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Influence of Chitosan and Alginate as Reinforcement Towards Sugar Palm Starch-Based Hydrogel

Tazkia Qonita Zahra[1], **Noverra Mardhatillah Nizardo**[1]*, **Witta Kartika Restu**[2]*

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Indonesia,
Depok, West Java 16424, Indonesia
[2]Research Center for Chemistry, National Research and Innovation Agency (BRIN), KST B.J Habibie,
Serpong, South Tangerang, Banten, 15314, Indonesia

*Corresponding author email: noverra.mardhatillah@sci.ui.ac.id; witt001@brin.go.id

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ABSTRACT. Nowadays, hydrogel has a wide range of applications, such as in the biomedical field, due to its excellent biocompatibility. Hydrogel may be synthesized using biomaterials that are abundantly found in the natural environment. This research involved the synthesis of a hydrogel using sugar palm starch as the base material, but hydrogel made from starch has lower chemical and physical properties. To enhance its chemical, physical, and mechanical properties, alginate and chitosan were incorporated as reinforcing agents because they can form strong gels, which might be due to the interaction of the amino (-NH_2) group from chitosan and carboxyl (-COO^-) group from alginate with the hydroxyl (-OH) group from starch. The objective of the study was to determine the best reinforcing agents for the formation of two sugar palm-based hydrogel formulations: sugar palm starch/chitosan hydrogel and sugar palm starch/alginate hydrogel, with good chemical, physical and mechanical properties, such as thickness, swelling ratio, water contact angle, functional group, mechanical properties including tensile strength, and elongation at break, surface morphology images, and thermal properties. The result showed that the hydrogel composed of sugar palm starch and chitosan as reinforcing agent exhibited better characteristics than alginate, indicating their potential for biomedical application as shown by its result in swelling ratio with value of 67.81±0.11%, average tensile strength of 0.113±0.04 MPa and elongation at a break value of 37.83±7.0%.

Keywords: Alginate, chitosan, hydrogel, Sugar palm starch

INTRODUCTION

Hydrogel is a polymer with a three-dimensional network it has a high capacity to absorb fluids without dissolving and maintaining the structure when it is in a swollen state due to the presence of polar hydrophilic functional groups such as -SO_3H, -OH, -NH_2, -COOH, -$CONH_2$ (Bashir et al., 2020; Han et al., 2022). Hydrogels may be synthesized in several ways; these include physically cross-linked and chemically cross-linked using both synthetic and natural polymers or biopolymers (Ahmed, 2015). Hydrogels have diverse applications in several fields, including biomedical, which are used as a medium to encapsulate active substances and control the release of drugs or active substances to prevent allergic or chronic inflammatory reactions and accelerate the recovery process (Debandi et al., 2016; Mantha et al., 2019) which might be due to their swelling ability, biocompatibility, flexible methods of synthesis, physical-chemical characteristics, and biodegradability (Das, 2013). One of the most promising materials for synthesized hydrogel for biomedical applications is polysaccharides obtained from plants. Nevertheless, hydrogels composed of a single polymer may exhibit some deficiencies in their chemical, physical and mechanical properties; therefore, synthesized hydrogels with two polymers showed better performance.

Starch is a biopolymer of the unit α-D-glucose (anhydroglucose) with the empirical formula $(C_6H_{10}O_5)_n$, consisting of amylose and amylopectin. The ratio of amylose and amylopectin present in starch depends on the source of starch (such as potatoes, wheat, and corn) and the crystallinity of polysaccharides. The proportion of amorphous and crystalline masses in starch is about 70 and 30%, respectively (Qamruzzaman et al., 2022). Chitosan is a polysaccharide that has a linear and crystalline structure, consisting of N-acetyl-D-glucosamine and poly-(1-4)-2-amino-2-deoxy-ß-D-glucan. Chitosan is derived through the process of deacetylating chitin from crustaceans, including clams, snails, shrimps, lobsters, crabs, and other arthropods. It can easily combine with other polymers and has excellent film or hydrogel-forming capabilities, good mechanical properties, and antibacterial activity (Ni'mah et al., 2019). Alginate is a linear anionic polysaccharide consisting of β-D-mannuronic acid (M-block) and 1–4 linked α-L-guluronic acid (G-block). Alginate is obtained by treating the marine brown algae (*Phaeophyceae*) with aqueous alkali solutions, usually using sodium hydroxide (NaOH). Alginate is a non-toxic, affordable, and biocompatible substance that is often used in the biomedical field (Tomić et al., 2023).

Starch, chitosan, and alginate have frequently been used as biopolymer materials to form hydrogel. Nevertheless, starch alone cannot serve as a base polymer because it fails to produce a stable hydrogel. In order to overcome this issue, starch is combined with other polymers that will enhance the physical and mechanical properties (Altaf et al., 2021). As studied by Delvari and Stiharu (2022) a biodegradable and water-soluble synthetic polymer, polyvinyl alcohol (PVA), is frequently mixed with starch to enhance its properties (Delavari & Stiharu, 2022). Akter et al. (2014) also studied this to overcome starch limitations by the blending of starch or its derivatives with various polymers. Chitosan has received significant attention due to its distinctive properties (Akter et al., 2014).

