

ORIGINAL ARTICLE

Development of Photosensitive Hydrogel-based 3-dimensional Bioprinting Using Locally Extracted Pectin From Durian Rind Waste and Cellulose for Pharmaceutical Application

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ABSTRACT

Introduction: Hydrogels have gained prominence in a variety of fourth industrial revolution applications, including three-dimensional (3D) printing. However, there are limitations to 3D manufacturing, such as deformities in the final product. This is a significant obstacle to adopting this technology in the pharmaceutical industry, as printed products may have insufficient mechanical properties and a high brittleness, making further processing of these dosage forms problematic. The objective of this study is to produce a new 3D bioink from a mixture of locally produced pectin-based material from durian rind waste and cellulose-based material (pectin/cellulose hydrogel) and to partially characterize the bioink hydrogel. **Methods:** Four formulations of pectin/cellulose-based hydrogel (3:1, 3:2, 4:1, and 5:3 ratio of pectin/cellulose) from durian rind waste and carboxymethyl cellulose (CMC) powder, as well as cross-linking agents, were developed and evaluated using a rheometer to evaluate viscoelastic properties, FTIR Spectroscopy to identify compounds, and thermogravimetric analysis (TGA) to evaluate thermal stability. **Results:** All bioink formulations exhibit outstanding shear-thinning behavior suitable for 3D printing. The viscosity of edible ink increases as the pectin/cellulose concentration increases. The formulation of 3:1 pectin/cellulose has greater heat resistance than others (highest thermal stability with 21.69% of residual weight) and the lowest percentage of weight loss (76.18%). **Conclusion:** The study of a pectin/cellulose hydrogel mixture provides an attractive outcome for the creation of bioink due to the effective synthesis of 3D printing shapes that are both smooth and uniform.

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and durability of the biomimetic functional tissue and organs as compared to the original tissue and organs (2). Additionally, bioinks commonly are based on hydrogel frameworks.

INTRODUCTION

In pharmaceutical area, 3-Dimensional bioprinting (3D bioprinting) or also known as additive manufacturing has been emerging as a fascinating new technology and alternative way in creating and producing biomimetic living constructs to match functional tissue and organs (1, 2) which can be used for many health treatment purposes. This 3D bioprinting provides researchers particularly tissue engineer, with bioinks-based biomaterials to print biologically relevant functional tissue or organs in 3D shape (2). Bioinks-based biomaterials have shown to increase the strength

The crucial properties of printability and biocompatibility for 3D bioprinting must be achieved by proper bioink characterisation. Biocompatible bioink is necessary to avoid toxicity or inflammation in the body. It should not contain any harmful substances or impurities that could cause adverse reactions. Loi et al. (3) have elucidated in detail the beneficial properties of a bioink that they developed by combining an extracellular matrix-like hydrogel with osteosarcoma cells. They stated that bio-ink must have a viscosity that is compatible with the printing procedure. It should be fluid enough to pass through the nozzle but not too thin that it will spread too much and

cause the loss of resolution. To guarantee a constant printing pressure, it was necessary to set the extruder to 37 °C. Bio-ink must be able to flow smoothly through a 3D printer nozzle and be deposited accurately in the desired pattern to create the desired tissue structure. Moreover, suitable mechanical properties such as elasticity, stiffness, and strength must match the properties of the intended tissue to ensure the successful printing of the tissue.

Hydrogels are made up of polymer networks that are cross-linked and can hold a considerable quantity of water. These cross-linked polymer networks form porous hydrogels structure with high containment capacity and widespread use in multiple industries such as waste water treatment, medical applications, pharmacies, other industrial and non-industrial applications (4,5). Pharmaceutical cross-linked hydrogels can be divided into chemically or physically cross-linked structures (6). A chemically crosslinked hydrogels are not reversible and have superior mechanical properties such as elastic modulus. This is made possible by the numerous covalent crosslinking pathways. To crosslink hydrogels, many processes such as heat polymerization, photopolymerization, enzyme crosslinking, and others can be used (7). Meanwhile, the choice of polymer is an important step in creating physical cross-linked hydrogel, and it depends on two main requirements. First, the network must be able to hold a significant amount of water molecules inside of it. Secondly, the interactions between the chains must be strong enough to create semi-permanent junctions in the molecular network (8). Hydrogen bonding, electrostatic and hydrophobic interactions between polymer chains are the three factors at work when a physical gel forms. Gel formation is reversible due to the purely physical network that results from all of these interactions (4).

