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The Development of a Pectin-Based Food Ink from Locally Sourced Durian Rind Waste for Possible Use as a 3D Printable Food Material

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ABSTRACT

Durian (*Durio zibethinus*) is a popular seasonal fruit in Southeast Asia. Pectin can be extracted from the rind. Pectin is an excellent source of fibre and is available in two forms: high and low methoxyl pectin. Both of these types of substances can be employed as gelling agents. As such, the purpose of this research is to partially characterise durian pectin in order to facilitate the development of a pectin-based edible ink formulation. Four formulations of pectin-based food ink were developed and evaluated using a rheometer to determine the viscoelastic properties, a Fourier transform infrared (FTIR) to determine the chemical functional groups available, and thermogravimetric analysis to determine the thermal stability using durian rind waste pectin and commercial pectin. The results indicated that durian pectin contains a low amount of methoxyl (LM) at 2.48 ± 0.31%, which is appropriate for the development of food ink, whereas commercial pectin has a high methoxyl (HM) content of 28.72 ± 0.47%. With viscosities of 31759.20 Pa/s and 7482.62 Pa/s, formulations 3 and 4 of LM pectin exhibited the highest viscoelastic properties. The third and fourth formulations of HM pectin, as well as the third and fourth formulations of LM pectin, contain components that include the alcohol, carbonyl compound, and carbonyl group are found in both pectin granules. The LM pectin formulation 2 offers the highest thermal stability (32.00% residual weight) and the lowest weight loss percentage (57.15%). Due to its capacity to form gel, the LM result demonstrated potential for use in the formulation of edible ink. Additionally, it can be used as a polymeric crosslinker in conjunction with other materials.

Keywords: Durian rind waste, food ink, HM pectin, LM pectin, physicochemical properties

INTRODUCTION

Three-dimensional (3D) food printing helps in the creation of food by depositing the food material following computer-aided designs. Food materials are anything that is in a paste or semi-liquid form or could be transformed into the right consistency for 3D food printing. There has been a revolution in food materials for 3D printing throughout the years, particularly in extrusion techniques, which they dubbed "food ink." Food ink can be classified as natively extrudable and non-natively extrudable. Natively extrudable food inks are inks with favorable viscoelastic properties that go through direct extrusion without the addition of any matrix ingredients such as gum or binder; nonetheless, additives were employed to increase print quality. Confectionery ink, dairy ink, and edible hydrogel ink are examples of natively extrudable food ink, whereas non-natively extrudable food ink is derived from plants and meat. In other word, 3D printability refers to the structure of the material that enables it to be treated and deposited while retaining its deposition (Feng *et al*., 2019). As can be seen, printability is material-dependent, but additive manufacturing is also material-dependent. In the case of 3D food printing, the 3D structure (as created by 3D food printing) may require post-processing to make it edible. Food materials that must be baked or cooked should possess properties that withstand post-treatment. They emphasized that by manipulating the rheological properties of materials, structural and mechanical properties, as well as post-processing, printability, applicability, and precision during 3D food

printing can be achieved. As a result, it is critical to comprehend the nature of food materials and the fundamental components of foods.

Recent research has emphasised the development of more complex inks that incorporate multiple hydrocolloids and conventional ingredients that have not been used in previous 3D printing tests, such as the food ink formulation of pectin gel, calcium chloride ($CaCl_2$), sugar syrup, bovine serum albumin (BSA), and β-cyclodextrin (Siew *et al*., 2019).

The primary concern in developing food ink is that the materials used in the formulation must be extrudable, flowable against stress or strain, and extremely stable in order to produce the geometrical shape design after the 3D printing process. The purpose of this research is to partially characterise durian pectin in order to aid in the development of an edible ink formulation based on pectin. The study's use of pectin concentration shown that increasing the mechanical strength and viability of the 3D food printing object were likewise dependent on the pectin concentration. The addition of pectin and sugar concentrations to this formulation shown that they have an effect on the build quality of the printed project by affecting the viscosity of the food ink. Meanwhile, the addition of BSA will stabilise and promote the porosity of the gel (Valerie *et al*., 2017). The addition of $CaCl_2$ demonstrates a considerable increase in the viscosity of the edible ink solution. The inclusion of β-cyclodextrin may aid in the synthesis of photosensitive hydrogels (Wenhui *et al*., 2020). Low methoxyl (LM) pectin might be recommended as a component suitable for food ink manufacturing due to its highly solidified gel feature.

