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The effects of extraction conditions on the yield of crude pectin extract from passion fruit (*Passiflora edulis* Sims.) and the application of the extract on jam forming ability

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ABSTRACT

The peel of purple passion fruit (*Passiflora edulis* Sims.) can account for up to 60% of the total weight and contains usable organic compounds such as polymeric sugars, including pectin. The influences of ripeness stages and the extraction conditions (solvents, peel to solvent ratio, temperature, time) on the extract of crude pectin yield were investigated. The results indicated that pectin yield was independent of fruit maturation. The extraction efficiency reached its peak when extracted with citric acid and demonstrated optimal pectin production yield with the ratio of 1:5 (w/v) and at 95°C for 80 minutes. The extracted crude pectin was then applied to mango jam production. Jam made from extracted pectin had a good structure, moderate hardness, nice color, and harmonious taste. The ability to form coagulation on mango jam of crude pectin was weaker than commercial pectin. This study found that the topic's practical applicability to the processing of food products was significant.

1. INTRODUCTION

Pectin is a natural fiber - polymer of polygalacturonic acid and methyl esters. Pectin can be found in the intercellular or middle lamella region of plant tissue (Tamaki et al., 2008) and is abundant in many kinds of fruits like apple, citrus fruit, tomato, and passion fruit.

Pectin can form gels under particular conditions and is widely used in the confectionery industry and manufacture of oral medicines, injections, cosmetics, and ointments (May, 1990). Pectin belongs to the gelling agent groups. Pectin is considered one of the safest and most accepted food additives. It is recorded as the "NOT SPECIFIED" ADI content by the JECFA (Joint FAO/WHO Expert Committee on Food Additives), SCF

(Scientific Committee for Food) of the European Union, and Generally Regarded (GRAS).

Many studies have shown that passion fruit peel contains high levels of pectin (Kulkarni & Vijayanand, 2010; de Oliveira et al., 2016). Currently, there are two main species of edible passion fruit, widely grown and commercially available: purple passion fruit (*Passiflora edulis* Sims.) and yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) (Pinheiro et al., 2008). Many authors examined and developed the pectin extraction procedure where the yellow passion fruit peel was treated as the research object (Pinheiro et al., 2008; Liew et al., 2014; de Oliveira et al., 2016). Some studies on extracting pectin from purple passion fruit peel selected extraction solvents such as HCl

(Yen, 2019) and citric acid (Mai & Nguyen, 2013). Therefore, in this research, the extraction solvents such as tartaric acid (Seixas et al., 2014; Quoc et al., 2015) and distilled water (de Oliveira et al., 2015), were reported efficient in extracting pectin from yellow passion fruit and citrus fruits, were applied in extracting pectin from purple passion fruit peel.

In addition, the studies of the effect of the maturation stages of fruit on the pectin extraction process and the application of this source of pectin are still limited.

The study aims to extract pectin safely and cost-effectively while maximizing the use of a largely unused source of passion fruit peel.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Passion fruit peel

The passion fruit of the *Đài Nông 1* variety supplied by Toan Nhien Company Limited was sorted into different stages of maturation (see 2.4.3). The fruit then was washed, removed epicarp and pulp. The clean peel was transported to and stored in the small bags at -18°C in the Laboratory of Food Engineering, Faculty of Chemical Engineering and Food Technology -Nong Lam University. The peel was naturally thawed before being used.

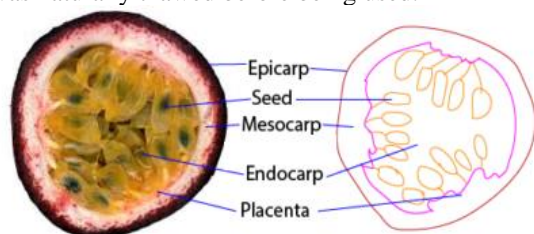


Figure 1. The cross-section of passion fruit

2.1.2. Mango

The Golden Cat Chu mango (*Mangifera indica* L.) was purchased from the local grocery store with yellow skin and without defects of physical injuries or rotten spots. The mangoes were stored in a cool place ($15 - 20^{\circ}\text{C}$) and used within a week.