Apart from that, there are two types of hydrogels which is conventional hydrogel that are not sensitive to the change of the environment and smart hydrogel which is sensitive to the change of an environment. It is called as smart hydrogel due to its ability to undergo physical and chemical changes as reactions to optical signal. The classifications of the smart hydrogel were pH-sensitive, temperature-sensitive, salt responsive or photosensitive hydrogels (9). A photosensitive hydrogel consists of a photoreactive moiety and a polymeric network, which is also referred to as a photochromic chromophore. The photosensitive materials have three responsive mechanisms, as shown in Figure 1. The mechanism is photosensitive molecule in a temperature sensitive material can transform the light energy into heat energy, and it will increase the temperature of the materials and when the internal temperature of the

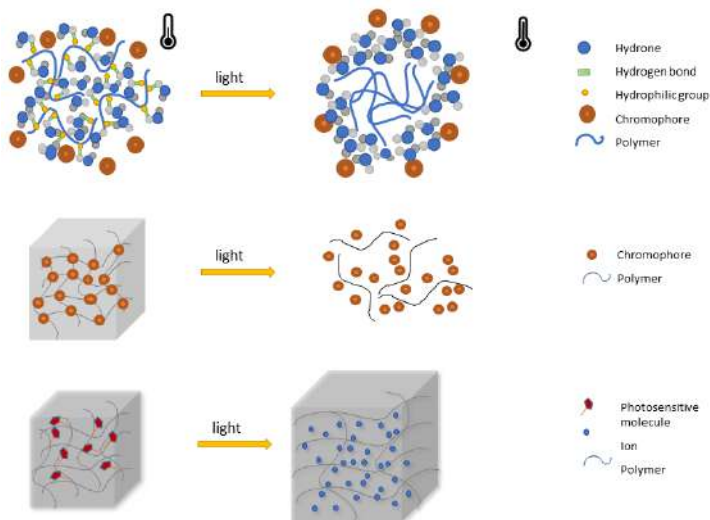


Figure 1 : The schematic diagram showing the responsive mechanisms of photosensitive materials.

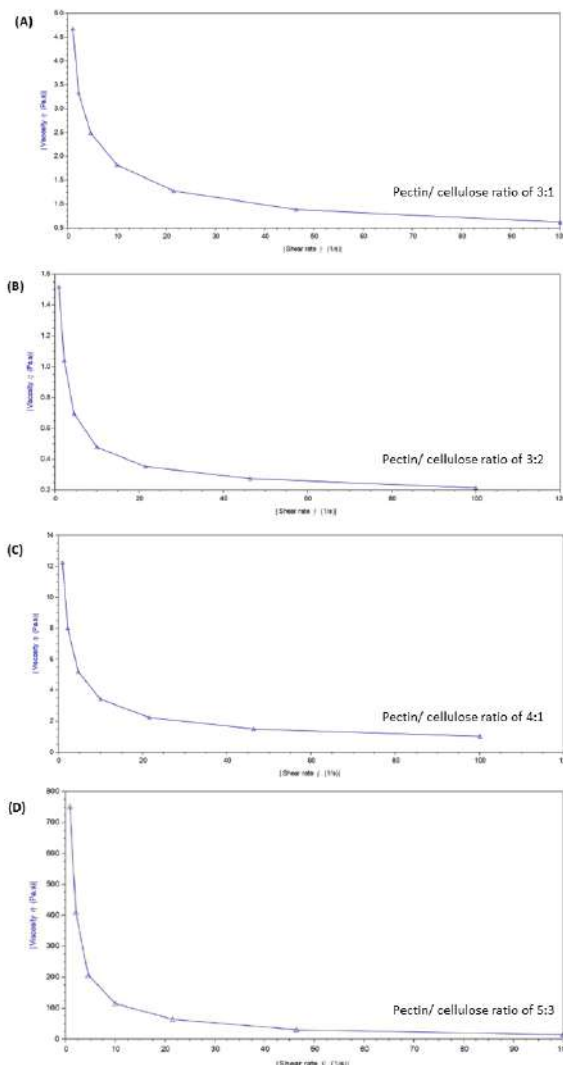


Figure 2 : The graph of viscosity against shear rate (A) Formulation consists of 3: 1 (pectin/ cellulose) (B) Formulation consists of 3: 2 (pectin/ cellulose) (C) Formulation consists of 4: 1 (pectin/ cellulose) (D) Formulation consists of 5: 3 (pectin/ cellulose).

gel meets a requirement for phase transition condition, the gel will provide a response (9). In response to light irradiation, the photosensitive molecules inside the gel cause ionisation, which is the following step. Additional ions are created when light is shone on the gel, which can disrupt the osmotic balance maintained by drifting ions and water molecules (9). Photochromic molecules are introduced into the hydrogel substance on the polymer backbone as side groups or cross linkers. The physicochemical features of these chromophores, such as dipole moment and geometrical structure, as well as the macroscopic hydrogel's properties, structure, and form, would be changed as a result of their photosensitivity. Azobenzene and cyclodextrin (CD) were examples of the materials that can be used to build the crosslinking points in the network of the hydrogel by host-guest interactions (10).