MATERIALS AND METHODS

Materials

Durian rinds were obtained from a fruit stall at Pontian, Johor. 1 N of hydrochloric acid (HCl) (Qrec, Malaysia), absolute ethanol (Hmbg, Malaysia), 95% ethanol (Hmbg, Malaysia), 0.1 N sodium hydroxide (NaOH) (Merck, German), sodium chloride (NaCl) (Merck, German), phenol red indicator (Denver-hill), $CaCl_2$ (Qrec, Malaysia), BSA (Sigma-Aldrich, United States), β-cyclodextrin (FUJIFILM, Japan) and consumer grade sugar syrup (Monin, France).

Extraction of Pectin

The extraction of pectin technique was previously mentioned by Wong *et al*. (2009). The powdered durian rind (104.78 g) was stirred in a mild acid aqueous solution in a 1:9 (w/v) ratio. The aqueous solution was made by adjusting the pH to 2.5 using 1 N HCl. The mixed solution was then incubated for 4 hours at 85 °C in a water bath. After that, the slurries formed were filtered through cheesecloth and allowed to cool 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 at room temperature for one hour. After that, it was centrifuged in a bench-top centrifuge (Kubota 5100, Fujioka, Japan) for 15 minutes at the speed of 4700 rpm. The solution was washed twice by using 95% ethanol with a ratio of 1:2 and centrifuged again for 15 minutes at the same speed. Then, the precipitate was filtered, collected and dried in an oven at 55 ℃ until constant weight was achieved (Voragen *et al*., 1995).

Methoxyl Content of Pectin

An amount of 0.1 g of pectin from durian rind was weighed into a 100 mL conical flask. It was dissolved with 1 mL of absolute ethanol. After that, 0.2 g of NaCl was added to the solution with 20 mL of distilled water, and six drops of phenol red indicator were added to the conical flask. The dried pectin must be fully dissolved in the solution. The solution was titrated with 0.1 N NaOH until noticing the colour changes that approximately occurred at pH 7.5. Then, 5 mL of 0.25 N NaOH was added to the solution and vigorously mixed. The solution was left at room temperature for about 30 minutes. After 30 minutes, 5 mL of 0.25 N HCl was added into the solution and then titrated with 0.1 N NaOH until the pink or purple colour appeared. The colour change of the indicator must be able to persist for at least 30 s (Sze *et al*., 2021). The methoxyl content of the pectin was determined and calculated as shown in Eq. (1),

$$\text{Methoxyl content (\%)} = \frac{(\text{mL of Alkali} \times \text{Normality of alkali} \times 3.1)}{\text{weight of sample}} \quad \text{Eq. (1)}$$

Production of Food Ink

Food ink was produced using five different formulations varying in the type of pectin and amount of sugar syrup, BSA, $CaCl_2$ and β-cyclodextrin. The control sample was prepared with pectin powder and calcium chloride, while the other four food ink formulations were made with pectin powder and calcium chloride plus sugar syrup, BSA, $CaCl_2$ and β-cyclodextrin. Table 1 shows the adapted and slightly modified formulation of each ingredient used (Aburto *et al*., 2015).

Table 1. Formulation of control, 1, 2,3 and 4 food ink

Ingredients	Control	1	2	3	4
Pectin* (g/L)	15	15	15	15	15
$CaCl_2$ (m/M)	12	17	12	12	12
Sugar Syrup %(v/v)	0	0	0	50	50
BSA (g/L)	0	0	5	5	5
β- cyclodextrin (% w/v)	0	1.7	0	0	1.7

*Either LM or HM pectin

Viscoelastic Properties of Food Ink

Prior to printing, the dynamic viscoelastic properties of food-inks were determined using a stress-controlled MCR501 rheometer with Direct Strain option (Mecomb Malaysia Sdn Bhd) and a cone-plate system with a diameter of 20 mm and a cone angle of 2°. The temperature was maintained at 25 °C throughout. The cone plate system was rinsed with distilled water before being used. The stage on which to place the formulations was also washed with distilled water. Following that, the formulation was placed on the stage and the machine was started. This machine will create a graph of viscosity vs shear rate. After the machine has completed running the formulations, the stage and cone plate will be thoroughly cleaned with distilled water before running the new formulation. This procedure was repeated with all prepared formulations. From the graph, the relationship between viscosity and shear rate may be deduced (Valerie *et al*., 2017).