2.1.3. Commercial pectin

The commercial pectin used in this project are pectin NH NAPPAGE (Louis François, France) and pectin HMP (GENU pectin - DE 72%, CP Kelco, Germany), which were stored in a dry, cool place (25°C) and away from direct sunlight.

2.2. Passion fruit peel preparation

The thawed peel was ground into small pieces, then passed through a stainless steel 1 mm mesh screen to gain uniform particles. The sample's moisture and dry matter contents were determined by using A&D MX-50 infrared moisture analyzer.

2.3. Experiment designs

2.3.1. Effects of ripening stages on pectin extraction yield

For extracting crude pectin, a sample of 10 g of prepared ground passion fruit peel (section 2.2) was soaked in a 5% citric acid solution (Thi et al., 2018) with the ratio of peel (W): acid solution (V) as 1:15. The mixture was hydrolyzed at 95°C for 50 minutes (Lanh, 2011), cooled to ambient temperature, and passed through filter cloths. The filtered solution was mixed with ethanol 96% (CEMACO) at a ratio of 1:3 and kept for 60 minutes (Quoc et al., 2015). The formed precipitate was then collected by filter paper and dried in a hot air oven at 65°C till reaching the constant weight (approx. 8% moisture content) to gain dried powder of crude pectin. The crude pectin was stored in a multilayer pack for the next steps. The extraction yield (%) of crude pectin for different ripening stages was calculated and compared to choose the appropriate ripening stage for further experiments.

2.3.2. Effects of extractants on pectin production yield

For investigating the appropriate solvents for pectin extraction, several types of extraction solvent were applied for a sample of 10 g of prepared peel. The type of solvents, concentrations, extracting ratios, and hydrolyzing conditions were shown in Table 1.

The gained precipitates were then dried to collect crude pectin powder. The appropriate solution with extraction protocol was then chosen for further experiments.

2.3.3. Effect of extraction conditions on pectin production yield

a. Peel to extractant ratio

The prepared peel to extractant ratios were kept at 1:5, 1:10, 1:15 and 1:20 (w/v) during extraction. The appropriate ratio was collected to be applied in the next experiments.

b. Extraction temperature

The extraction temperature applied for the chosen ratio was conducted at 75, 85, 95, and 100°C. The trial that gave the highest extraction yield was chosen for the next step of the experiment.

c. Extraction time

The effects of time on extraction efficacy were carried out and varied for 20, 50, 80, and 110 minutes to determine the time of extraction needed for maximum yield of pectin (%).

Table 1. Type of extractants, ratios, and hydrolyzing conditions applied for crude pectin extraction

Extraction solvents	Concentration	Ratio (w/v) (Peel: solution)	Hydrolyzing condition	References
Citric acid	5%	1:15	95°C, 50 mins	Thi et al., 2018
Tartaric acid	0.25%	1:40	95°C, 50 mins	Quoc et al., 2015
Hydrochloric acid	0.1 N	1:30	95°C, 60 mins	Mai & Nguyen, 2013; Roy et al., 2018
Distilled water	-	1:30	85°C, 30 mins	de Oliveira et al., 2015

2.3.4. Effect of the extracted pectin on mango jam properties in comparison with commercial pectins

The gel-forming ability of extracted pectin was conducted when applied to mango jam production. Mango jam was produced with the application of 5% extracted pectin with/without calcium lactate 1% as the supplement. Besides, 2% pectin NH NAPPAGE and 5% HMP pectin (DE=72%) were applied as the comparison factors.

The method proposed by Kopjar et al. (2009) was applied to make mango jam with several modifications. The Cat Chu mangoes were washed, peeled, and removed from pits. The pulp was ground by a blender and then passed through a 1 mm sieve mesh. The total soluble solid of mango paste (29 - 31°C, pH 3.9 - 4.1) had a value of 15°Bx by refractometer (ATAGO 0 - 100°Brix, Japan).

