as the perfect materials for generating fluorescent light because of their unique electrochemical characteristics, two-photon excitation, and adjustable emission wavelength [20]. Fig. 4 shows the photographs of the $CuInS_2$/ZnS (QDs) solution observed under daylight and under UV radiation. The $CuInS_2$/ZnS (QDs) present red fluorescence under UV radiation. This indicates that $CuInS_2$/ZnS

QDs spectrum exists in the red spectrum range that is close to the visible light spectrum ranging from 600 to 1100 nm [11]–[14].

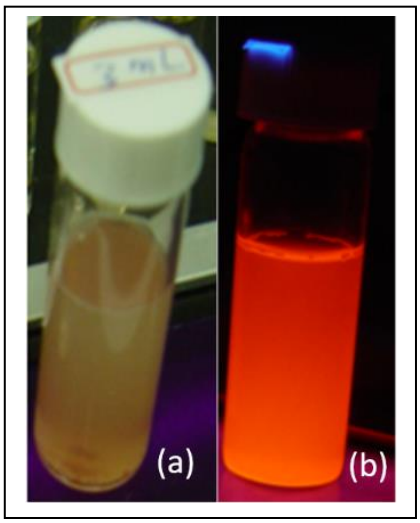

Fig. 4. The photographs of $CuInS_2$/ZnS (QDs), a) observed under daylight and b) observed under UV radiation

The photographs of $CuInS_2$/ZnS hydrogel and their fluorescent characteristic are presented in Fig. 5a-d. Fig. 5a and d show that the $CuInS_2$/ZnS hydrogel experienced swelling after 1 day of immersing. Meanwhile, Fig. 5b reveals that the $CuInS_2$/ZnS hydrogel leads to the red spectrum of photoluminescent emission at the overall pH range. Photoluminescence emission in Fig. 5c, after 5 days of immersing, presents a blue spectrum at pH of 1, 3, 5, and 13, and presents red spectrum at pH of 7, 9, and 11. The results show that immersing the $CuInS_2$/ZnS QDs hydrogel for 5 days at pH 1, 3, 5, and 13 changes the color spectrum from red to blue. This emission spectrum makes $CuInS_2$/ZnS hydrogel applicable for biological purposes [12]. The gel fluorescence at pH 1, 3, and 13 appeared very weak before absorbing water. After absorbing water (after 5 days), the gel fluorescence showed the same results. Even gels with a pH of 5 look weak. However, the QDs gel at pH 7, 9, and 11 showed strong fluorescence, especially at pH 11 which looks very strong. This result is different from the study conducted by Wang et al. [44], which uses glutathione as the main material in QDs synthesis. The results of this study show that optimum fluorescence occurs at pH 3, whereas in our study optimum fluorescence occurs at pH 11. This probably occurs because glutathione is an anionic group of the tripeptide, whereas in our study we used amino alkyl methacrylate which is a cationic group. The unique characteristic of QDs hydrogel is had strong fluorescence at a pH level of 7, in the range of the biological fluid of the human body (7.35-7.45), shows the suitability of the application of QDs hydrogel as a biosensor. QDs hydrogel can be used as active sensors by changing their fluorescence properties when reacting with analytes, or by conjugating antibodies to the dot surface to act as passive label probes. The idea is that light is absorbed by this QD hydrogel at a certain wavelength (excitation) and emitted at a different, higher wavelength (emission). For example, fluorophore-coupled particular antibodies or proteins are introduced into the cell to visualize the target molecule. After that, the specimen is exposed to light at the excitation wavelength and seen via a filter that only lets light that indicates the presence of the structure of interest pass through [45].

This research is limited to analyzing the characteristics of QDs hydrogel based on pH parameters only, other parameters such as temperature and time are still open to research. Likewise, the synthesis of QDs with different core and shell structures, and the using of different hydrogel materials is a new opportunity.