Identification of Chemical Functional Groups in the Food Ink

The Fourier transform infrared (FTIR) spectrum was used to confirm the chemical functional groups available in the edible ink formulations. A 2 mL sample of the food inks formulations was placed on the small, thin disc which was then inserted into the FTIR instrument. The spectrum's resolution was adjusted to 4 cm^{-1}, and the recorded wavenumbers ranged between 450 cm^{-1} and 4000 cm^{-1} (Eleftherios *et al*., 2020).

Thermal Stability of Food Ink

The thermal stability of the food ink was tested via thermogravimetric analysis (TGA). TGA was performed on the TGA module of the Q5000 series thermal analysis equipment (TA Instruments, West Sussex, UK). The quantity for formulation food ink of between 0.5 and 5 mg was placed in an open pan which is platinum 100 μL attached to a microbalance. The food ink must be properly dried by utilising a hot air oven before undergoing thermal analysis. The samples were heated at 20 °C/min in the standard mode with a ramp test from 25 ℃ to 500 ℃ with a flow rate of 10 mL/min under dry nitrogen (Muhammad *et al*., 2018).

RESULTS AND DISCUSSION

Methoxyl Content of Commercial Pectin and Durian Rind Pectin

According to Table 2, it is shown that the reading methoxyl content of pectin extracted from durian rind and commercial pectin derived from citrus is 2.48 ± 0.31% and 28.72 ± 0.47%. According to Rury *et al*. (2017), pectin with a methoxyl content ranging from 2.50% to 7.12% is categorised as LM pectin, whereas methoxyl content greater than 7.2% is categorised as HM pectin. Therefore, commercial pectin derived from citrus is classified as HM pectin whereas pectin derived from durian rind is classified as LM pectin.

According to Agarwal *et al*. (2021), four key attributes to consider when formulating pectin-based inks for 3D printing applications were summarised. 3D printing of pectin-based inks is influenced by factors such as degree of methylation (DM) value, crosslinking technique, rheological behavior, and chemical structure of the backbone. Various crosslinking procedures can be used depending on the DM value, which will affect the shape fidelity and stability of 3D printed objects. Pectin solutions can be solidified by divalent ions (the most common), positively charged biopolymers (polyelectrolyte complex), hydrogen bonding, and hydrophobic interactions in combination with a high sugar content, depending on the DM. The rheological properties of the inks are critical for optimal extrusion and shape retention. Other than that, it is possible to improve specific elements of the printed product, such as mechanical or cell-adhesion capabilities, as well as create new crosslinking techniques, such as UV polymerization, by inserting specified moieties.

Viscoelastic Properties of the Food Ink

From figures 1 & 2, the trends for all of the formulations using LM and HM pectin show a decrease in viscosity with the increase of the shear rate. The decrease in viscosity is due to the effect of the materials used in the formulation, for example $CaCl_2$, β-cyclodextrin, bovine serum albumin and sugar syrup. The different concentration of the $CaCl_2$ also affects the viscosity of the food ink. From all of the formulations for both HM and LM pectin, the graph first showed the viscosity was at its highest point, and then it decreased and continued stably which shows shear-thinning behaviour in all complexes. This result is consistent with the study by Sarraf *et al*. (2021), which found that the rheological properties of hydrocolloids were influenced by pH and salt concentration. In these cases, the polymer molecule chains are arbitrarily positioned when the sample solutions are at rest, but are aligned in the same flow direction when shear forces are applied. In shear-thinning fluids, hydrodynamic forces distort aggregates, which finally disintegrate, resulting in decreased viscosity.

The differences between the rheometer graph of the formulation using HM pectin and LM pectin are that the viscosity of the formulation for LM pectin is higher compared to the HM pectin. The viscosity of the LM pectin starts at the higher points compared to the HM pectin in all formulations. This is mainly due to the different types of pectin and the presence of galacturonic acid. Pectin concentration affects the viscosity of food-ink mostly through changing the density of the polymer network, which slows intermolecular movement in gel and network crosslinking by calcium ion (Ca^{2+}) (Diana *et al*., 2018). From this, it can be concluded that the viscosity of LM pectin is higher compare to the viscosity of HM pectin formulations. The higher viscosity will give a better result for the food ink for 3D food printing. For LM pectin, the viscosity shown by formulations 3 and 4 in figures 3 and 4 is higher compared to other formulations with the viscosities of 31759.2 Pa/s and 7482.62 Pa/s which make them the suitable candidates for the best potential food ink for 3D printing (Valerie *et al*., 2017).