Mango paste (150 g) was mixed thoroughly with 107 g of sucrose (Bien Hoa, Viet Nam) at the ratio of 1:1 (w/w). A prepared mixture of sugar (10 g) - pectin powder was added and heated to reach the temperature of 106°C (and kept for 6 min) for jam forming. The mango jam was then hot-filled into a glass container (71.5 mm height, 43 mm top internal diameter, 52 mm bottom internal diameter) to reach the height of 20 mm from the bottom, covered with a lid, and placed at ambient temperature for 24 hours.

2.4. Analytical methods

2.4.1. Physicochemical properties of passion fruit peel

Physicochemical properties of passion fruit peel sample were analyzed in the Laboratory of Eurofins

Sac Ky Hai Dang Company Limited. All experiments were recognized by ISO/IEC 17025:2017 VILAS 238.

2.4.2. Production yield of pectin

The production yield (%) of crude pectin was calculated based on the dry mass of passion fruit peel.

Yield (%) = [mass of obtained crude pectin (g)/ mass of dry matter of passion fruit peel (g)]x100 (%)

2.4.3. Color measurement

The colorimetric parameters of the fruit were determined at three random points on the surface by a Konica Minolta CR-400 colorimeter (Konica Minolta, USA). The color values were expressed in the color scale of CIE L*a*b* (Luo, 2015).

2.4.4. Evaluate of reddish-purple area for ripening stage sorting

The ripening status of passion fruit can be determined by the area of the reddish-purple on the surface of the peel. For separating ripening stages, the square-rid method (FAO, 1998) with 2 mm x 2 mm square-ruled paper was applied to evaluate the reddish-purple area per the entire area of a single fruit.

The passion fruits were selected according to maturation stage and classified into four groups based on the percentage of purple peel area over the total surface area of passion fruit peel: less than 30% (A), 30-50% (B), 50-80% (C), and more than 80% (D) were shown on Figure 2.

2.4.5. Texture analysis for mango jam

The Bloom test method was used to evaluate the gel strength of mango jam by a texture analyzer

(Zwick/Roell Z1.0, Germany). Which, the sample was plunged by a cylinder (15 mm diameter) with a vertical reciprocating movement at a constant speed

of 5 cm per min and a depth of 10 mm. The gel strength was shown as gam force (g).

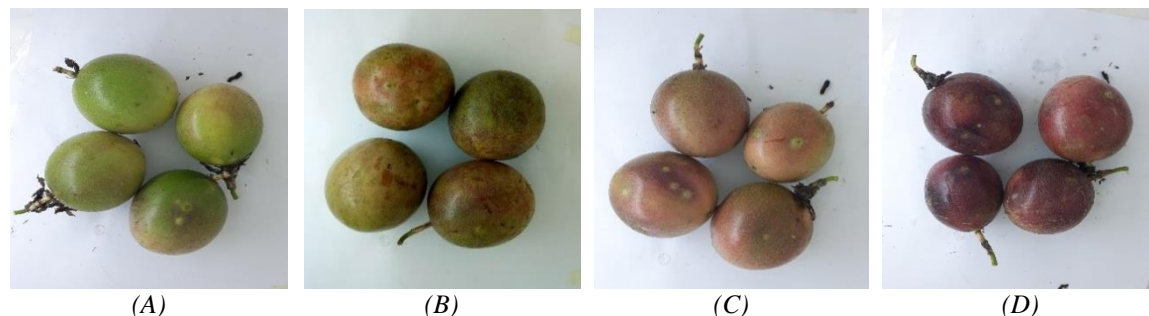


Figure 2. The four ripening stages of passion fruits

The maturation stage was classified into four groups based on the percentage of purple peel area over the total surface area of passion fruit peel: less than 30% (A), 30-50% (B), 50-80% (C), and more than 80% (D).

2.5. Statistical analysis

The collected data were analyzed using Excel 2013 and JMP software (version 10, SAS Institute, Cary, NC). Differences between means were evaluated

using ANOVA, followed by the LSD test. Statistical significance was considered at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Physicochemical properties of passion fruit peel

Table 2. Physicochemical properties of passion fruit peel

Physicochemical properties	Unit	Analytical methods	Results
Carbohydrates	%	AOAC 986.25 mod	7.52
Lipid	%	EVN-R-RD-2-TP-3498 (Ref. FAO Food 14/7-1986)	Not detected (LOD=0.1)
Protein	%	TCVN 10034:2013 (ISO 1871:2009)	1.17
Moisture	%	EVN-R-RD-2-TP-3496	90.5

LOD: low of detection

3.2. The correlation between ripening stages and colorimetric values

Figure 3 showed the correlation of the four ripening stages to evaluate the colorimetric values of fruit. Along with the stage of ripening, the values of L^* and b^* showed that the passion fruits became darker and less yellow. The red color value of a^* increased remarkably as maturation advanced.

