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THE EFFECT OF HYDROLYZED PECTIN AS A SUGAR SUBSTITUTE ON THE PHYSICOCHEMICAL PROPERTIES OF PINEAPPLE SPREAD

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Abstract. *Pineapple is a fruit that is widely produced in Indonesia. Pineapple can be processed into jam or fruit spread to extend its shelf life. However, jam contains high amount of sugar. High consumption of sugar in the diet can contribute to a high kilojoules diet or known as 'energy dense' and thus contribute to the development of health problems like obesity or diabetes. Sugar has important role in the jam. It binds water molecules to build spreadable product. Pectin can also bind water molecules and cannot be digested by human body but addition of pectin as sugar replacement cannot build spreadable product because pectin molecules is much longer than sucrose. Thus, depolymerization through hydrolysis is needed on pectin molecule. Therefore, the purpose of this research was to determine the effect of different hydrolyzed pectins as substitute for sucrose in a pineapple spread. The physicochemical properties of hydrolyzed pectin (viscosity and color value) and pineapple spreads made from the hydrolyzed pectin (color value, degree of brix, water activity, syneresis and spreadability) were investigated. The results showed that pectin treated with 0.05 M of HCl for 96 h produced the best pineapple spreads based on water activity and percentage of syneresis results. Moreover, pineapple spreads made from pectin treated with 0.05 M of HCl for 96 h has the most similar color and spreadability to the one with sucrose.*
Keywords: *fruit spreads; hydrolyzed pectin; pineapple; sugar substitute*

1. Introduction

Food preferences have changed considerably in the last decade, with high-nutritive-value foods and bioactive substances becoming increasingly popular (Dubey *et al*., 2021). Pineapple is a tropical fruit that is high in vitamin C and manganese, among other nutrients. In 2019, over 26 million tons of pineapples were produced globally (Hadidi *et al*., 2020). However, more than 30% are lost during and after they leave the farm gate, resulting in significant annual economic losses (Dereje & Abera, 2020). Besides that, pineapple is also easy to perish due to its high moisture content. Hence, pineapples must be processed into shelf-stable products such as jam to reduce postharvest losses and extend shelf life.

Jams are high-sugar-content fruit preserves. These are made by heating fruit pulp, sugar, pectin, acid, and other ingredients (preservatives, coloring, and flavoring agents) to a thick gel consistency (Belović *et al*., 2017). However, in recent years, rising health concerns, as well as a larger incidence of diabetes and obesity, have increased the demand for reduced or no-sugar-added products, encouraging producers to develop healthier alternatives to sugar-based foods using

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artificial sweeteners (Peinado *et al.*, 2012). Sugar has an important role in the jam. It binds water molecule to build matrix together with pectin into spreadable product and made water unavailable for microbial growth (Belkacem *et al.*, 2021). Replacing sugar with artificial sweeteners can give a sweet taste to pineapple jam but cannot produce spreadable product. Therefore, short chain hygroscopic molecules can be used as fillers to make jam without sugar (fruit spread).

Pectin is a high molecular weight polysaccharide, found as a component of the primary cell wall and middle lamella of fruits and vegetables, and is commonly found in conjunction with cellulose, hemicellulose, and lignin (Adetunji *et al.*, 2017). Pectin consists of three polysaccharide types, which are homogalacturonan, rhamnogalacturonan I and rhamnogalacturonan II. Homogalacturonan represents the backbone chain of the pectin, containing α-1, 4-linked D-galacturonic acid units, which cannot be depolymerized by human body's enzyme (Chen *et al.*, 2015). Pectin can be depolymerized by either acid, enzymatic hydrolysis, β-elimination or mechanical degradation into smaller molecules which can act as sugar replacement in the food product (Chen *et al.*, 2015), while acid hydrolysis is the simplest and cheapest way to produce depolymerized pectin (oligogalacturonides) (Locatelli *et al.*, 2019). Thus, in this study, different hydrolyzed pectins are studied for their potential as sugar replacement in pineapple spreads.