Mixed starch or its derivatives with other polymers as a reinforcing agent is being investigated because it can improve starch's lower chemical, physical, and mechanical properties. The glucose units in starch joined by glycosidic bonds and the hydroxyl (-OH) groups form starch and interact with amino (-NH_2) groups in chitosan. When chitosan is added, it will improve the hydrogel chemical, physical, and mechanical properties by inducing the polymer intermolecular or intramolecular cross-linking (Akter et al., 2014). Also, the hydroxyl (-OH) groups form starch and interact with the alginate carboxyl (-COO^-) group via intermolecular hydrogen bonding (Onyido et al., 2012).

Several research on hydrogel for biomedical applications have been reported. There was a study from Rudyardjo & Wijayanto (2017) that synthesized chitosan-alginate hydrogels with variations of lauric acid as a plasticizer for wound dressing application. The compositions increased thickness, elongation at break, and swelling ratio, while the tensile strength value was decreased (Rudyardjo & Wijayanto, 2017). Then, Liu et al. (2018) synthesized an alginate-chitosan hydrogel, and the results showed that it was mechanically robust and could achieve compression stress at a value of 1.4 MPa without breaking, indicating the hydrogels were promising when used as scaffolds for tissue engineering (Liu et al., 2018). Then, a study by Chopra et al. (2022) synthesized a chitosan/PVA-based hydrogel, and the result showed that the hydrogel had good performance for wound healing application, based on the significant value of the swelling ratio, moisture uptake, and mechanical properties (Chopra et al., 2022). Then, a study by Wang et al. (2023) synthesized hydrogel from methacrylic anhydride gelatin, acrylamide, and acrylic acid N-hydroxysuccinimide ester. The result showed that the hydrogel had good performance in swelling ratio and mechanical properties for healing infected diabetic wounds (Wang et al., 2024).

In this research, the sugar palm starch-based hydrogel was prepared by incorporating chitosan or alginate as reinforcing agents to improve its chemical and physical properties. This is because other research groups used two or more reinforcing agents in order to synthesize the hydrogel. Therefore, this research observed the utilization of a single biomaterial as a reinforcing agent. We used chitosan water-soluble instead of chitosan non-water soluble because chitosan is just soluble in an acid solution and may have side effects for sensitive skin. Various tests were conducted to investigate the chemical and physical properties of the hydrogel, such as thickness, swelling ratio, water contact angle, functional group characterization using a Fourier transform infrared spectroscopy (FTIR). While tensile strength and elongation at break measurement using a universal testing machine (UTM), surface morphological analysis using a scanning electron microscope (SEM), and thermal analysis using differential scanning calorimetry (DSC).

The novelty in this research lies in utilizing a single biopolymer reinforcing agent to produce hydrogel with good properties as well as hydrogel with the combination of two or more synthetic or biopolymer reinforcing agents in previous studies. To the best of our knowledge, research dealing with sugar palm starch-based hydrogel using a single biopolymer reinforcing agent needed to be improved.

EXPERIMENTAL SECTION

Materials

Sugar palm starch (*Arenga pinnata*) with an amylose content of 23.19% was obtained from the local industry in Klaten, East Java, Indonesia. Alginate was obtained from the local industry in Jogjakarta, Indonesia. Water-soluble chitosan was obtained from collaborative research with North Sumatera University with a deacetylation degree of 50-60%. Citric acid monohydrate for analysis (CAS: 5949-29-1) were purchased from Merck, Germany. Glycerol United States Pharmacopeia (USP) grade was used. Distilled water (aquadest) used as a solvent was obtained from the Millipore water purification system.

Preparation of Hydrogel

The process was started by dissolving sugar palm starch, chitosan water-soluble or alginate, glycerol, and citric acid in 25 mL of aquadest. The mixture was stirred at 80 °C for 2 hours with a magnetic stirring hotplate at 200 rpm speed. The final solutions were poured onto a 3 cm×3 cm silicone mold and dried at 80 °C for 5 hours in an oven. The hydrogels were peeled off from the silicone mold and stored in a desiccator at room temperature before further tests. The composition of hydrogel F0 (sugar palm starch hydrogel), F1-F3 (sugar palm starch/chitosan hydrogel), and F5-F6 (sugar palm starch/alginate hydrogel) were stated in **Table 1**.

Characterization of Hydrogel

Thickness Measurement

The hydrogel thickness was measured at five different positions using a digital thickness gauge (TGH000125-Syntek, China) with a measuring range of 0-25.4 mm and a resolution of 0.001 mm.

Functional Group Characterization

Fourier transform infrared (FTIR) analysis was conducted on the hydrogel samples to identify the presence of functional groups within the hydrogel. The spectra were acquired using an FTIR spectrometer (Tensor II-Bruker, Germany) in the Attenuated Total Reflectance (ATR) method at the wavenumber range between 4000 to 500 cm^{-1}, resolution of 4 cm^{-1}, and number of sample scans was 45 seconds.