Valorization of pectin-rich plant biomass residues has received a great deal of attention from the industrial biotechnology and pharmaceutical communities recently. The current 'take-make-dispose' production paradigm must be replaced with a circular economy model (11,12). Using biomass-derived agro-industrial waste streams as source materials for the production of functionalized biomaterials can significantly contribute to this concept. Durian is an immensely popular fruit in Southeast Asia, particularly in Malaysia, the Philippines, Thailand, and Indonesia. This fruit, whose scientific name is *Durio zibethinus*, is known as the "king of fruits." Durian has been easily and extensively cultivated in the food processing industries, such as durian ice cream, chocolates and jams. However, only one-third of the durian fruit is edible, as the seeds and rinds are discarded (13). Previously, we were able to extract the methoxyl content of durian peel pectin using the titration method, in Mashhor et al. (14). The extracted pectin from durian rind has a methoxyl content of 3.22 ± 0.07 %, which classifies it as low methoxyl pectin. Pectin with a methoxyl content between 2.50 % and 7.12 % is classified as low methoxyl pectin, whereas pectin with a methoxyl content greater than 7.2 % is classified as having a high methoxyl content (15). Pectin with a high methoxyl content has been used to form a matrix for drug delivery, according to a 2018 study by Lara Espinoza et al. However, due to its high molecular weight and poor solubility in water, it causes drug migration, early release, and erosion of the cover (16). Maciel et al. (17) found that low methoxyl pectin is preferable for encapsulating bioactive compounds due to its low molecular weight and capacity to form water-insoluble cross-linked polymer. These characteristics can aid in the formation of stable matrices that effectively regulate the release of bioactive compounds.

On the other hand, carboxymethyl cellulose or sodium carboxymethylcellulose (CMC) is an anionic cellulose derivative that shows amphiphilic properties as it has a hydrophobic polysaccharide backbone and surrounded hydrophilic carboxyl groups which also means it can be dissolved in water when compared to cellulose which naturally is not soluble in water (18). Besides, CMC also has properties of biocompatible and biodegradable. CMC is synthesized in a non-aqueous monochloroacetic acid/sodium solvent medium and carboxymethylated to get a degree of substitution. It is soluble in water of at any temperature. Solubility of CMC depends on degree of polymerization and degree of substitution. Solubility will be greatest with lesser polymerization and more CMC substitution (19).

The purpose of this research is to prepare a new bioink hydrogel from a mixture of pectin-based durian rind waste solution and cellulose-based powder and to partially characterize the bioink hydrogel for suitable 3D printing finished product. The use of a locally extracted pectin and cellulose powder to create bioink for 3D printing is a relatively new concept. Apart from that, the addition of food-grade cross-linking agents to formulate hydrogels improve mechanical properties and allows the introduction of specific physical-chemical reactions. In particular, Ca^{2+} ions are the most suitable option for mild reaction conditions. Similarly, it has been used as a crosslinking agent to form 3D networks by Eivazzadeh-Keihan et al. (20).

MATERIALS AND METHODS

Sample preparation

Durian rind waste was obtained from local market in Pagoh, Johor and commercial CMC powder was purchased in online market (EvaChem, EvaChem & Trusol, Selangor, Malaysia). A sharp knife was used to cut open the durian fruit and separate the arils from the rinds. The durian rind was thinly cut into pieces with a thickness of around 0.5 cm to aid in the drying process (21). The smaller durian rinds were then spread out on aluminium sheets and dried in a hot air oven (Memmert, D06836, Germany) at 60 °C for about 24 hours, or until a constant weight was attained. The rinds were turned into powder using a grinder (Micro Universal Bench Top Grinder; Retsch ZM 100, Haan, Germany) after drying.

Extraction of pectin

Prior to this, Mashhor et al. (14) described the extraction of pectin. Initially, 40 g of durian rind powder in a 1:9 (w/v) ratio of a mild acid aqueous solution. Using 1 N HCl, the pH level of the aqueous solution was adjusted to 2.5. The whole mixture was then incubated in a water bath at 85 °C for four

hours. The slurries were filtered through cheesecloth and allowed to settle at room temperature. The filtrate was then combined with acidified ethanol, which consisted of 4% HCl mixed with 95% ethanol in a 1:4 ratio (v/v), and the mixture was left for one hour at the room temperature. The sample was then centrifuged for 15 minutes at 4700 rpm in a bench-top centrifuge (Kubota 5100, Fujioka, Japan). The solution was rinsed twice with ethanol containing 95% at a ratio of 1:2, and then centrifuged for 15 minutes at the same speed. The precipitate was then filtered, collected, and desiccated in an oven at 55 °C until its weight was constant.

The formulation of pectin-cellulose bioink

Pectin solution and cellulose solution at different concentrations were dissolved in distilled water. Then, the pectin solution and cellulose solution were mix for an hour using a magnetic stirrer. After that, 3% (w/v) glycerol was added to the prior blended solution and left to stir for another one hour. A 1% (w/v) calcium chloride (CaCl₂) solution was added to the polymer mixture solution drop by drop. The hydrogel was formed as a result of CaCl₂ cross-linking. The method was repeated but, using another formulation while ascorbic acid and sodium nitrate were added, as shown in Table I. The method conducted was referring to the method published by Ghosh et al. (6).