2. Methods

2.1. Preparation of hydrolyzed pectin

The production of hydrolyzed pectin refers to the method used by Garna *et al.* (2006) with slight modifications. Pectin (100 g) was mixed with 1 L of 0.025 M or 0.05 M HCl. The solutions were incubated for 24, 48, 72, and 96 h at 80°C and neutralized with NaOH 0.025M and 0.05M to reach a pH level of 6. The solutions were then dried for 10 h in the convection oven at 85°C, cooled to room temperature, and powdered using grinder. Produced pectin powder was washed three times using 80% ethanol solution then centrifuged at 3,500 rpm for 10 min. The supernatant was taken and dried using convection oven at 90°C for 4 h.

2.2. Preparation of pineapple spread

Pineapple spread production was made referring to the method used by Chauhan *et al.* (2013) with a few modifications. The pineapple was peeled up and blended until it became a pulp. Pectin and citric acid were mixed until a pH of 3.1 – 3.5 is obtained. Spread was made from 9 different treatments. The first spread was given sucrose as a control, and the second spread was given hydrolyzed pectin from treatment with different concentrations of HCl and time as follows, 0.025 M for 24 h; 0.05 M for 24 h; 0.025 M for 48 h; 0.05 M for 48 h; 0.025 M for 72 h; 0.05 M for 72 h; 0.025 M for 72 h; 0.05 M for 72 h; 0.025 M for 96 h; and 0.05 M for 96 h. The cooking process was carried out by stringing at 80°C until all the ingredients are mixed.

2.3. Physiochemical analysis

2.3.1. Viscosity analysis

Viscosity analysis was carried out by referring to Garna *et al.* (2006). In this study, viscosity testing was carried out on hydrolyzed pectin using a digital Brookfield viscometer (DVEERVTJ0 Brookfield, America). Before testing, hydrolyzed pectin solutions were cooled down until it reached 30°C. Viscosity testing was carried out before acid hydrolysis and after acid hydrolysis.

2.3.2. Color analysis

Color analysis was carried out by referring to the method used by Smith (2014). Color measurements were carried out using the CIELAB method (L*, a*, b*) using a chromameter (NH310 3nh, China). Color was measured 3 times with different points from one another.

2.3.3. Spreadability

The spreadability test was carried out by taking 1 teaspoon of each spread and then smearing the spread on the surface of the white bread. Furthermore, the spread that has been tested for its spreadability will be given the following values: bad (+), poor (++), fair (+++), good (++++), and excellent (+++++) following sensory evaluation principle described by Meilgaard (2007).

2.3.4. Degree of Brix and water activity analysis

The ºBrix was determined using an ATAGO refractometer. The water activity was determined using AQUALAB4 QTE aw meter.

2.3.5. Syneresis

Syneresis was measured using Whatman paper. Samples were left in the refrigerator for 24 h, then the water on the surface was absorbed using Whatman paper and weight using an analytical balance following method described by Dipowaseso *et al.* (2018) with modification.

2.4. Statistical analysis

All analyses were done in triplicate and reported as mean±SD. Data within each group were analyzed by one-way ANOVA followed by the Duncan test using SPSS software. $P \leq 0.05$ was considered to indicate a statistically significant difference.

3. Results and Discussion

3.1. Effect of acid hydrolysis on the physicochemical properties of pectin

3.1.1. Effect of acid hydrolysis on viscosity and pH

The effect of hydrolyzed pectin on its viscosity and pH were shown in Table 1. Results showed that an increase in acid concentration and incubation time decreased the viscosity and pH significantly. Acid hydrolysis will cause the glycosidic bond in pectin break, resulting a shorter molecules which make viscosity decrease (Sandhu *et al.*, 2007). The acid will accelerate the hydrolysis process, which allows the number of electrons to be attacked by free electron pairs present in oxygen in the C-O-C from glycosidic and resulting in oxygen with a positive charge (Mosier *et al*., 1999). Oxygen with a positive charge will take electrons from hydrogen and release free protons as a catalyst. This whole process breaks the glycosidic linkages and leaves carbohydrate monomers. The finding in this study is also supported by Nikolic and Mojovic (2007) that reported hydrolysis of pectin reduced the viscosity of pectin.

Table 1. Effect of hydrolysis on viscosity and pH of pectin.