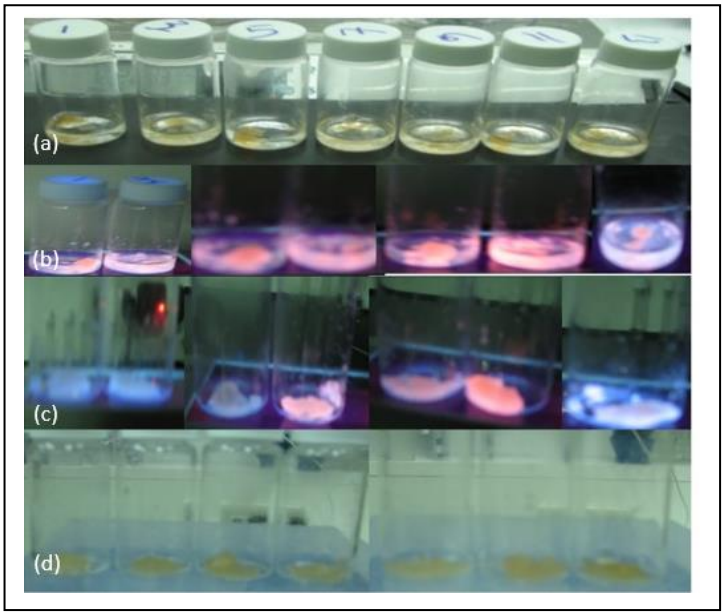

Fig. 5. The photographs of $CuInS_2$/ZnS hydrogel a) QDs hydrogel appearance under daylight b) QDs hydrogel appearance under UV lamp radiation c) QDs hydrogel appearance under UV lamp radiation after 5 days immersed in water d) QDs hydrogel appearance under daylight after 1 day immersed in water

4. Conclusion

$CuInS_2$/ZnS hydrogel has important applications in biosensors and waste processing industries. Swelling ratio and fluorescence are important characteristics of QDs hydrogel. In the waste processing industry, the swollen hydrogel provides a more porous matrix than the shrunken hydrogel. The porous matrix of the hydrogels allows the heavy metal to penetrate the hydrogels via passive diffusion and immobilize the matrix. Hence, on-site heavy metal ion detection is made possible by the QDs hydrogel. In the biosensor application, QDs hydrogel can be used as active sensors by changing their fluorescence properties when reacting with analytes, or by conjugating antibodies to the dot surface to act as passive label probes. The results show that pH affects the swelling ratio and fluorescence of $CuInS_2$/ZnS hydrogel. The increase of pH from 1 to 5 increases the swelling ratio of the hydrogel, for pH above 5 the hydrogel tends to shrink. $CuInS_2$/ZnS QDs hydrogel has a maximum swelling ratio at a pH of 5 and provides a red spectrum at the initial state and strong fluorescence at pH of 7, 9, and 11. The result also revealed that $CuInS_2$/ZnS hydrogel has sufficient fluorescence and swelling ratio characteristic at the pH level of the biological fluid of the human body namely 7.35-7.45, this suggests the biological application of $CuInS_2$/ZnS hydrogel such as for drug delivery and biosensing.

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The Effect of Adding Bread Yeast, Tempeh Yeast, and Tape Yeast on The Process of Making VCO from Coconut

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ABSTRACT

Coconut (*Cocos nucifera* L.) is one of Indonesia's agricultural products with potential. The most valuable coconut product is coconut oil, which can be obtained from the flesh of fresh coconuts or copra. Virgin Coconut Oil (VCO) or pure coconut oil is the result of processing from the coconut plant in the form of a clear liquid with a distinctive coconut smell and has a long shelf life. Pure coconut oil or VCO has many benefits for body health, such as natural antibacterial, antiviral, anti-fungal, and anti-protozoa properties. This study aims to compare the effect of Tempeh yeast, bread yeast, tape yeast, and without yeast on variations in the ratio of grated coconut and water are 1: 1; 1:1,5, and 1:2, on the amount of VCO produced. The experiment was carried out again and then the average volume of VCO produced by each yeast was taken. The average VCO results obtained ratio of 1:1 were 209.5 ml for adding bread yeast, 153.5 ml for not using yeast, and 150.5 ml for adding Tempeh yeast. Meanwhile, adding tape yeast produces the smallest yield of 89 ml. Based on the ratio of grated coconut and water (1 kg: 1 liter) the optimum yield is 1:1. While the minimum yield is 1:1,5 ratio. The most VCO obtained is by adding bread yeast to thick coconut milk in the second fermentation process. The order of highest yield based on the addition of yeast is bread yeast, tempeh yeast, and tape yeast. The yield of VCO yeast tempeh was slightly better than the yield without adding yeast. The organoleptic results and some of the VCO test results based on SNI 7783:2008 have fulfilled the specified requirements.