Reid (1992) stated that the color change accompanying maturation in many fruits was widely used as a maturity index. In several fruits, the color change involves the loss of chlorophyll, the synthesis of new pigments such as carotenoids and/or anthocyanins, and the unmasking of other

pigments that were previously formed during the development of the fruit (Ferrer et al., 2005).

In Figure 3, the slope value of a^* (10.865) was much higher than the parameter of the other colorimetric values of L^* (-5.5179) and b^* (-7.2899). The same results were recorded in the study of Dobrzański (2002) in an apple ripening study.

On the other hand, the result displayed a strong correlation of a^* value to the ripening stages of fruit. Chelpiński (2019) and Łysiak et al. (2012) also confirmed that the value of a^* was the best parameter to represent the ripening of cherries and apples, respectively.

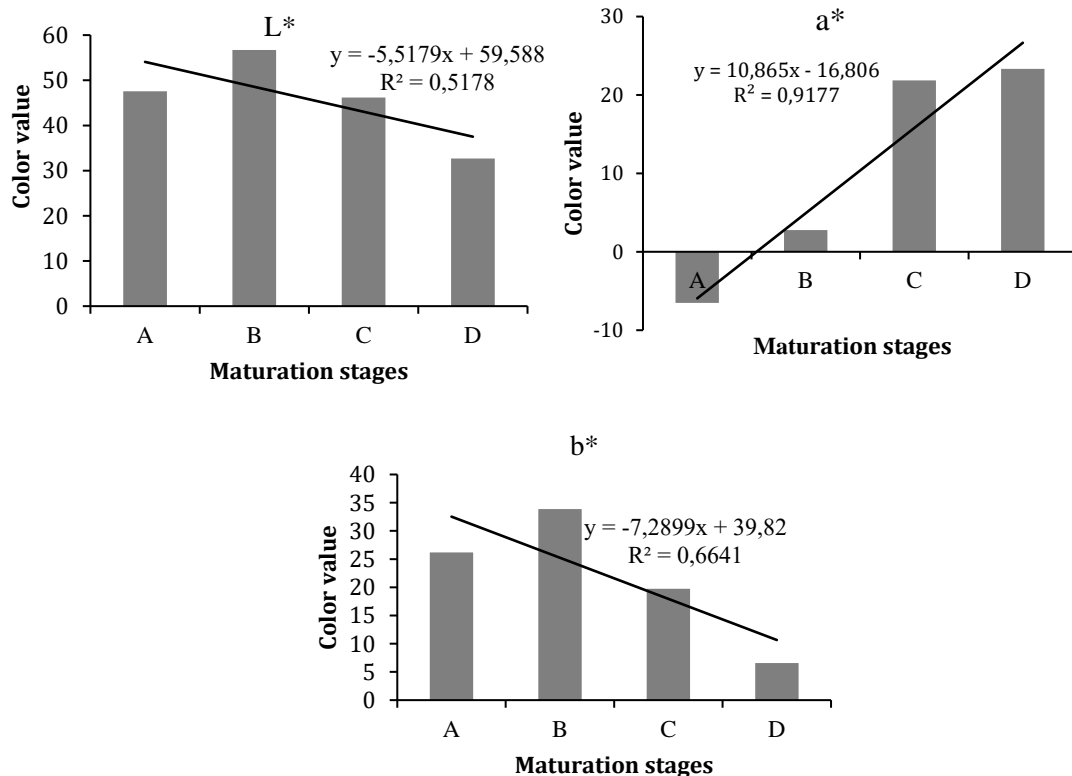


Figure 3. The color value (L*, a*, b*) at different maturation stages

3.3. The extraction yield of crude pectin at different ripening stages

Citric acid was widely chosen for pectin extraction (Pinheiro et al., 2008; Liew et al., 2014; Thi et al., 2018). In this research, citric acid was applied to extract crude pectin from peel at different ripening stages. Table 3 displayed the influence of the ripening stage on the crude pectin content. The yields slightly increased with ripening but decreased when the fruit got overripen (>80% maturation stage).