Table 2. Methoxyl content of durian rind and commercial pectin

Sample	Reading	Volume of NaOH (ml)	Methoxyl content	Average ±SD
Commercial pectin	1	9.10	28.21	28.72 ± 0.47%
	2	9.40	29.14	
	3	9.30	28.83	
Durian rind	1	0.90	2.79	2.48 ± 0.31%
	2	0.80	2.48	
	3	0.70	2.17	

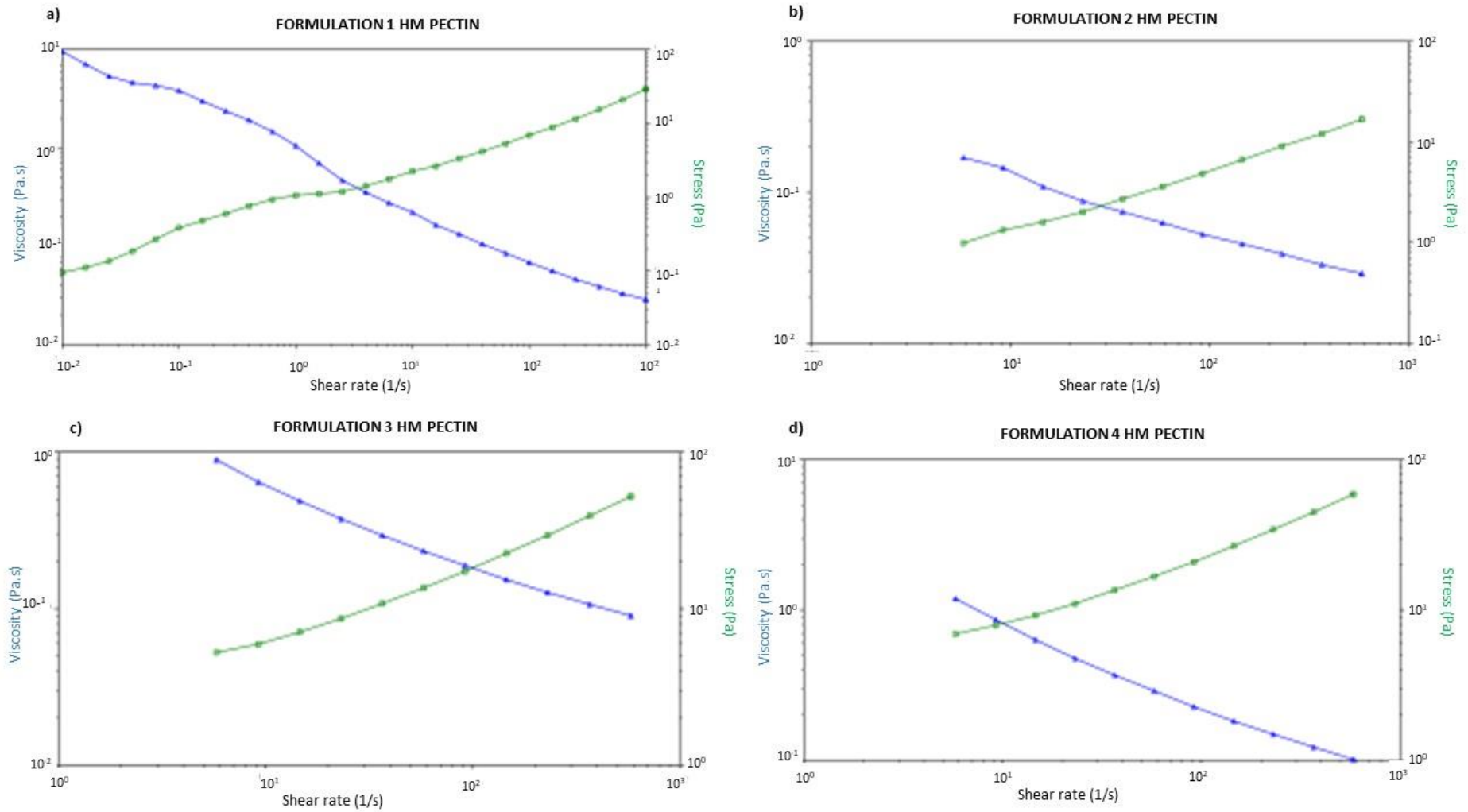

Figure 1. The viscosity of formulations (a), (b), (c) and (d) for HM pectin decreased with increasing shear rate