1. Introduction

Coconut oil is one of the processed coconut products that is in high demand; it accounts for around 10% of the global market's oil and fat requirements [1]. Meanwhile, virgin coconut oil (VCO) is defined as oil obtained from the meat of fresh old coconuts (Cocos Nucifera L.), processed by pressing with or without water, and heated to a maximum of 60°C. It is safe for consumption, according to Indonesian National Standards (SNI) 7381-2008 concerning VCO [2]. VCO contains around 66% oil, 6-7% protein, 48% water, 5% crude fiber, and ±2% ash. Virgin coconut oil contains fatty acids, sterols, vitamin E, and phenolic acid. These chemical components exhibit antioxidant activity in plant materials, food products, and biological systems.[3] VCO has numerous benefits, including antiviral, antibacterial, antifungal, and antiphrastic properties. It can also aid in the treatment of metabolic disorders, such as digestive issues, absorption of amino acids and soluble vitamins in fat, diabetes management, and improved blood circulation. VCO is commonly used in

beauty treatments to retain skin suppleness. VCO is also utilized as a raw material in soap production [4]. When VCO is absorbed into the skin and the tissue cell structure, it helps to build connective tissue. In this way, VCO can minimize tissue damage caused by overexposure to sunlight.[5]

Researchers are interested in VCO due to its potential health benefits, such as lowering the risk of cancer, preventing viral infections, boosting the immune system, maintaining soft and smooth skin, being cholesterol-free, and not contributing to obesity [6].VCO contains alkaloids and saponins, which are effective anti-inflammatory and antibacterial agents [7].

Five processes are used in the production of VCO: gradual heating, fishing methods oil, fermentation, enzymatic, centrifugation, and ultrasonic approaches. The microbe Saccharomyces cerevisiae, also known as Bread yeast, is used in the fermentation process to create VCO. The conditions of pH, temperature, energy source, and free water all affect the growth of bread yeast bacteria. At 30 ºC, Saccharomyces cerevisiae can grow to their maximum potential. The method of gradual heating involves heating coconut milk to a temperature below 90 ºC, and then returning the heated oil to a low temperature (below 65 ºC). Using a specific ratio, fishing oil is added to coconut milk to employ the oil fishing method. Whereas, to carry out the fermentation process, yeast is added to coconut milk [8][9]. The oil-protein linkages in the coconut milk emulsion phase can also be broken down with the aid of enzymes in the enzymatic technique. In this case, protein rather than fat is harmed. Inside-out protein protease enzymes are a class of protein enzymes that break down lipoprotein connections. Enzymes of the following kinds can be employed to dissolve inner lipoprotein bindings in fat emulsions: papain, bromelain, and protease enzyme. Centrifugal force was used to spin coconut milk in an attempt to disrupt the fat-protein linkages therein. Since oil has a lower specific gravity than water, centrifugation will force the two to separate on their own. 20,000 rpm was the rotational speed employed [10]. Meanwhile, the ultrasonic technique of producing VCO employs direct ultrasonic waves to break down the VCO and water emulsion, to increase yield and improve VCO quality[11].

The addition of yeast to coconut milk emulsion can also be accomplished by the activities of microorganisms that produce proteolytic enzymes, such as *Rhizopus Oligosporus*, which is present in tempeh yeast. Protease enzymes are hydrolases that can break down proteins into simpler molecules. A class of hydrolases known as proteases is capable of easily breaking down proteins into other molecules. Protease enzymes are used to break down the lipoprotein links that hold the proteins in coconut milk together. When these lipoproteins are destroyed, the oil that is held by the bonds breaks free and becomes one [12]. Coconut milk's lipoprotein linkages are broken down by protease enzymes, releasing the oil attached to them. This bacterium can create protease enzymes, which can degrade the protein bonds that surround the fat globules in coconut milk cream emulsions.[13] Adding bread yeast to VCO affects peroxide value, iodine value, acid number, percentage free fatty acid (FFA) or acid-free fat, and organoleptic test of VCO aroma. However, the substrate does not impact all VCO analyses.[14] Tape yeast can be used to make virgin coconut oil (VCO), which is produced by fermentation. The amount of tape yeast used to create the most virgin coconut oil (VCO) is 160.2 ml (yield 16.1%) [15]. The reason why yeast tape is utilized in VCO processing is because it contains microflora, such as yeast, which can produce lipase, which breaks up the coconut milk emulsion. Yeast tape is typically employed in tape production. Thus, chemical bond breakage will occur during the fermentation process [16]. Bread yeast contains Saccharomyces cerevisiae, which produces proteolytic and amylolytic enzymes when grown in an emulsion. Amylolytic enzymes degrade carbs to generate sour. The presence of acid lowers the pH of coconut milk until it reaches the protein's isoelectric point, causing it to coagulate. The coagulated proteins are then broken down by enzymes called proteolytics, and they may be easily removed from the oil [12].