Swelling Ratio

The hydrogel samples in dry state were weighed (W_0) and immersed in 50 mL of distilled water at 25 °C. At various time intervals, the hydrogel samples were weighed (W_t) in a swollen state after blotting with tissue paper to remove the surface water. The swelling ratio was calculated by Equation **1**:

$$\text{Swelling ratio (\%)} = \frac{W_t - W_0}{W_0} x100 \quad (1)$$

Water Contact Angle

The hydrogel wettability was assessed by a water contact angle observation using a CMOS camera (VHX-5000, Keyence, Japan) integrated into a three-dimensional (3-D) digital microscope. The hydrogels were sectioned into dimensions of 50 mm in width x 30 mm in length, then placed onto a glass slide and positioned centrally on the microscope stage while the machine moved in a left-to-right direction. The hydrogels were subjected to a droplet of deionized water, about 4 µL in volume and the contact angle pictures were taken at intervals times.

S*urface Morphology Analysis*

The hydrogel was morphologically examined by a scanning electron microscope (SU3500, Hitachi, Japan). Before analysis, the hydrogel samples were lyophilized in a vacuum environment for 24 hours. The hydrogel samples were affixed onto carbon sample holders and then coated with gold (Au) through sputtering for 2 minutes. The examination ran at an acceleration voltage of 5 kV and was taken at 1000x magnification.

Mechanical Properties

The mechanical properties of the hydrogel samples were evaluated using the ASTM D882–10 standard, using a Universal Testing Machine (UTM) (AG-X Plus 50kN-Shimadzu, Japan) equipped with a 50 kN load cell. To assess the tensile strength (TS) and elongation at break (EB) of various hydrogel types, a total of five test specimens were fabricated for each hydrogel variant. These specimens were precisely measured to have dimensions of 25 mm in width, 150 mm in length, and 0.1 mm in thickness. Before characterization, the hydrogel samples underwent a conditioning process lasting a minimum of 40 hours at a temperature of 23 ± 2 °C (73.4 ± 3.6 °F) and a relative humidity of 50 ± 10%. The crosshead speed used was 10mm/min, and the grip distance was set at 10 mm.

Thermal Properties

The thermal characteristics of the hydrogel were analyzed by Differential scanning calorimetry (DSC 4000-Perkin Elmer, United States). The hydrogel samples weighing approximately 5 mg were carefully measured and securely enclosed in aluminium pans. These hydrogel samples were subjected to thermal analysis within a temperature range of 25 to 300 °C, utilizing a scan rate of 10°C/min. The thermal analysis process involved a cyclic heating procedure, commencing from an initial temperature of 25 °C and gradually increasing to 300 °C. The samples were then maintained at this temperature for 10 minutes. Subsequently, the cooling phase commenced, starting from 300 °C and gradually decreasing to 25 °C. This was followed by another heating phase, starting from 25 °C and increasing to 300 °C. Finally, the hydrogel samples were subjected to a cooling phase, commencing from 300 °C and gradually decreasing to 25 °C.

Table 1. Composition of Hydrogel.

Formulation	Code	Sugar Palm Starch (g)	Chitosan (g)	Alginate (g)	Citric acid (g)	Glycerol (ml)
Sugar palm starch hydrogel	F0	0.6	-	-	0.1	1
Sugar palm starch/chitosan hydrogel	F1	0.6	0.2	-	0.1	1
	F2	0.6	0.4	-	0.1	1
	F3	0.6	0.6	-	0.1	1
Sugar palm starch/alginate hydrogel	F4	0.6	-	0.2	0.1	1
	F5	0.6	-	0.4	0.1	1
	F6	0.6	-	0.6	0.1	1

RESULTS AND DISCUSSION

Thickness Measurement

The thickness measurement results of the sugar palm starch-based hydrogels were shown in **Table 2**. The thickness of sugar palm starch-based hydrogel (F0-F6) is in the range between 0.436-0.663 mm. This result is similar to a previous study from Simões, et al. (2020) that prepared hydrogel for starch/xanthan gum cross-linked with citric acid. Hydrogel in this study has a thickness in the range between 0.344-0.525 mm (Simões et al., 2020). The thickness of sugar palm starch-based hydrogel with reinforcing agent (F1-F6) was higher than the thickness of sugar palm starch hydrogel (F0). The thickness value tends to increase along with the increase of the reinforcing agent concentration addition to the sugar palm starch-based hydrogel, and the highest thickness value was sugar palm starch/alginate compared to sugar palm starch/chitosan because alginate prevents water from evaporating throughout the drying process. As studied earlier by Rudyarjo and Wijayanto (2017) the more lauric acid is added to the hydrogel, the more water is trapped in the starch granules. This is because lauric acid inhibits the evaporation of water during the drying so that the resulting hydrogel gets thicker (Rudyardjo & Wijayanto, 2017). Then, Samani et al. (2023) as stated in earlier findings about chitosan and agar film, differences in film thickness depend on the way the films were prepared, and the amount of additive used. Due to strong intermolecular interactions between chitosan and agar (Samani et al., 2023). Similar to the research by Sondari, et al. (2022) film thickness is a physical attribute that is influenced by the concentration of dissolved particles in the solution as well as the size of the casting plate. (Sondari et al., 2022).