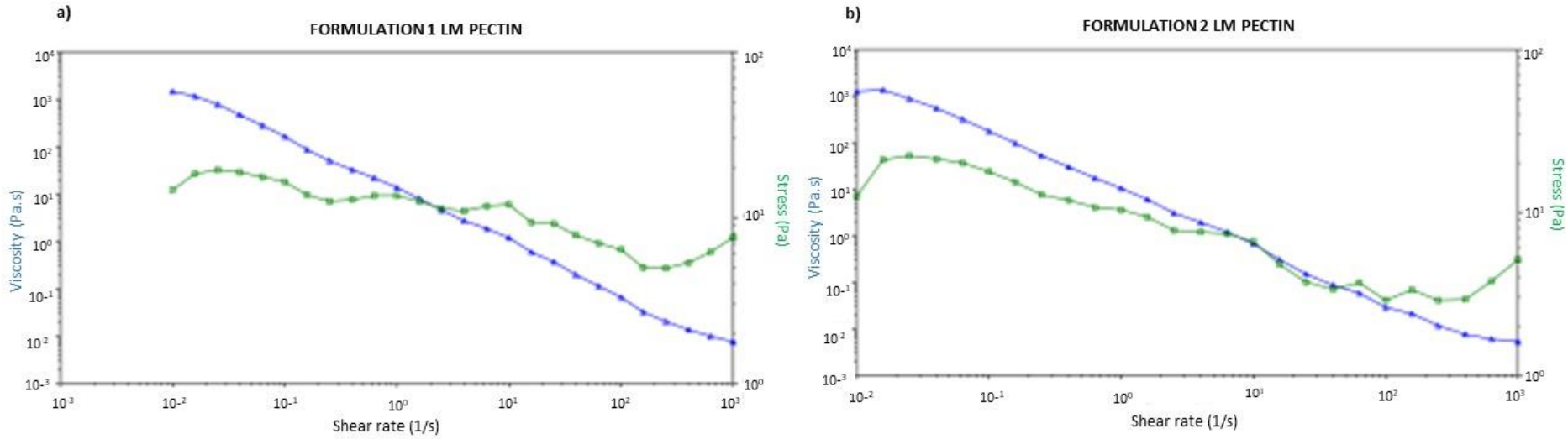

Figure 2. The viscosity of formulations (a) and (b) for LM pectin decreased with increasing shear rate