Good quality VCO appears clear as crystal, does not smell rotten, and has a unique coconut flavor. This indicates that it is not blended with any other substances, such as water. The presence of water in oil can promote hydrolysis or oxidation events that produce a rotten odor. The hydrolysis reaction

converts oil into free fatty acids and glycerol.[17] This research aims to determine the amount of VCO produced from variations in the use of yeast and the ratio between the weight of grated coconut and water. This fermentation method uses tape yeast, tempeh yeast, and bread yeast.

2. Research Methodology

Materials

The ingredients used in this research were grated coconut, water, Tempeh yeast, tape yeast, and bread yeast. The tools used in this research are a coconut milk filter, measure, basin, small filter, 2 kg plastic bag, plastic bottle, measuring cup, digital scale, and small straw.

Procedures

The process begins with obtaining grated coconut from the Giwangan market, which is then combined with warm water in specific weight ratios (w/w) of 1:1, 1:1.5, and 1:2. This mixture is kneaded and filtered to yield smooth coconut milk free of any impurities. Subsequently, the coconut milk is placed in a transparent plastic bag and allowed to sit for 3 hours. After this resting period, two distinct layers form: the upper layer consists of concentrated coconut milk, while the lower layer is primarily water. The concentrated coconut milk is carefully separated from the water. The concentrated coconut milk is then transferred to a new clear plastic bag, and 1.5 grams of yeast starter is added. Thoroughly stirring the mixture, it is left to ferment for 12 hours in a warm room.

After the fermentation period, the concentrated coconut milk is separated into four layers: residual water, sediment of coconut cream (blondo), Virgin Coconut Oil (VCO), and a floating sediment of coconut milk. To extract the VCO, a container is prepared, and the volume of the obtained VCO is measured. This meticulous process ensures the production of high-quality Virgin Coconut Oil with distinct layers for precise extraction.

Table 1. VCO quality requirements are based on SNI 7381:2008

No	Test type	Units	Requirement
1.	Condition :		
	1.1 Smell		Typical fresh coconut, not rancid
	1.2 Taste		Normal, typical coconut oil
	1.3 Color		Colorless to pale yellow
2.	Water and evaporated compounds	%	Max 0,2
3.	Iodine number	g iod/100 g	4,1 – 11,0
4.	Free Fatty Acid (calculated as lauric acid)	%	Max 0,2
5.	Peroxide number	mg ek/kg	Max 2,0

VCO Quality Testing According to SNI 7381-2008

Testing of VCO results was carried out at the Chemix Pratama Bantul Yogyakarta laboratory, where the samples were repeated once (duplo) and then averaged.

a. Organoleptic test

Color test

Analyze samples organoleptically using the sense of sight (eyes). VCO colors that fulfill SNI 7381:2008 specifications (table 1) range from colorless to pale yellow.

Smell test

Conduct organoleptic analysis of samples using the sense of smell (nose). The odor of the VCO sample meets the specifications outlined in SNI 7381:2008 for VCO odor requirements. It should smell normal, that is, typical for oil. Fresh coconut with no rancid scent.

Taste test

Perform organoleptic examination on samples using the sense of taste (tongue). The VCO samples meet the SNI 7381:2008 criterion for VCO taste, which requires a characteristic coconut oil flavor with no alien taste.

b. Water content

The weighing bottle is cooked in an internal oven at 105˚C for one hour. After cooling in a desiccator for ½ hour, record the weight. After adding 5 grams of pure coconut oil (VCO) to the bottle, weigh the result. Preheat oven to 105˚C for 1 hour, then chill in a desiccator for 30 minutes. Weigh the weighing bottle with the sample inside. Repeat heating and weighing until a consistent weight is achieved. Water content is represented as a percentage of weight per weight, calculated to two decimal places using the following formula:

$$Water\ content\ (\%) = \frac{w1-w2}{w1}\ x\ 100\%$$

Whereas :