In this study, any cubic photon 3D, a commercial 3D printer was used. By directing a UV laser along a predetermined path on the resin’s surface, the initial layer was hardened to a defined depth onto a building platform (22). The platform travels away from the photopolymerization surface after the first layer was cured to start polymerizing the second layer of the prescribed pattern, and so on, until the three-dimensional object was finished. In comparison to conventional 3D printing methods, this technique offers several benefits, including quicker print times, heat-free printing, improved surface polish, and higher resolution (23).

Rheology of pectin-cellulose bioink hydrogel

The dynamic viscoelastic characteristics of bioinks were examined prior to printing using a MCR501

rheometer with Direct Strain option (Mecomb Malaysia Sdn Bhd) and a cone-plate device with a 40 mm diameter and 1° cone angle, as previously described by Mashhor et al. (14). The temperature was constantly kept constant at 25 °C. Before the cone plate operation, it was rinsed with distilled water. In addition, the platform where the compositions will be placed was rinsed with distilled water. The machine started immediately after the formulation was placed on the stage. Graphs viscosity versus shear rate was created. After the machine have completed all formulations, the machine’s stage and cone plate were properly cleaned with distilled water.

Thermogravimetric Analysis (TGA)

Using a thermogravimetric analyzer, a thermal analysis will be carried out. TGA analysis will be carried out on the Q5000 series thermal analysis system’s TGA module (TA Instruments, West Sussex, UK). A quantity ranging from 10-20 mg was placed in an open pan of platinum 100 L that was connected to a microbalance. The sample was heated at 20 °C/min from 25 to 500 °C under dry nitrogen with a flow rate of 10 mL/min.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

The ink formulation’s chemical structure was described and verified by the FTIR spectra. In the tiny, thin disc, 2 mL of the ink formulations were placed and inserted in the FTIR instruments. The spectrum’s resolution was set at 4 cm⁻¹ and average wavenumbers ranging from 450 to 4000 cm⁻¹.

RESULTS

Rheology properties of the pectin-cellulose bioink hydrogel

From the results obtain, the trends for all formulations using pectin from durian rinds and CMC shows a decrease in viscosity with the increase of shear rate. As shown in Figure 2, viscosity fell dramatically from 4.680 Pa.s to 0.618 Pa.s, 1.518 Pa.s to 0.216 Pa.s, 12.271 Pa.s to 0.998 Pa.s and 752.867 Pa.s to 14.439 Pa.s, respectively. Meanwhile, in Figure 3, the yield stress rose as the shear rate increased, for all formulations. The components in the formulation

Table I : The formulation used in the pectin-cellulose-based bioink solution

Formulation	Pectin %(w/v)	CMC %(w/v)	Ascorbic acid (g)	Sodium Nitrate (g)	CaCl ₂ solution (%)	Glycerol (%)	Epoxy (g)
1	3	1	0	0	1	3	60
2	3	2	1	0.5	1	3	60
3	4	1	2	1	2	3	60
4	5	3	4	2	2	3	60

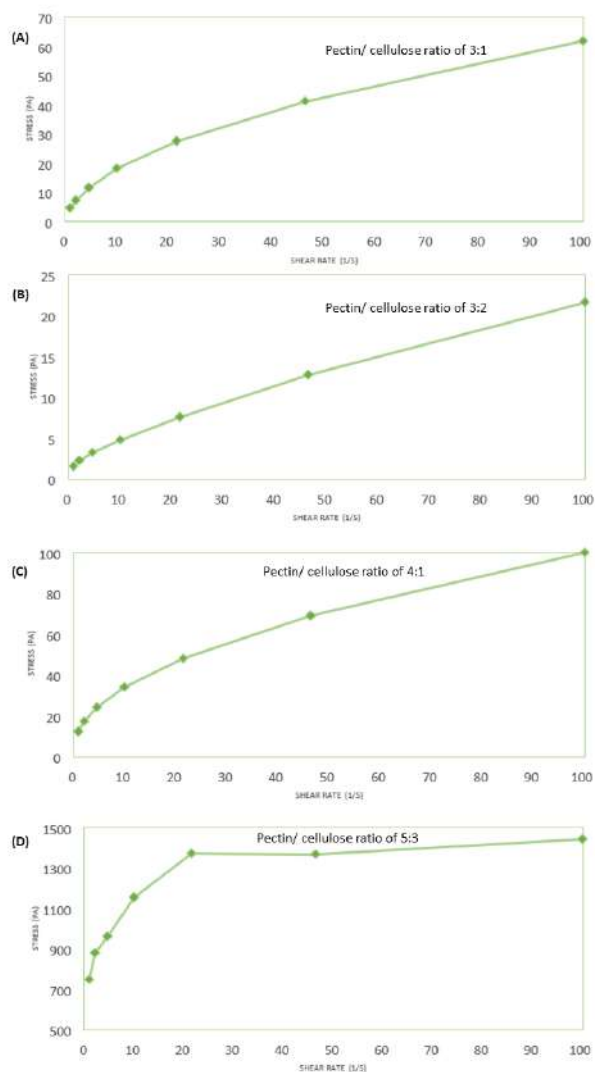


Figure 3 : The graph of stress against shear rate (A) Formulation consists of 3: 1 (pectin/ cellulose) (B) Formulation consists of 3: 2 (pectin/ cellulose) (C) Formulation consists of 4: 1 (pectin/ cellulose) (D) Formulation consists of 5: 3 (pectin/ cellulose).