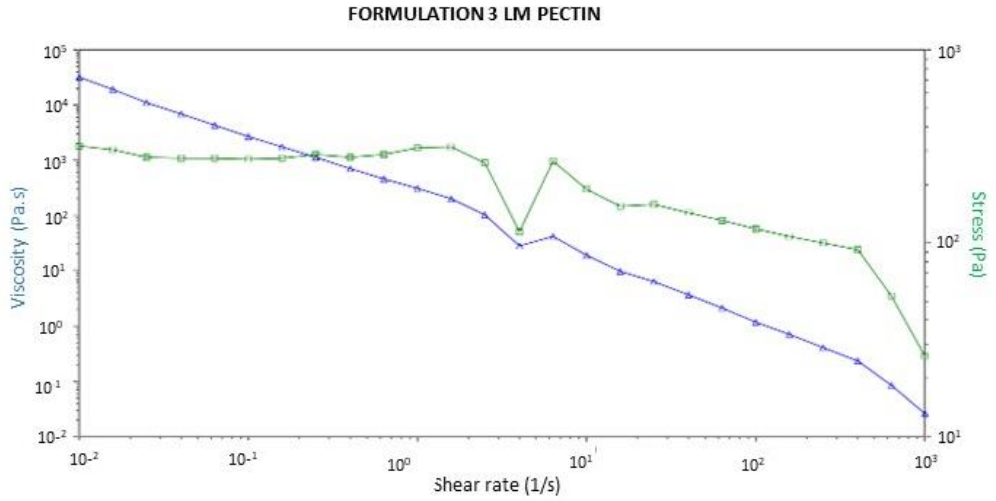

Figure 3. Viscosity versus shear rate formulation 3 LM pectin

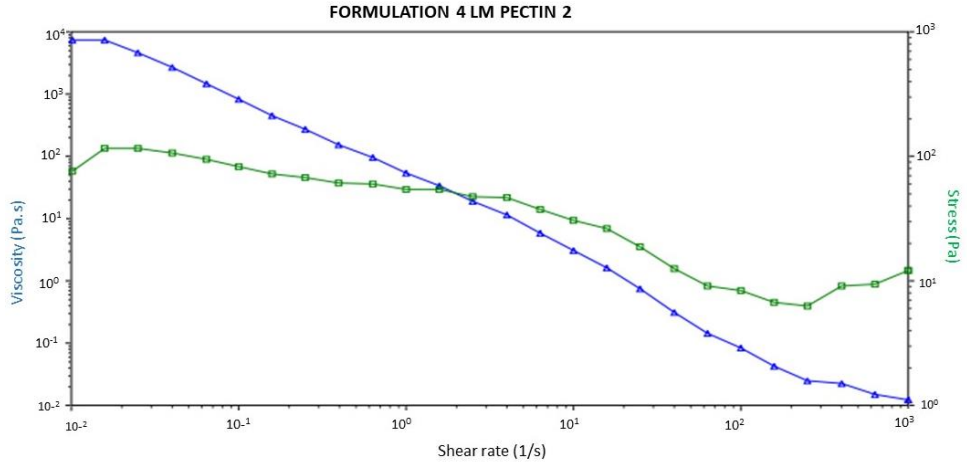

Figure 4. Viscosity versus shear rate formulation 4 LM pectin

Identification of Chemical Functional Groups in the Food Ink

The characteristics of the pectin bands can be found in the region regarding C-H stretching for the carbohydrate backbone. The sample of pectin is also mainly characterised by the wavenumber of 1145 cm^{-1}, 1105 cm^{-1}, 1014 cm^{-1}, and 952 cm^{-1}, where the 1105 cm^{-1} and 1014 cm^{-1} bands are diagnostic wavenumbers of pectic polysaccharides rich in uronic acid (Carolina *et al*., 2009), mode with ramp test (Muhammad *et al*., 2018).

The FTIR of HM pectin powder is shown in Table 3. The absorption of the spectrum starts with the absorption at 3600 cm^{-1} to 3100 cm^{-1} causing IR absorption of the hydrogen-bonded O-H stretch. Next, the band position was at 2850 cm^{-1} to 2960 cm^{-1} with medium to strong intensity for functional group class alkanes and alkyl group C-H. The band position is at 1670 to 1780 cm^{-1} with a strong intensity which represents the functional group class C=O carbonyl compound. The range of 1101.87 cm^{-1} suggests the stretching pectin is presented in the FTIR graph and the corresponding vibration of alcohols, carboxylic acid and esters. For control, formulation 1 and formulation 2 of HM pectin, the absorption of the spectrum starts with the absorption of 1730 cm^{-1} to 1650 cm^{-1} causing the IR absorption of C=O stretch which form functional groups of

Table 3. The compound found in HM and formulation food ink

Sample	Functional group class	Band Position (cm^{-1})	Intensity
HM pectin powder	Alcohol, hydrogen-bonded O-H stretch	3600-3100	Strong, broad
	Alkanes and alkyl group, C-H	2850 - 2960	Medium to strong intensity
	Carbonyl compound, C=O	1670 - 1780	Strong intensity
Control (HM pectin), 1 (HM pectin), 2 (HM pectin).	Alcohol, hydrogen-bonded O-H stretch	3600-3100	Medium to strong intensity
	Carbonyl compound, C=O	1750-1625	Strong intensity
3 (HM pectin), 4 (HM pectin).	Alcohol, hydrogen-bonded O-H stretch	3600-3100	Medium to strong intensity
	Carboxylic acid with C=O stretch	1730-1650	Strong intensity
	Ester or ether C-O bond	1300-1000	Strong intensity

carboxylic acid. Next, the absorption of the spectrum is the absorption at 3600 cm^{-1} to 3100 cm^{-1} causing IR absorption of the hydrogen-bonded O-H stretch. The absorption of the spectrum is 1300 cm^{-1} to 1000 cm^{-1} where the functional group is ester or ether that causes the IR absorption of the C-O bond. For formulation 3 and 4 HM pectin, the absorption of the spectrum starts with the absorption of the spectrum is 1300 cm^{-1} to 1000 cm^{-1} where the functional group is ester or ether that causes the IR absorption of the C-O bond. Next, the absorption of the spectrum is the absorption at 3600 cm^{-1} to 3100 cm^{-1} causing IR absorption of the hydrogen-bonded O-H stretch. The other peaks were observed in the 1750 cm^{-1} to 1625 cm^{-1} range for ketone group adsorption of C=O.