Sample	Viscosity (cP)	pH before hydrolysis	pH after hydrolysis
0.025 M for 24 h	2117.67 ± 0.00^{e}	3.49 ± 0.03^{b}	2.58 ± 0.01^{f}
0.05 M for 24 h	1798.67 ± 4.43^{a}	2.10 ± 0.01^{a}	2.05 ± 0.01^{b}
0.025 M for 48 h	2302.83± 5.78^{f}	3.54 ± 0.01^{c}	2.54 ± 0.01^{e}
0.05 M for 48 h	1861.50 ± 2.83^{b}	2.06 ± 0.02^{a}	2.04 ± 0.01^{ab}
0.025 M for 72 h	2541.33 ± 10.11^{g}	3.8 ± 0.04^{b}	2.46 ± 0.01^{d}
0.05 M for 72 h	1892.17 ± 1.60^{c}	2.09 ± 0.01^{a}	2.04 ± 0.01^{ab}
0.025 M for 96 h	2552.50 ± 7.28^{g}	3.45 ± 0.01^{b}	2.42 ± 0.02^{c}
0.05 M for 96 h	1930.00 ± 1.97^{d}	2.10 ± 0.01^{a}	2.01 ± 0.01^{a}

$^{a-g}$Same superscript indicate there is no significant different among the data in the same column at $p<0.05$ using one way ANOVA with Duncan's Post Hoc

3.1.2. Effect of hydrolysis on color

The color analysis of hydrolyzed pectin was shown in Table 2. Results showed that hydrolyzed pectin with longer incubation time and higher HCl concentration has a darker color (browner) which represented with higher L*, a* dan b* value. In the other words, the longer the pectin hydrolyzed, the browner the color would be. This was due to the acidic environment that cut the homogalacturonan bonds into smaller monomers. It resulted in an increase in the brownish color of the product, due to the formation of furfural acid which made the caramelization reaction (Golon & Kuhnert, 2012). Acid hydrolysis will increase the amount of glucose or galactose (Garna *et al*., 2004) and when hydrolyzed pectin is dried in the oven at 80°C for 8 h, it caused a caramelization reaction because glucose and galactose are reducing sugars which allow caramelization to occur during drying. This will have an impact on the degradation of the color of hydrolyzed pectin powder (Quintas *et al*., 2007). In addition, Einhorn-Stoll and Kunzek (2009) also reported that degradation of dried pectin during storage was also followed with visible browning.

3.2. Effect of hydrolyzed pectin on the pineapple spread properties

3.2.1. Effect of hydrolyzed pectin on ºBrix degree, water activity, and syneresis

The effect of hydrolyzed pectin on ºBrix degree, water activity, and syneresis of pineapple spreads were shown in Table 3. According to the results, increased acid concentration and incubation time resulted in a significant increase in ºBrix degree and a decrease in water activity and syneresis. Compared to jam made from sucrose, jam made from pectin hydrolyzed at 0.05 M for 96 h had similar ºBrix and water activity values. This was due to the increase of shorter oligogalacturonide content with the increase in acid concentration and incubation time. The high reducing sugar will help bind water to spread so the osmotic pressure and water activity (Aw) decrease. The resulting reducing sugar also increased the number of dissolved solids in the jam which caused the ºBrix value to increase along with increasing acid concentration and incubation time (van der Sman, 2017). This finding is supported by the finding of Vilela *et al.* (2015) reporting that jam with oligosaccharide (fructose oligosaccharide) addition has higher water activity compared to jam made only with mono- and disaccharides (sucrose, fructose, and sorbitol).

Table 2. Effect of hydrolysis on color of pectin.