Functional Group Characterization

Functional groups of sugar palm starch, chitosan, alginate, citric acid, sugar palm starch/chitosan hydrogel, and sugar palm starch/alginate were investigated on the FTIR, and the spectra are depicted in **Figure 1** for sugar palm starch/chitosan hydrogel and in **Figure 2** for sugar palm starch/alginate hydrogel. The presence of a new band, corresponding to the C=O stretching showed in the range between 1709-1716 cm^{-1} for sugar palm starch/chitosan hydrogel (F1-F3) and 1716-1718 cm^{-1} for sugar palm starch/alginate hydrogel (F4-F6), which confirmed esterification process that indicated chemical links between the hydroxyl groups of starch and the carboxyl groups of citric acid as cross-linkers.

Table 2. Thickness Measurements Result.

Formulation	Code	Thickness (mm)
Sugar palm starch hydrogel	F0	0.419 ± 0.028
Sugar palm starch/chitosan hydrogel	F1	0.436 ± 0.014
	F2	0.448 ± 0.012
	F3	0.504 ± 0.024
Sugar palm starch/alginate hydrogel	F4	0.428 ± 0.005
	F5	0.570 ± 0.018
	F6	0.663 ± 0.028

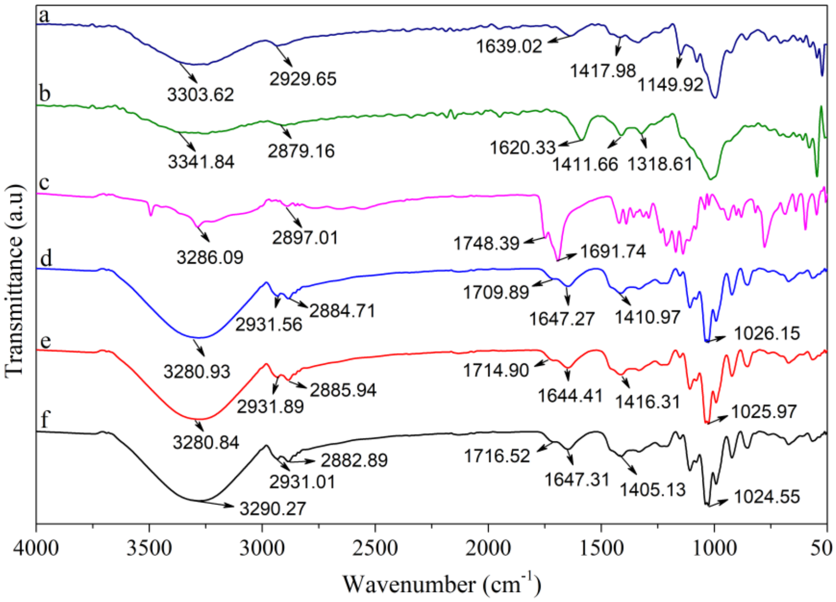

Figure 1. FTIR spectra of sugar palm starch (**a**), chitosan water-soluble (**b**), citric acid (**c**), and sugar palm starch/chitosan hydrogels: F1 (**d**), F2 (**e**) and F3 (**f**).

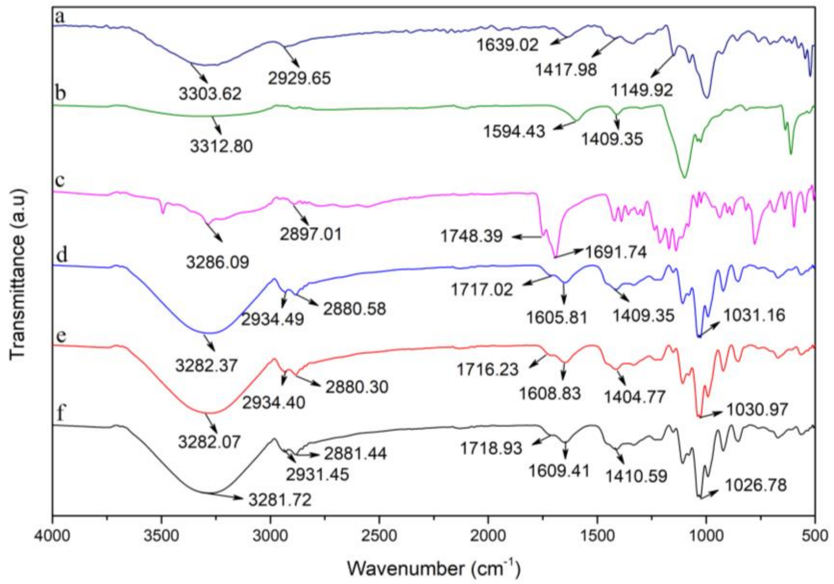

Figure 2. FTIR spectra of sugar palm starch (**a**), alginate (**b**), citric acid (**c**), and sugar palm starch/alginate hydrogels: F4 (**d**), F5 (**e**) and F6 (**f**).