During fruit development, protopectin was laid down in primary cell walls and accumulates to high concentrations in the fruits (Kozłowski & Pallardy, 1997). As fruit ripened, protopectinases degraded the insoluble protopectin which was present in unripe fruits and gave rise to highly polymerized soluble pectin (Patidar et al., 2018). Furthermore, the high concentration of polygalacturonase and pectinesterase in overripen fruits was believed to induce the softening due to the breakdown of starch, and degradation of pectin in fruit (Kozłowski & Pallardy, 1997). Consequently, the crude pectin yields showed increased when started ripening and

decreased at the final ripening stage (>80%). The results were also similar to the conclusion of The Editors of Encyclopaedia Britannica (2018) that at the ripening stage, the precursor substance protopectin converted to pectin which helped fruits to remain firm and retain their shape, and pectin was broken down to simple sugars when overripe, leading to become soft and begin to lose its shape.

Table 3. Production yields of pectin extracted with different maturation stages

Maturation stages	Production yields of pectin (%)
<30%	31.87 ± 0.95 ^a
30-50%	32.24 ± 0.46 ^a
50-80%	32.49 ± 0.99 ^a
>80%	31.91 ± 0.61 ^a

a Means in a column are not significantly different from each other ($p < 0.05$). Data are presented as mean ± standard deviation with $n = 3$.

Although the yields for stages showed Table 3 was suitable for the explanation proposed by the above studies, there was statistically an insignificant

difference between yields because the pectin extracted from passion fruit peel in the study was crude pectin. In fact, the maturation stage of 50-80% is the common ripeness stage of fruit that is used in processing manufacture. Therefore, the maturation stage of 50-80% was chosen for further experiments.

3.4. Comparison of extraction solvents for higher pectin extraction yields

The extraction of pectin was influenced by the extraction solvents used. Table 4 showed the production yields of crude pectin from passion fruit peel by citric acid, tartaric acid, HCl, and distilled water.

Table 4 showed that the highest yield was 33.11% from HCl whereas the lowest yield was 9.43% from distilled water. While citric acid and tartaric acid also gave a quite high extraction yield of 32.58% and 24.07%, respectively.

Several studies noted that organic acids had a lower dissociation constant in comparison to mineral acids, and those had a less hydrolyzing capacity (Kermani et al., 2014). Furthermore, the yield of pectin by acid extraction increased as the dissociation constant of acid increased (Sudhakar & Maini, 2000).

A similar result, reported by Tu et al. (2002), showed that protopectin was converted to soluble pectin in acidic conditions. This is caused by the broken bonds of the polysaccharide chains in the cell wall leading to the release of pectin into the environment. However, in a more acidic environment, not only the large molecular weight substances were hydrolyzed, but also the bonds in the polygalacturonic acid chains were also strongly severed, part of pectin was decomposed, resulting in the extraction yield of pectin decreased (Thi et al., 2018).

Table 4. Production yields of pectin extracted with different types of extraction solutions

Extraction solutions	Production yields of pectin (%)
HCl	33.11 ± 0.36 ^a
Citric acid	32.58 ± 0.54 ^a
Tartaric acid	24.07 ± 1.76 ^b
Distilled water	9.43 ± 1.01 ^c

^{a, b, c} Means with different superscripts in a column differ significantly ($p < 0.05$). Data are presented as mean ± standard deviation with $n = 3$.

In this experiment, the extraction yield that applied HCl had no significant higher value than that used by citric acid. Castillo-Israel (2015) also reported a similar result in the study. Nevertheless, in terms of peel-to-solution ratio, the amount of citric acid used was less than that of HCl (Table 1), resulting in enhanced economic value. Therefore, citric acid was chosen for the next experiments.