w1 = sample weight

w2 = sample weight after drying

c. Iodine number

The determination of the iodine number through the Wijs technique involves a meticulous procedure requiring several reagents, such as chloroform or carbon tetrachloride, sodium solution, 0.1 N thiosulfate standard, 15% KI solution, starch indicator, and the crucial Wijs reagent. Employ a covered Erlenmeyer flask to collect samples weighing up to 0.5 grams. Initiate the process by adding 10 mL of chloroform and 25 mL of iodine-bromide reagent to the collected samples. Allow the mixture to stand undisturbed for 30 minutes in a darkened room. Following this incubation period, introduce 10 mL of 15% KI solution and proceed to titrate with 0.1 N $Na_2S_2O_3$ until the solution's color transforms into a vibrant yellow hue. To refine the determination, incorporate 3 drops of starch indicator and continue titrating until the blue color dissipates. The iodine number, indicative of grams absorbed per decimal, is then expressed using a formula as below:

$$Iod\ number = \frac{12{,}69\ x\ T\ x\ (V0 - V1)}{w}$$

Whereas :

T = Normality of sodium standard solutions thiosulfate 0.1 N

V0 = Volume of 0.1 N Na thiosulfate solution required for blank screening (ml)

V1 = Volume of 0.1 N Na thiosulfate solution required for sample titration (ml)

w = weight sample (gram)

d. Peroxide number

Accurately weigh a sample ranging from 0.3 to 5 grams. Dissolve the sample by vigorously shaking the Erlenmeyer flask with 10 mL of chloroform. Following this, introduce 15 mL of glacial acetic acid and 1 mL of a saturated potassium iodide solution. Seal the Erlenmeyer immediately and shake the mixture vigorously for 5 minutes in a dimly lit room at a temperature between 15-25°C. Conduct titration using a standardized sodium thiosulfate solution (0.02 N), employing starch solution as an indicator to detect the endpoint. It is essential to perform a blank determination for calibration. The peroxide number, expressing the concentration in milligrams of active oxygen per kilogram, is then

reported based on the precise titration results. Calculated the number to two decimal places with the formula:

$$Peroxide\ number\ (mg/kg) = \frac{N\ x\ (V1 - V0)}{w} x\ 1000$$

Whereas:

V0= The volume of $Na_2S_2O_3$ is 0.02 N which is required for blank titration (mL)

V1= The volume of $Na_2S_2O_3$ is 0.02 N which is required for sample titration (mL)

N = Normality of $Na_2S_2O_3$

w = weight sample (gram)

e. Fe metal contamination

The sample is placed in 100 ml glass cups and shaken to ensure homogeneity. After that, 5 ml of nitric acid was added and heated in an electric heater until the solution was nearly dry. Add 50 ml of distilled water to this solution, then strain it through filter paper into a 100 ml measuring flask and fill to 100 ml with distilled water. Dilute to desired heavy metal concentrations (0.0 mg/l, 1.0 mg/l, 5.0 mg/l, 10.0 mg/l, 15.0 mg/l, and 20.0 mg/l). Heavy metal levels were detected using SSA at a wavelength of 283.3 nm [18].

3. Results and Discussion

1. Yield VCO

The most optimal yield was obtained with a 1:1 ratio of shredded coconut to water of 209.5 ml using Bread yeast (Table 2). Overall, bread yeast produces the highest output when compared to other yeasts or no yeast. In general, a 1:1 ratio of grated coconut to water results in greater VCO than other ratios. On average, tempeh yeast outperforms sans yeast by a small margin and tape yeast. The addition of tape yeast resulted in the lowest yield of 77 ml. The ratio of grated coconut to water has an optimum value at a ratio of 1:1 and a minimum value at a ratio of 1:1.5 in this study.

Table 2. Yield VCO with variations of yeast and grated coconut to water ratio.

The ratio of grated coconut to water	Experiment	Original	Tape yeast	Tempeh yeast	Bread yeast
1: 1	1	114	87	100	198
	2	193	91	201	221
	Average	153,5 ml	89	150,5	209,5
1: 1,5	1	130	57	145	111
	2	128	55	132	168
	Average	129	73	138,5	139,5
1: 2	1	130	68	155	143
	2	131	70	140	171
	Average	130,5	69	147,5	157
Average		137,6	77	136,5	168,6

2. Organoleptic Test Result

The VCO produced is clear in color, smells like coconut oil, and tastes bland (Figure 1). This meets the requirements for virgin coconut oil set by the Indonesian national standard. According to SNI 7381-2008, the standard VCO must be clear to pale yellow and smell like fresh coconut oil, not rancid. Based on Table 3, the results are presented organoleptic properties of odor in coconut oil. This is the typical smell of fresh coconut oil, not rancid. According to SNI 7381: 2008, scent VCO shall not be rancid and contain the aroma of coconut oil. This signifies that the odor of coconut oil

already fits the criteria. The organoleptic results of the taste investigation match the standards of SNI 7381:2008, namely the usual taste of fresh coconut and not rancid.