The following characteristic peaks were identified. Wavenumber in the range between 2882-2885 cm^{-1} due to N-H stretching vibrations, the shifted peak in C=O stretching (amide I) from 1584 cm^{-1} in chitosan to 1644-1647 cm^{-1} in sugar palm starch/chitosan hydrogel (F1-F3) indicated that interactions were present between the hydroxyl group of starch and the amino group of chitosan, and 1405-1416 cm^{-1} belong to N-H bending (amide II). For sugar palm starch/alginate hydrogel (F4-F6), the shifted peak in COO- asymmetric stretching vibrations of 1594 cm^{-1} in alginate to 1608-1650 cm^{-1} sugar palm starch/alginate hydrogel (F4-F6) indicated that interactions were present between the hydroxyl group of starch and the carboxyl group of alginates. Also, intense bands attributed to symmetric stretching vibrations of COO- in the range between 1404-1410 cm^{-1}. Furthermore, the vibrations of the C–O bonds for alcohol (glycerol) appeared in the range between 1024-1031 cm^{-1} and the wide band between 3280-3290 cm^{-1} may be ascribed to the stretching and vibration of hydroxyl (–OH) bonds for F1-F6 hydrogel. All the characteristic bands were described in the previous publication about hydrogel from starch (Hernández et al., 2023; Restu et al., 2020; Wilpiszewska et al., 2019), chitosan (Chopra et al., 2022; Wu et al., 2019), and alginate (Gajić et al., 2023; Liu et al., 2018).

Swelling Ratio

Swelling is the ability to absorb fluid and create pores when it is in a swelling state. The swelling ratio curves of the prepared hydrogel formulations are depicted in **Figure 3**. Based on the results, all of the hydrogels have reached the maximum swelling ratio at 4 hours. Moreover, the swelling ratio gradually increased from 1 to 4 hours and then decreased after 4 hours, as also from the study of Ishfaq et al. (2023) this phenomenon might be due to polymer erosion (Ishfaq et al., 2023). The swelling ratio of the sugar palm starch/chitosan hydrogel (F1-F3) and starch/alginate hydrogel (F4-F6) was higher compared to the sugar palm starch hydrogel (F0). The maximum swelling ratio that reached after 4 hours was 41.44% for sugar palm starch-based hydrogel (F0), 67.81% for sugar palm starch-based hydrogel with chitosan as reinforcing agent (F1) and 50.77% for sugar palm starch-based hydrogel with alginate as reinforcing agent (F4). This result is similar to a previous study by Hu et al. (2023) that prepared hydrogel from carboxymethyl cellulose and genipin as a cross-linker. Hydrogel in this study has a swelling ratio in the range between 19.40-60.80% (Hu et al., 2023).

In general, the addition of reinforcing agents increased the sugar palm starch-based hydrogel swelling ratio. The higher swelling ratio was sugar palm starch/chitosan hydrogel compared to sugar palm starch/alginate. Based on statistical analysis, the chitosan-reinforced hydrogel exhibited a better swelling ratio than alginate-reinforced hydrogel, which has a significant value ($p < 0.05$). Chitosan as a reinforcing agent improves hydrogel water absorption, resulting in a highly wettable surface, and sugar palm starch/alginate hydrogel indicates tighter-networked hydrogels than sugar palm starch/chitosan hydrogel. This result is similar to that of a previous study by Susanthy et al. (2016) this phenomenon might be due to the abundant number of free water molecules in the chitosan solution, which can reduce the amount of hydrogen bonding, which might be due to less tight bonding (Susanthy et al., 2016). The swelling ratio depends on several factors, such as the material, cross-linker, environment, and swelling medium (Chelu et al., 2023).

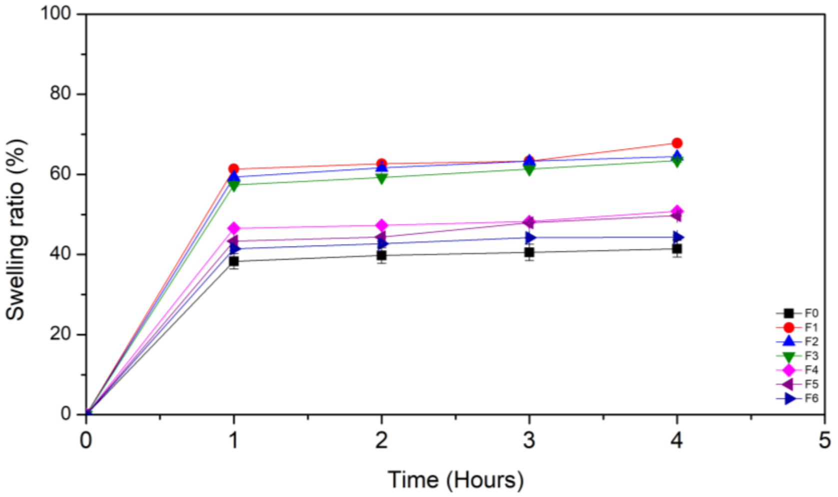

Figure 3. Swelling ratio curve of sugar palm starch-based hydrogel (F0-F6).

Water Contact Angle

The wettability of the hydrogels was assessed by a contact angle (θ) to observe the resistance of the hydrogel against water and to determine the hydrophobic properties of its surface (Sondari et al., 2022). The water contact angle measurement uses water as the polar solvent. The water contact angle obtained from 20-120 seconds for sugar palm starch/chitosan hydrogel (F1) was 23-32° (**Figure 4**), and for sugar palm starch/alginate hydrogel (F4) was 42-51° (**Figure 5**), as we can see the contact angle value decreased as well as the time increased. The water contact angle values obtained from sugar palm starch/alginate were higher than those obtained from sugar palm starch/chitosan hydrogel. It can be concluded that alginate was more hydrophobic than chitosan due to the fact that it is related to the swelling ratio result, which is less swelling than chitosan. Syahara et al. (2015) stated that the polarity of the sample will be correlated with the value of the contact angle (Syahara et al., 2015).