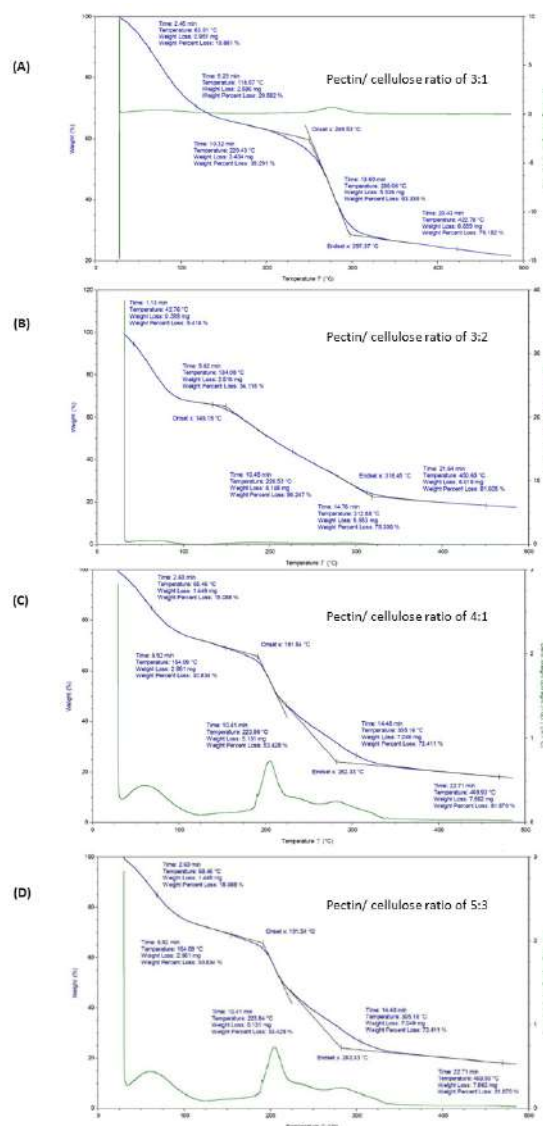


Figure 4 : The graph of TGA for (A) Formulation consists of 3: 1 (pectin/ cellulose) (B) Formulation consists of 3: 2 (pectin/ cellulose) (C) Formulation consists of 4: 1 (pectin/ cellulose) (D) Formulation consists of 5: 3 (pectin/ cellulose).

have an impact on the viscosity reduction, for example, CaCl_2 , ascorbic acid, sodium nitrate and glycerol. This was referred to as shear thinning. Their deposition recovery time was reasonable, and their viscosity reduces as yield stress increases. Meanwhile, Figure 2(D) shows a higher initial value of viscosity with 752.867 Pa.s than other formulations. It is expected that ascorbic acid may cause viscosity increases in regions where a liquid crystalline phase is occurred, as mentioned by Ji et al. (9).

Thermogravimetric analysis for pectin-cellulose bioink hydrogel

From Figure 4 the absorbed water in the pectin/cellulose formulations evaporated as the temperature rose, resulting in a small mass loss 10 %, 5 %, 15 % and 15 % respectively in the first zone (20-100 °C). The

weight reduced dramatically (by about 29 %, 34 %, 30 % and 30 % respectively) in the second zone (100-200 °C) as a result of the pyrolytic polysaccharide breakdown (24). Third, the temperature range of 200 to 500 °C represented the continuous weight loss (63 %, 75 %, 73 % and 73 %) caused by ash thermal decomposition. The TGA plot of the graph was reflect the thermal stability with relation to temperature change. The TGA profile for pectin/cellulose hydrogel was reveal that the initial degradation begins at 160 °C and finishes at 390 °C. In term of thermal stability, formulation 3:2 pectin/cellulose, 4:1 pectin/cellulose and 5:3 pectin/cellulose have lower thermal stability with the residual weight less than 20.00 % compare to formulation for 3:1 pectin/cellulose. From this, it can be concluded that the formulation of 3:1 pectin/cellulose has better

heat resistance than others as the highest thermal stability with 21.69 % of residual weight and has the lowest percentage of weight loss which was 76.18 % compared to others.