Table 4. The compound found in LM and formulation food ink

Sample	Functional group class	Band Position (cm^{-1})	Intensity
LM pectin powder	Alcohol, hydrogen-bonded O-H stretch	3600-3100	Strong, broad
	Alkanes and alkyl group, C-H	2850 - 2960	Medium to strong intensity
	Carbonyl compound, C=O	1670 - 1780	Strong intensity
	Alcohol, carbonyl group C-O	1050 -1150	Strong intensity
Control (LM pectin), 3 (LM pectin), 4 (LM pectin).	Alcohol, hydrogen-bonded O-H stretch	3600-3100	Medium to strong intensity
	Carbonyl compound, C=O	1750-1625	Strong intensity
	Alcohol, carbonyl group C-O	1050 -1150	Strong intensity
1 (LM pectin), 2 (LM pectin).	Alcohol, hydrogen-bonded O-H stretch	3600-3100	Medium to strong intensity
	Carboxylic acid with C=O stretch	1800–1500	Strong intensity

From Table 4, the spectrum IR absorption at the range of a broad band around 3700 cm^{-1} to 3000 cm^{-1} for LM pectin powder and all formulations, typical for polysaccharides, is attributed to the O–H stretching vibration (OH). The other absorption found is at 1050 cm^{-1} to 1150 cm^{-1} which was attributed to the stretching vibration of the carbonyl group C-O in LM pectin powder, control (LM pectin), 3 (LM pectin) and 4 (LM pectin). Regarding the spectrum of pectin formulation, two bands were detected between 1800 cm^{-1} to 1500 cm^{-1}, which were attributed to the stretching vibrations of the carbonyl group (C=O) for all formulations [6]. The band located at 1733 cm^{-1} corresponds to the C=O of the methyl ester group ($COOCH_3$) and to the non-dissociated carboxyl group (COOH), while the band located at 1624 cm^{-1} is assigned to the asymmetric stretching vibration of the carbonyl group of the carboxyl ion (COO^-). From both tables, it showed that formulations 3 and 4 for HM pectin and control, and formulation 3 and 4 for LM pectin consist of stable components compared to the other formulations. All of them can be detected as classes of alcohol, carbonyl compounds and carbonyl groups that can be found in both types of pectin powder.

Thermal Stability of the Food Ink

The increase in the temperature causes a slightly mass loss during the stage of 40 ℃ to 150 ℃. This is caused by the water evaporation in the samples. A previous study found that, as the temperature increased from 150 ℃ to 400 ℃, the pectin decomposed and the quality of the pectin dropped rapidly by more than 60%. The

galacturonic acid chain for pectin was also hyperthermally cleaved at this point, releasing the functional groups and chain breaking, which resulted in the formation of different gases and solid substances as a result of ring opening. The loss of mass became slow as the temperature approached 400 °C, probably due to additional thermal disintegration of the solid substances generated in the second stage (Aburto *et al*., 2015). The aliphatic and ketone groups were present in solid carbon, which partially disrupted and firmly aggregated the structure of grafted polyaromatic hydrocarbons.

In terms of the thermal stability, the formulations 1, 3 and 4 of HM pectin in Table 5 show that they have lower thermal stability compared to control and formulation 2 HM pectin with a residual weight of less than 30.00% compared to other formulations. Meanwhile, the thermal stability for control and formulation 2 of LM pectin in Table 6 has higher thermal stability than the residual weight of more than 30.00% and formulation 1 with less than 30.00% residual weight. As a result, it can be stated that LM pectin has greater heat resistance than HM pectin as the highest thermal stability is formulation 2 (32.00% residual weight) and the lowest percentage of weight loss (57.15%). Because the dissolved molecules in the aqueous solution are free to migrate and unfold or the chemical structure undergoes a conformation transition, the formulation preparation had a minor impact on thermal stability (Wan-ling *et al*., 2022).