Surface Morphology Analysis

Surface morphology analysis of sugar palm starch, sugar palm starch hydrogel (F0), sugar palm starch/chitosan hydrogel (F1), and sugar palm starch/alginate hydrogel (F4) were observed using SEM. The surface morphology was observed using 1000x magnifications. The morphological characteristics of the prepared hydrogels were considered an appropriate method for checking the homogeneity and uniformity of the prepared hydrogels. **Figure 6a** showed that sugar palm starch was spherical in shape and heterogeneous in size. Morphology images of the sugar palm starch in hydrogel form after cross-linking of starch with citric acid and lyophilized (F0, F1 and F4) depicted in **Figures 6b**, **6c** and **6d** showed uniform surface morphology, homogeneous material distribution, porous and rough surface. Porous, rough-surfaced spherical particles were produced during the cross-linking of the polymer (Sathiyaseelan et al., 2023). As studied by Demeter et al. (2023) the porosity in the hydrogel is crucial for biomedical applications such as wound dressing because it enables the absorption of the fluid or wound exudate, delivery of the drug and helps keep the wound dry and promotes oxygen supply to the injury (Demeter et al., 2023).

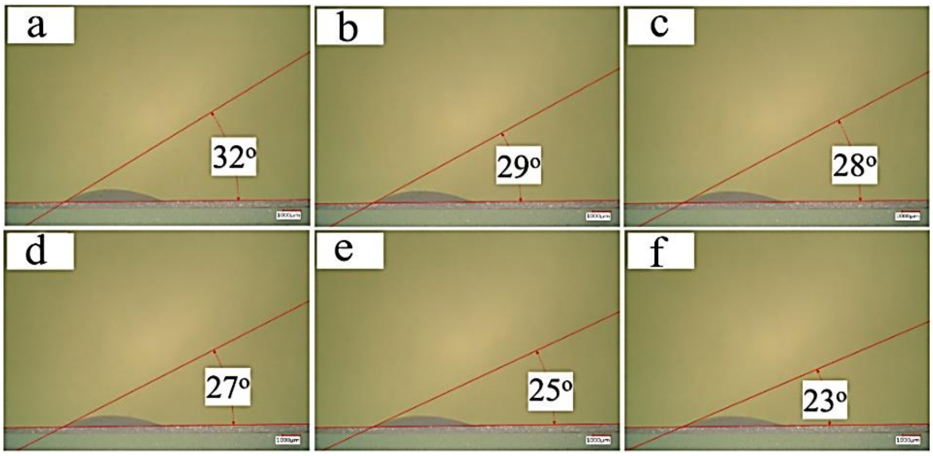

Figure 4. The water contact angle of sugar palm starch/chitosan hydrogel (F1) at 20 seconds (**a**), 40 seconds (**b**), 60 seconds (**c**), 80 seconds (**d**), 100 seconds (**e**), and 120 seconds (**f**).

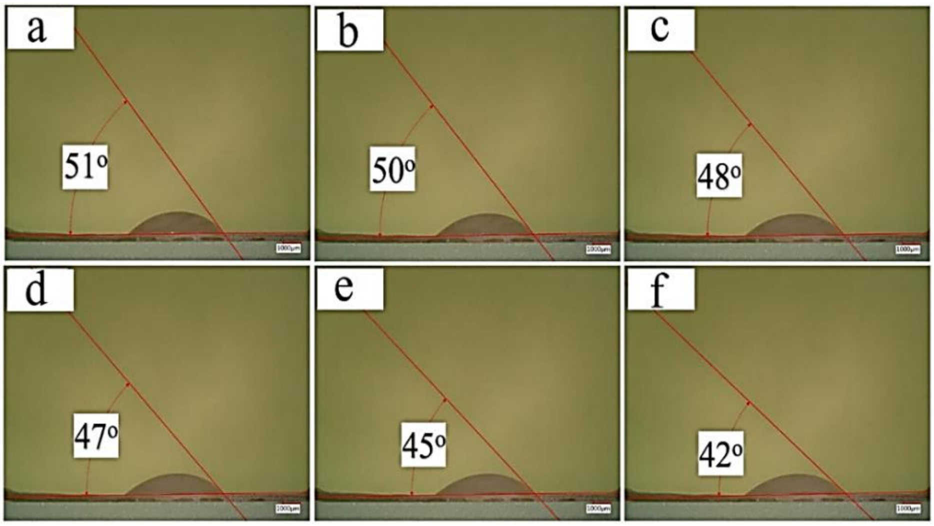

Figure 5. The water contact angle of sugar palm starch/alginate hydrogel (F4) at 20 seconds (**a**), 40 seconds (**b**), 60 seconds (**c**), 80 seconds (**d**), 100 seconds (**e**), and 120 seconds (**f**).