Sample	**L***	**a***	**b***	**c***	**H***
0.025 M, 24 h	76.62 ± 0.06[h]	5.34 ± 0.02[b]	17.98 ± 0.04[c]	18.77 ± 0.05[c]	101.63 ± 0.04[a]
0.05 M, 24 h	72.26 ± 0.13[g]	8.28 ± 0.12[e]	20.76 ± 0.25[e]	22.36 ± 0.27[e]	107.16 ± 0.04[c]
0.025 M, 48 h	72.52 ± 0.04[f]	11.26 ± 0.03[h]	27.64 ± 0.02[h]	29.85 ± 0.01[h]	110.98 ± 0.03[e]
0.05 M, 48 h	70.20 ± 0.08[e]	4.26 ± 0.03[a]	14.18 ± 0.03[b]	14.80 ± 0.01[b]	106.58 ± 0.07[b]
0.025 M, 72 h	69.24 ± 0.10[d]	7.06 ± 0.02[c]	21.32 ± 0.03[f]	22.46 ± 0.05[e]	109.89 ± 0.09[d]
0.05 M, 72 h	68.06 ± 0.03[c]	9.58 ± 0.02[f]	23.91 ± 0.12[g]	25.76 ± 0.03[f]	112.46 ± 0.06[f]
0.025 M, 96 h	63.65 ± 0.19[b]	7.53 ± 0.06[d]	18.51 ± 0.12[d]	19.98 ± 0.14_d	114.27 ± 0.16[g]
0.05 M, 96 h	57.06 ± 0.05[a]	10.24 ± 0.02[g]	24.09 ± 0.02[h]	26.18 ± 0.02[g]	122.59 ± 0.05[h]
Sucrose	88.44 ± 0.42[i]	9.46 ± 0.17[f]	-52.64 ± 0.24[a]	-51.79 ± 0.28[a]	139.34 ± 0.24[i]

[a-i]Same superscript indicate there is no significant different among the data in the same column at $p<0.05$ using one way ANOVA with Duncan's Post Hoc

Table 3. Effect of hydrolyzed pectin on ºBrix degree, water activity, and syneresis of pineapple spread.

Sample	**ºBrix**	**Aw**	**Syneresis (%)**
0.025 M for 24 h	44.50 ± 0.71[a]	0.926 ± 0.00[h]	0.286 ± 0.00[g]
0.05 M for 24 h	47.50 ± 0.71[bc]	0.917 ± 0.00[fg]	0.271 ± 0.00[g]
0.025 M for 48 h	47.00 ± 0.00[b]	0.919 ± 0.00[g]	0.254 ± 0.01[f]
0.05 M for 48 h	50.00 ± 0.00[d]	0.892 ± 0.00[d]	0.228 ± 0.01[e]
0.025 M for 72 h	48.00 ± 0.00[d]	0.914 ± 0.00[f]	0.193 ± 0.00[d]
0.05 M for 72 h	52.00 ± 0.00[e]	0.862 ± 0.00[c]	0.138 ± 0.00[b]
0.025 M for 96 h	49.50 ± 0.71[d]	0.899 ± 0.00[e]	0.156 ± 0.01[c]
0.05 M for 96 h	54.00 ± 0.00[f]	0.847 ± 0.00[a]	0.091 ± 0.00[a]
Sucrose	54.00 ± 0.00[f]	0.8506 ± 0.00[b]	8.301 ± 0.01[h]

[a-g]Same superscript indicate there is no significant different among the data in the same column at $p<0.05$ using one way ANOVA with Duncan's Post Hoc

The syneresis rate of hydrolyzed pectin is much lower than that of sucrose. This is because even though it has undergone hydrolysis, not all of the glycoside linkage in pectin molecules are cut off, so it could havehigher degree of polymerization than sucrose which resulting in higher cross-linking in the matrix formation. By increasing the degree of cross-linking, the gel will swell less and reach equilibrium at a smaller volume (Lara-Espinoza *et al.*, 2018; Mizrahi, 2010). In addition, the amount of reducing sugar released due to hydrolysis also has an important role in increasing the cross-links that occur, such as xylitol which has properties like sucrose in increasing cross-links.

3.2.2. Effect of hydrolysis on pineapple spread

The effects of different hydrolyzed pectins on the color of pineapple spreads were shown in Table 4. Results showed that spread with hydrolyzed pectin 0.05 M for 96 h became the darkest, and 0.025 M for 24 h became the lightest as shown in lower L* value, higher a* value and b* value. Meanwhile, sucrose has a darker color compared to hydrolyzed pectin. This was due to the amount of reducing sugar from hydrolyzed pectin being less than sucrose, thus affecting the caramelization reaction (Garna *et al.*, 2004). The commercial jam has a darker color when compared to the nine samples used. In addition, the commercial jam adds 60% sucrose to the total jam, whereas in the samples tested the use of sugar or sugar substitutes only 50% of the total weight so it affects the caramelization reaction during the jam cooking process (Hu *et al.*, 2016).