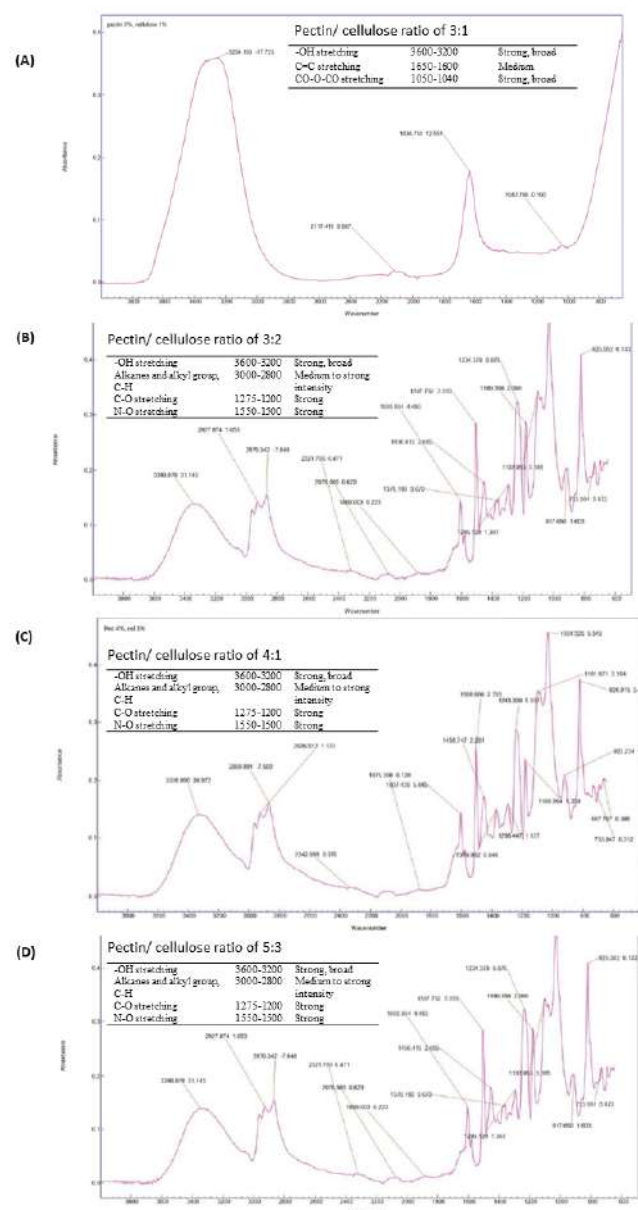


Figure 5 : The graph of FTIR Figure 4. The graph of FTIR for (A) Formulation consists of 3: 1 (pectin/ cellulose) (B) Formulation consists of 3: 2 (pectin/ cellulose) (C) Formulation consists of 4: 1 (pectin/ cellulose) (D) Formulation consists of 5: 3 (pectin/ cellulose).

FTIR analysis for pectin-cellulose bioink hydrogel

The FTIR spectra for the durian pectin, cellulose and all of the cross-linked between CaCl₂, glycerol, ascorbic acid and sodium nitrate were taken to validate the cross-linking between the polymer pectin, cellulose CMC and the cross-linkers used in the formulation. From Figure 4, the FTIR spectra of pectin/cellulose formulations display absorption peak at 3254 cm⁻¹ which represents hydroxyl group (-OH stretching). Meanwhile, the medium peaks at 1636

cm⁻¹ and a strong peak at 1042 cm⁻¹ represents C=C stretching and CO-O-CO stretching. The FTIR spectra graph of Figure 5(A) only contain fingerprint for pectin/cellulose, epoxy and glycerol. On the other hand, FTIR spectra for Figure 5(B), 5(C) and 5(D), display absorption peak at 3336 cm⁻¹ and 3346 cm⁻¹ which were owing to -OH stretch vibration peaks.