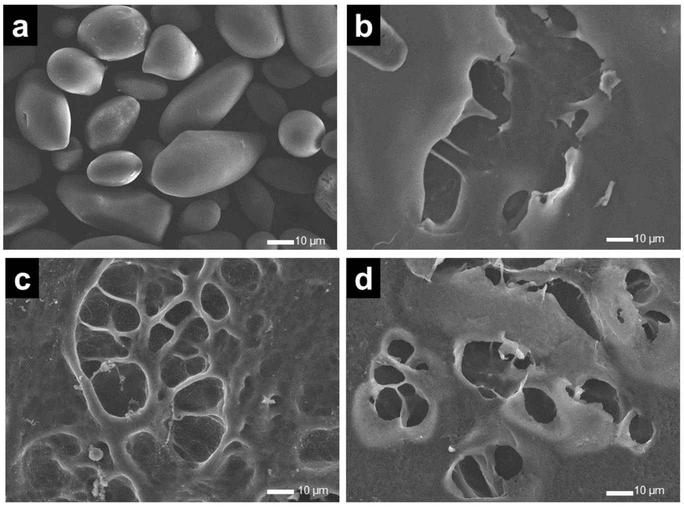

Figure 6. SEM images of sugar palm starch (**a**), F0 (sugar palm starch hydrogel) (**b**), F1 (sugar palm starch/chitosan hydrogel) (**c**), and F4 (sugar palm starch/alginate hydrogel) (**d**).

Mechanical properties

Mechanical properties are essential in the application of hydrogels for flexibility and durability. The selected parameters to represent the mechanical properties of the hydrogel were tensile strength and elongation at break, as shown in **Table 3**. The tensile strength for sugar palm starch-based hydrogel with chitosan (F1) and sugar palm starch-based hydrogel with alginate (F4) was 0.113 and 0.235 MPa, respectively and for elongation at break was 37.83 and 57.13%, respectively. This result is similar to a previous study from Sobczak-Kupiec et al. (2023) prepared hydrogel from polyvinylpyrrolidone (PVP) and gelatine has a tensile strength in the range between 0.17-0.26 MPa and elongation at break in the range between 11-23% (Sobczak-Kupiec et al., 2023).

The tensile strength and elongation at break for sugar palm starch hydrogel (F0) were lower than sugar palm starch/chitosan hydrogel (F1) and sugar palm starch/alginate hydrogel (F4). It can be concluded that synthesized hydrogel from sugar palm starch with a reinforcing agent has better mechanical properties than sugar palm starch hydrogel without a reinforcing agent. Chopra et al. (2022) stated that chitosan could create a superior network, which might be attributed to strong physical contact and networking between chitosan and other polymers (Chopra et al., 2022). Also studied by Restu et al. (2020) the mechanical properties of starch nanoparticles in the development of edible films were improved by the incorporation of chitosan (Restu et al., 2020).

The interactions between the hydroxyl (-OH) in starch and amino (-NH_2) groups in chitosan, exhibit the capacity to augment the density and elevate the tensile strength of the film. Nevertheless, the tensile strength and elongation at break value for sugar palm starch/chitosan hydrogel were lower than sugar palm starch/alginate hydrogel. Based on statistical analysis, alginate-reinforced hydrogel exhibited better tensile strength than chitosan-reinforced hydrogel with a significant value ($p < 0.05$). However, in terms of elongation at break, the value was not statistically significant ($p > 0.05$). Sugar palm starch/chitosan hydrogel was weaker against external pressures and has less tight bonding, which might be due to free water molecules in the chitosan solution reducing the amount of hydrogen bonding, as well as studied by Liu et al. (2016) the increase in hydrogen bonds will improve the mechanical properties (Liu et al., 2016).

Thermal properties

A differential scanning calorimeter (DSC) was used to investigate the thermal properties of a hydrogel produced from sugar palm starch and cross-linked with citric acid. The thermal data of glass-transition temperature (*Tg*) and melting temperature (*Tm*) obtained from the DSC was presented as a thermogram in **Figure 7**. A shift in the slope is related to the amorphous phase represented by *Tg*, which is the transition of the glass state to an elastic state (Kheirabadi et al., 2015), and the area indicates an endothermic peak related to T_m, the crystalline structure under the endothermic peak (Soliman & Furuta, 2014).

The glass-transition temperature (T_g) for sugar palm starch/alginate hydrogel (F4) is shown at 144.77 °C (**Figure 7a**), for sugar palm starch/chitosan hydrogel shown at 146.70 °C (**Figure 7b**), and for sugar palm starch hydrogel was shown at 229.38 °C (**Figure 7c**). Characteristic endothermic peaks were exhibited at 71.48 °C for sugar palm starch (**Figure 7d**), as stated by Ghozali et al. (2023) it correlates with the temperature gelatinization of sugar palm starch (Ghozali et al., 2023).