Table 4. Effect of hydrolyzed pectin on color of pineapple spread

Sample	**L***	**a***	**b***	**c***	**H***
0.025 M, 24 h	75.48 ± 0.16[j]	12.69 ± 0.15[a]	58.57 ± 0.50[d]	42.37 ± 0.37[de]	1.36 ± 0.00[g]
0.05 M, 24 h	72.47 ± 0.40[i]	17.02 ± 0.20[e]	60.93 ± 0.28[e]	44.74 ± 0.22[g]	1.20 ± 0.00[e]
0.025 M, 48 h	69.21 ± 0.26[h]	18.51 ± 0.48[g]	58.40 ± 0.47[d]	43.32 ± 0.25[f]	1.26 ± 0.01[c]
0.05 M, 48 h	67.20 ± 0.07[g]	17.60 ± 0.12[f]	53.75 ± 0.68[c]	39.99 ± 0.46[c]	1.25 ± 0.00[b]
0.025 M, 72 h	65.52 ± 0.19[f]	15.59 ± 0.42[d]	46.98 ± 1.52[b]	35.00 ± 1.06[b]	1.25 ± 0.01[b]
0.05 M, 72 h	63.23 ± 0.27[e]	17.39 ± 0.29[ef]	57.90 ± 0.33[d]	42.75 ± 0.17[ef]	1.28 ± 0.01[d]
0.025 M, 96 h	60.38 ± 0.14[d]	14.92 ± 0.09[c]	46.81 ± 0.20[b]	34.74 ± 0.12[b]	1.26 ± 0.00[g]
0.05 M, 96 h	57.75 ± 0.14[c]	19.53 ± 0.23[g]	56.52 ± 0.26[c]	41.38 ± 0.13[c]	1.32 ± 0.01[a]
Sucrose	55.41 ± 0.13[b]	13.61 ± 0.01[b]	57.66 ± 0.08[d]	41.89 ± 0.08[d]	1.34 ± 0.00[f]

[a-g]Same superscript indicate there is no significant different among the data in the same coloumn at $p<0.05$ using one way ANOVA with Duncan's Post Hoc

Table 5. Effect of hydrolyzed pectin on spreadability of pineapple spread

Sample	**Spreadability**
0.025 M for 24 h	+
0.05 M for 24 h	+
0.025 M for 48 h	+
0.05 M for 48 h	+
0.025 M for 72 h	+
0.05 M for 72 h	++
0.025 M for 96 h	++
0.05 M for 96 h	+++
Sucrose	+++++

+: bad, ++: poor, +++: fair, ++++: good, ++++++: excellent

3.2.3. Effect of hydrolysis on spreadability of pineapple spread

Based on Table 5, pineapple spread made with hydrolyzed pectin treated with 0.05 M HCl for 96 h had a closer spreadability to sucrose compared to those of other. With increasing HCl concentration and incubation time, the texture and spreadability of spread substituted with hydrolyzed pectin will improve. This was due to pectin degrading in an acidic environment and losing viscosity. The lower the viscosity of hydrolyzed pectin, the higher the gel strength (Li *et al.*, 2014). The increased gel strength was caused by the huge amount of H_2O molecules trapped in the jam, which causes the gel to become viscous (Tiwari & Bhattacharya, 2011). This finding is similar to the research done by Vilela *et al.* (2015) showing that jam made by fructose oligosaccharide addition has lower spreadibility compared to jam made by mono- or disaccharide (fructose, sucrose, and sorbitol) because oligosaccharide has longer structure compared to mono- or disaccharide.

4. Conclusions

The present study showed that pectin hydrolysis decreased the viscosity, pH value, and L* value. The higher the concentration of HCl and incubation time, the lower the viscosity, pH value, and lightness of the solution. Pineapple spreads produced from different hydrolyzed pectins had different characteristics. The higher the concentration of HCl and incubation time, the lower the water activity, syneresis, and lightness of pineapple spread but the higher the ºBrix degree and spreadability. Hydrolyzed pectin at 0.05 M for 96 h had the potential to become a sugar substitute because it had water activity below 0.85, higher spreadability, and color similar to commercial pineapple jam.

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