Table 5. Thermal stability of control, 1, 2, 3 and 4 of HM pectin formulations

Sample	Weight loss at 400 °C, %	Residual weight, %	Temperature of steps in TGA, °C	
			Step 1	Step 2
Control	62.19	30.86	223.68	262.36
Formulation 1	72.86	21.96	274.84	343.97
Formulation 2	72.77	31.87	188.69	255.14
Formulation 3	76.87	23.18	188.15	321.63
Formulation 4	76.87	21.61	211.50	326.47

Table 6. Thermal stability of control, 1, 2, 3 and 4 of LM pectin formulations

Sample	Weight loss at 400 °C, %	Residual weight, %	Temperature of steps in TGA, °C	
			Step 1	Step 2
Control	62.65	33.33	134.91 °C	298.62 °C
Formulation 1	73.65	23.57	234.38 °C	342.74 °C
Formulation 2	57.15	34.00	187.80 °C	368.67 °C

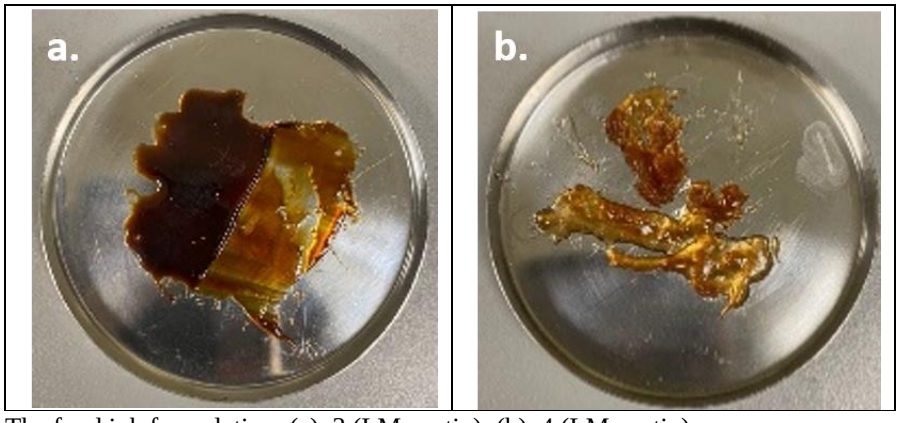

Figure 5. The food ink formulation: (a). 3 (LM pectin); (b). 4 (LM pectin)

From the observation, the similarities between formulation 3 and formulation 4 in Figure 5 are due to both using the same formulation for the amount of sugar syrup which is 22.5 mL and $CaCl_2$ at 12.5 mM. Both of these materials have contributed to the same physical properties in that they are sticky and hard to dry.

Both of the formulations are easily dissolved in water although they have a sticky structure.

According to Diana *et al*. (2018) the binding process between calcium and zinc ions with pectin in a neutral solution such as water is a result of electrostatic interaction between the charged carboxyl group of the pectin and calcium and zinc ions. Because both LM pectin and the polymer have formed a crosslink, the viscosity will increase as a result of this interaction. Moreover, the viscosity of the modified cell wall polysaccharide matrix (MPS) increases with the presence of calcium ions linked to the LM ion via the "eggbox" model. The addition of sugar syrup also contributes to the formulation's viscoelastic characteristics being improved. This is supported by the fact that LM pectin gels with a high degree of esterification are formed by the formation of calcium crosslinked with free carboxyl groups, whereas HM pectin gels with a high degree of esterification are formed by hydrophobic force and hydrogen bonds with a low pH and a high sugar content (Muhammad *et al*., 2018).

Agarwal *et al*. (2021) investigated partially crosslinked pectin inks with viscoelastic qualities and good printability (through the addition of 12.5 mM Ca^{2+} ions); nonetheless, the printed objects had a poor porosity (1.7%). When sugar syrup (50% v/v) was added to the inks, the porosity of the object increased to 3.1%, and the texture improved, compared to the results obtained with 12.5 mM Ca^{2+} ion content. BSA was used to provide the objects more porous architecture by stabilizing the air bubbles created during the stirring process. These objects exhibited porosities as high as 21.9%.

CONCLUSION

The addition of pectin to $CaCl_2$, BSA, sugar syrup, and β-cyclodextrin aided in the formation of a strong and rigid gel. Furthermore, due to their structural ability to form a gel, both HM and LM pectin can be used as food ink. Formulation 3 and 4 LM pectin have higher viscoelastic qualities, with viscosities of 31759.20 Pa/s and 7482.62 Pa/s, respectively. For determining the components, formulations 3 and 4 of HM pectin, as well as controls 3 and 4 of LM pectin, consist of stable components that can be detected as a class of alcohol, carbonyl compounds, and carbonyl groups in both pectin powders. In terms of thermal stability, 2 LM pectin has the highest thermal stability at 32.00% residual weight and the lowest weight loss percentage at 57.154%. Thus, sugar syrup, $CaCl_2$, BSA, and β-cyclodextrin can be combined with LM pectin from durian rind to enhance viscoelastic qualities while maintaining thermal stability and identifying a stable molecule.

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