Based on this study, adding chitosan and alginate as reinforcing agents in the synthesized sugar palm starch-based hydrogel causes a decrease in the peak and glass-transition temperature (T_g) attributed to the decrease in water content and breakdown of the gel network (Polez et al., 2022). This phenomenon makes hydrogel suitable for biomedical applications such as wound dressing because a decrease in *Tg* value makes hydrogel more elastic, and the *Tm* value improves the thermal stability of the hydrogel with a reinforcing agent, compared to sugar palm starch hydrogel without a reinforcing agent. The glass-transition temperature was also influenced by glycerol as a plasticizer, as studied by Ghozali et al. (2023) the plasticization of starch by glycerol occurs through the reduction and exchange of intermolecular and intramolecular bonds via a glycerol-starch hydrogen bonding mechanism. Glycerol effectively reduces intramolecular hydrogen bonding while simultaneously increasing intermolecular spacing, perhaps resulting in a decrease in the glass-transition temperature (Ghozali et al., 2023).

Table 3. Result of mechanical measurement of hydrogels.

Formulation	Code	Mechanical Parameters	
		Tensile strength (MPa)	Elongation at break (%)
Sugar palm starch hydrogel	F0	0.070±0.06	21.68±8.0
Sugar palm starch/chitosan hydrogel	F1	0.113±0.04	37.83±7.0
Sugar palm starch/alginate hydrogel	F4	0.235±0.05	57.13±8.0

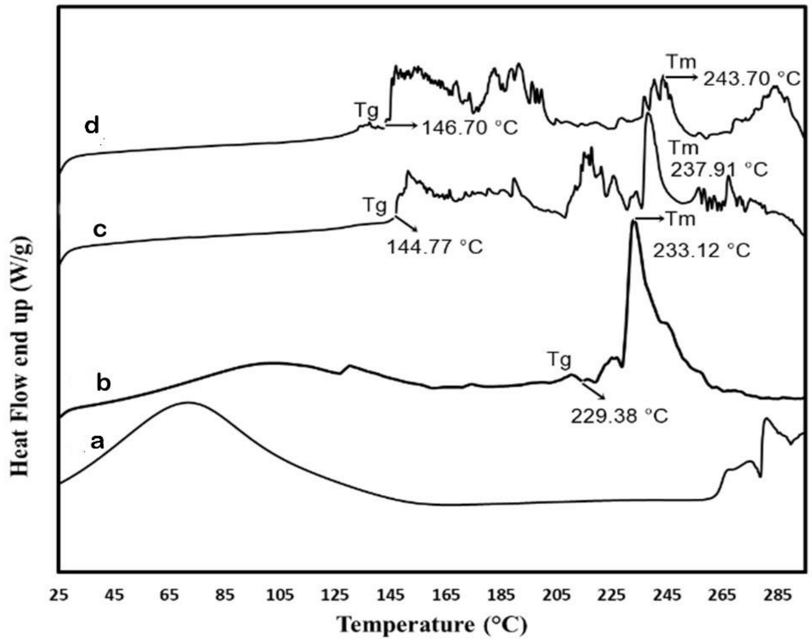

Figure 7. DSC thermograms of sugar palm starch (**a**), sugar palm starch hydrogel/F0 (**b**), sugar palm starch-chitosan hydrogel (**c**), sugar palm starch-alginate hydrogel (**d**).

Table 4. Calculation of crystallinity from sugar palm starch-based hydrogels.

Sample	T_m (°C)	ΔH T_m ($^J/_g$)	X_c (%)
F0 (sugar palm starch hydrogel)	233.12	97.40	785.48
F1 (sugar palm starch/chitosan hydrogel)	237.91	29.98	241.77
F4 (sugar palm starch/alginate hydrogel)	243.70	27.10	218.55

In terms of the crystallinity of the materials, a novel approach has been devised to assess the material's crystallinity using data from DSC. The degree of crystallinity was calculated using melting temperature data with the following Equation **2**:

$$X_c\% = \frac{\Delta H_{Tm}}{\Delta H^{\circ}_{Tm}} x100 \qquad (\mathbf{2})$$

Where:

ΔH$_{Tm}$ = enthalpy of fusion from the melting point of the sugar palm starch hydrogel

ΔH°$_{Tm}$ = enthalpy of fusion of 100% native starch based on the literature (12.40 $^J/_g$)
(Tawil et al., 2011).

Based on **Table 4**, there is a decrease in the degree of crystallinity from sugar palm starch hydrogel without a reinforcing agent (F0) to sugar palm starch hydrogel with chitosan (F1) and alginate (F4) as a reinforcing agent. The incorporation of a reinforcing agent could decrease the crystallinity compared to the hydrogel without a reinforcing agent, representing the complete synthesis and interaction between materials. That result is similar to a previous study by Soliman and Furuta (2014) that the degree of crystallinity of amylopectin soluble starch film is lower than gelatin and amylopectin soluble starch as raw material (Soliman & Furuta, 2014).

CONCLUSIONS

The hydrogel in this study was produced by combining sugar palm starch with reinforcing agents such as chitosan and alginate, glycerol as a plasticizer, and citric acid as a cross-linking agent. Chitosan was appropriate as a reinforcing agent in sugar palm starch-based hydrogel for future biomedical applications, as demonstrated by its higher swelling ratio than alginate-reinforced hydrogel. Although the tensile strength and elongation at break value are lower than alginate-reinforced hydrogel, they are still within the acceptable range obtained in earlier studies. The incorporation of a reinforcing agent gives hydrogels better characteristics that statistically significant.

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COMPETING INTEREST

The authors have declared that they have no competing interests.

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