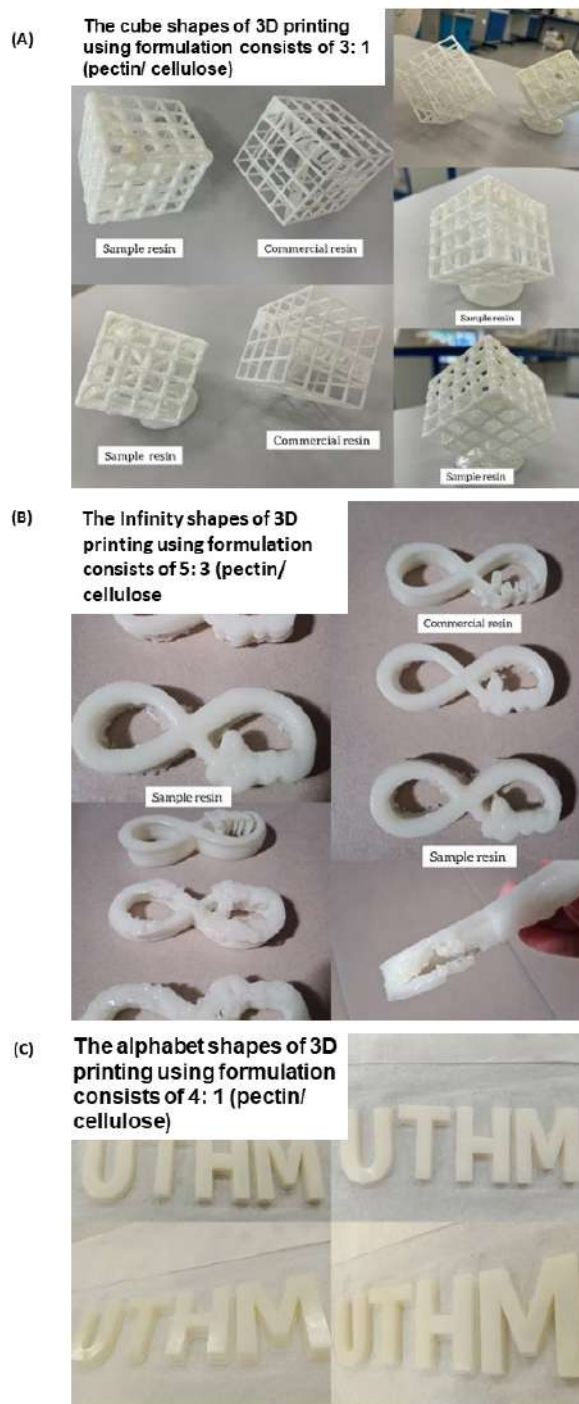


Figure 5 : (A) The cube shapes of 3D printing using formulation consists of 3: 1 (pectin/ cellulose) (B) The Infinity shapes of 3D printing using formulation consists of 5: 3 (pectin/ cellulose) (C) The alphabet shapes of 3D printing using formulation consists of 4: 1 (pectin/ cellulose)

The strong peaks at 1456 cm^{-1} and 1374 cm^{-1} represents CH_2 and $-\text{OH}$ bending vibration peaks respectively. The peaks between 1706 to 1770 cm^{-1} represents carboxymethyl group ($-\text{COOCH}_3$). The strong peaks of 1234 cm^{-1} and 1243 cm^{-1} represents the C-O stretching vibration peak in the hydrogel. The peaks may have shifted as result of the hydrogel's considerable crosslinking by Ca^{2+} ions.

Formation of 3D shapes using developed hydrogel

In Figure 6(A), the resultant shape resembles an impermeable hydrogel, while in Figure 6(B), several of the shapes are fractured. This was possibly due to the high concentration of ascorbic acid in the resin formulation. For Figure 6(C), the alphabet shapes were uniform due to the use of a straightforward shape relative to others. Moreover, hydrochloric acid was a powerful acid with a high ionic strength, using it in this study to extract pectin would result in a larger pectin yield. Due to their strong attraction for cations like Ca^{2+} , which stabilized pectin molecules, acids with higher ionic strengths have a propensity to precipitate pectin more quickly. As a result, a significant amount of pectin may precipitate out given enough time. In comparison to other waste, the durian rind was larger and contains elements that were more closely connected together, including 85 % total carbohydrates, 27.81 % crude fibre, and 73.45 % cellulose. The hydrogel can tolerate freezing when glycerol was added because of the strong cooperative hydrogen bonding that can occur between the water and the polymeric network (25).

DISCUSSION

In this study, pectin and cellulose composites have been created to replicate the structure of plant cell walls and the networks of pectin/cellulose were linked by either CaCl_2 , sodium nitrate or reducing agent, ascorbic acid. Koosha et al. (26) claims that viscosity of the solution at various pH levels was caused by the molecular cross-linking of hydrocolloids with calcium ions, and that the addition of salt minimizes this viscosity. Depending on the CaCl_2 concentration, the viscosity of the edible ink formulation will also change. When calcium ions were present in the presence of pectin, synergistic impact would be identified, resulting in increased rheological properties of the composites. The pectin network help to strengthen the pectin/cellulose composite gel. This was important for the dependability and reproducibility of 3D-printed structures. Printability was affected by ink concentration and molecular weight, which were mostly dictated by shear. Low-viscosity inks can improve printing quality while also making it easier to remove an unreacted component. It was important to understand how ink properties such as (viscosity, cross-linking kinetics, and oxygen inhibition) and practical parameters such as (intensity and irradiation

wavelength) affect printing resolution (25-27).

Rheology was useful for polymer characterization because of its ability to detect even minute changes in polymer structures. The relationship between rheology and structure was critical for the development of new materials. This was because the procedure was sensitive to the polymer structure, the material must be kept within tight tolerances to ensure optimal processability. Rheology was an excellent tool for process and quality control. The relationship between structure and rheology was ambiguous because rheological behaviour does not accurately reflect the polymer structure in complex materials. Additional measurements must be coupled with the rheological data in this case to account for contributions from other structure elements. It was critical to use techniques like as optical, thermal, dielectric, and others in conjunction with rheological data (28).