Fin 1. Original VCO appearance and with the addition of yeast
(left to right: original, tempeh yeast, tape yeast, and bread yeast)

Table 3. Results of organoleptic testing of coconut oil

Parameter	Original	Tempehh yeast	Tape yeast	Bread yeast
Color	Clear white	Clear white	Clear white	Clear white
Smell	The distinctive smell of fresh coconut oil and no rancid smell	The distinctive smell of fresh coconut oil and no rancid smell	The distinctive smell of fresh coconut oil and no rancid smell	The distinctive smell of fresh coconut oil and no rancid smell
Taste	Typical taste of fresh coconut oil	Typical taste of fresh coconut oil	Typical taste of fresh coconut oil	Typical taste of fresh coconut oil

3. VCO Quality Testing Results According to SNI 7381-2008

Water content testing is done to determine oil resistance. Internal water content. Coconut oil (VCO) has a significant influence on quality. Oil with high water content typically has a short shelf life. The table displays the water content of coconut oil (VCO). The average water content of the VCO produced is 0.074%, meeting Indonesian National Standards (SNI) 7381: 2008 for oil quality criteria for pure coconut oil (VCO) of no more than 0.2%. VCO's high water content reduces its durability. Apart from that, the presence of water in VCO causes a hydrolysis process. If there is water in the oil, it will hydrolyze, resulting in free fatty acids and glycerol, which will turn the oil rancid [19].

Table 4. VCO Quality Testing Results

Parameter	Water content (%)	Iod number (g I_2/100g)	Peroxide number (mg ek/kg)	Fe metal contamination (mg/kg)
SNI Standar	Maximal 0,20	4,1 – 11,0	Maximal 2,0	Maximal 5,0
Original	0,092	0,3554	$1{,}231 \times 10^{-4}$	1,342
Tempehh yeast	0,078	0,3456	$8{,}49 \times 10^{-3}$	1,377
Tape yeast	0,062	0,3456	$8{,}67 \times 10^{-3}$	1,620
Bread yeast	0,062	0,3355	$8{,}81 \times 10^{-3}$	1,614

The results of water content, iod number, Peroxide number, and Fe metal contamination are shown in Table 4. The iodine number reveals how unsaturated the fatty acids in oils and fats are. Unsaturated fatty acids can bind iodine, forming saturated molecules. The amount of iodine attached reflects the number of double bonds found in the oil. When it combines with iodine, it forms a saturated molecule. The experiment yielded 3.455 mg/g of coconut oil iodine (VCO). These data show that the produced coconut oil has a lower value than the determined SNI. Heating or frying can cause the breaking of unsaturated bonds in oil to become saturated, lowering the iodine content.

The peroxide number analysis is used to evaluate how oxidized the oil is. Oil quality improves with a lower peroxide number, while a higher peroxide number leads to lower quality. The data obtained shows a peroxide number below 2,0 g iod/100g, which indicates a very low peroxide level. This means that the VCO produced is of very high quality and more difficult to oxidize.

According to the Fe content test findings, the four types of VCO were extremely low, with an average of 1.488 mg/l. This value fulfills SNI 7381:2008. Given the significance of the Fe element as an oxygen binder in the blood, the presence of Fe is required for VCO, which is used to treat a variety of disorders. However, it is important to note that if it exceeds the limit, it will undoubtedly become poison for the body.

4. Conclusion

According to the findings of the research, the highest yield of Virgin Coconut Oil (VCO) was obtained by adding bread yeast, Tempeh yeast, and tape yeast in order of size. The highest average VCO results obtained ratio of 1:1 were 209.5 ml for adding bread yeast, 153.5 ml for not using yeast, and 150.5 ml for adding Tempeh yeast. Meanwhile, adding tape yeast produces the smallest yield of 89 ml. Organoleptically, the VCO results met the standards of SNI 7381-08. The results of the quality test revealed that the water content (<0,20%), peroxide number (<2,0 mg ek/kg), and Fe metal contamination (<5,0 mg/kg) met SNI 7381-2008 standards. Meanwhile, the iodine number was beyond the established standard limits (4,1-11,0 g iod/100g). A small iodine number suggests a high level of saturation. The smaller the amount of iodine indicates the less unsaturated fatty acids.

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