The intensity of the absorption band is one of the important variables that can be investigated using FTIR in hydrogels. The OH bond of an alcohol group usually has absorption in the range of $3200\text{--}3600\text{ cm}^{-1}$, while the OH bond of the carboxylic acid group occurs at about $2500\text{--}3300\text{ cm}^{-1}$. In the present study, the FTIR spectrum of hydrogel with pectin/cellulose ratios of 3:2, 4:1, and 5:3 reveals a decrease in the intensity of the $-\text{OH}$ stretching vibration peak at $3600\text{--}3200\text{ cm}^{-1}$ and much broader shape compared to the spectrum of hydrogel with a pectin/cellulose ratio of 3:1. This may be due to the formation of intramolecular and intermolecular hydrogen bonds and $-\text{OH}$ stretching (29,30). It is hypothesized that the crosslinking of low methyl pectin and CMC with ascorbic acid and sodium nitrate disrupted the intramolecular hydrogen bonds of purified components, resulting in structural rearrangement. Less intensity also reflects the decrement of the crystalline nature of the hydrogel (31). The result is consistent with the findings of Tuan Mohamood et al. (32) and Sdrobis et al. (33), who demonstrated that the hydrogen bonding parameter is the most significant factor in the swelling of cellulose fiber. Sdrobis et al. (33) stated that a decrease of the H-bond energy after swelling in higher alkaline solutions shows that the structural order has been disturbed. Alternatively, in a 3:1 pectin/ cellulose formulation, no additional peak can be observed in the hydrogel. This could be due to the fact that crosslinking only involved Ca^{2+} from CaCl_2 as the crosslinking agent. However, blending CMC with low methyl pectin and CaCl_2 caused minimal shifting but increased absorption in the $-\text{OH}$ stretching. The $-\text{OH}$ band of 3:1 pectin/ cellulose has shifted to lower wavenumber at 3254 cm^{-1} expressing complexation with CaCl_2 salt and is primarily responsible for self-associated hydrogen bonding of neat polymers. This result was correlated with FTIR spectra of cassava

starch CMC films by Tongdeesontorn et al. (29). and Seslija et al. (34).

On the other hand, small weight losses of formulation pectin/ cellulose ratio of 3:1, 3:2, 4:1, and 5:3 hydrogels were observed at temperatures of 63.91 °C, 42.76 °C and 68.46 °C, as depicted by TGA thermograms. It is important to note that initial moisture discharge is typical of natural fibers. According to Mohtar et al. (35), any degradation or decomposition occurring at temperatures below 100 °C is not regarded as a significant thermal event in the TGA study. The second stage of decomposition was the most important for all four samples. The higher temperature at which degradation begins may be associated with the more tightly packed chains, which are more resistant to degradation. As mentioned by Khalid et al. (36), it is important to note that CMC is less susceptible to degradation than pectin; therefore, the inclusion of CMC increases the thermal stability of hydrogel even further.

In addition, only one major degradation profile can be detected in pectin/CMC hydrogels, which occurs between 200 °C and 315 °C. Strong physical interactions between the polar groups of pectin and CMC are confirmed by the presence of a single major degradation peak. Formulation pectin/cellulose of 3:1 ratio has the highest thermal stability, with thermal degradation beginning at 220.43 °C. Consequently, based on the TGA analysis, the investigated low methyl pectin/CMC hydrogels have sufficient thermal stability for use in pharmaceutical application. This result is consistent with the previous research conducted by Jantrawut et al. (37). Akar et al. (38) stated that the second stage of CMC decomposition may be due to the degradation of the cellulose backbone and the elimination of CO₂ due to decarboxylation. In their thermal study, found that the inclusion of Ca²⁺ ions into the CMC structure through ionic crosslinking, which made the CMC hydrogel backbone more stable. Seki et al. (39), in their study on different crosslinking reagents of CMC/HEC hydrogel, stated that the crosslinked CMC hydrogel has better T_{max} (temperature at which maximum weight loss occur) than CMC, which delayed thermal decomposition.

CONCLUSION

In conclusion, the new bioink was created by combining pectin from durian rinds and carboxymethylcellulose. The incorporation of pectin/cellulose with CaCl₂, glycerol, ascorbic acid, sodium nitrate and epoxy were help in terms of producing strong and rigid 3D structure. In addition, pectin with cellulose formulations can be used to as bioink to their ability and structural characteristics from viscoelastic properties, thermal stability and

compound determination. All formulations using pectin from durian rinds and carboxymethyl cellulose shows a decrease in viscosity with the increase of shear rate and the yield stress for all formulation were increases with the increasing of shear rate. This was referred to as shear thinning. Their deposition recovery time was reasonable, and their viscosity reduces as yield stress increases. In term of thermal stability, 3 : 2 (pectin/ cellulose), 4 : 1 (pectin/ cellulose) and 5 : 3 (pectin/ cellulose) respectively have lower thermal stability with the residual weight less than 20.00 % compare to other formulations. However, 3 : 2 (pectin/ cellulose) and 4 : 1 (pectin/ cellulose) formulations respectively show the higher potential as bioink because of their neat and smooth of 3D printing shape. Hence 3 : 2 (pectin/ cellulose) formulation enhances the properties of hydrogel. For future researcher, bioink may only be used for 24 hours at a time, hence the formulations for each characterization method must be made in little quantities in order to obtain reliable results. A substitute technique called differential scanning calorimetry (DSC) and scanning electron microscopy (SEM) can be used to test the formulation.

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