3.5. Effect of extraction conditions on pectin production yield

3.5.1. Effect of peel: acid ratio

In the experiment, the ratios were conducted from 1:5 (w/v) to assure that the wet material can be thoroughly immersed in the solution. Figure 4 showed that the pectin yield was maximum when the peel: the acid ratio was 1:5 (38.16%). There was a statistically significant difference and decreased gradually when the peel: acid ratio decreased from 1:5 to 1:15 and tended to increase slightly when down to 1:25. However, at the lower ratios, the pectin yield did not show significant differences between the results.

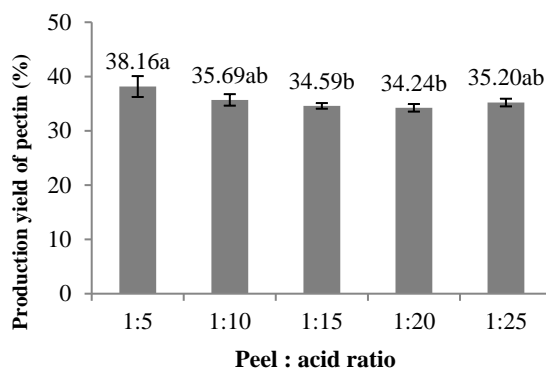


Figure 4. Effect of peel: acid ratio on pectin production yield

^{a, b} Means with different superscripts differ significantly ($p < 0.05$). Data are presented as mean with $n = 3$ and standard deviation on top of each column.

The ratio between the solvents and sample had a significant impact on the rate of diffusion (Bin et al., 2005). This ratio boosted the diffusion rate and provided the capacity to thoroughly separate the dispersed molecule, but it required more energy to have the effect in the solvent. For an extraction process, an appropriate ratio must be chosen. The solvent must be provided enough to penetrate the capillaries of the passion fruit peel and dissolve protopectin to the outside during the extraction process. In the case that the ratio is too low, the

resources could be wasted and reduce efficiency. Consequently, the 1:5 ratio of passion fruit peel to solvent extract was chosen as the ideal rate for the extraction process and had become a fixed parameter for further experiments.

3.5.2. Effect of extraction temperature

Figure 5 showed the crude pectin extraction yields increased from 23.82% to 37.74% with the increase in extraction temperature from 75°C to 95°C. Nevertheless, the obtained pectin tended to decrease for the trial of 100°C. Figure 5 showed that the yield at 85°C, 95°C, and 100°C was not significantly different; but the yield at 95°C had a significant difference from that of 75°C.

Temperature plays an important role in solvent extraction. High energy can accelerate the rate of diffusion of extracting compounds to the environment and affect efficient yield. For the research, the yield reached a peak at 95°C and declined at a higher temperature. Pectin can be denatured at very high temperatures and reduce extracting efficacy.

Therefore, the extracting temperature of 95°C was chosen for extracting pectin from passion fruit peel in further steps.

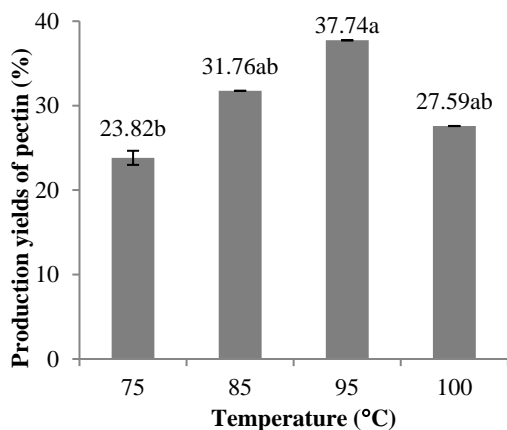


Figure 5. Effect of extraction temperature on pectin production yield

a, b Means with different superscripts differ significantly (p < 0.05). Data are presented as mean with n = 3 and standard deviation on top of each column.

3.5.3. Effect of extraction time on pectin production yield

The result in Figure 6 showed that the pectin yield increased from 29.82% to 38.77% with the extension of extraction time from 20 to 80 minutes, then decreased with the longer extraction time.

Figure 6 showed that the yield in 50, 80, and 110 minutes was not significantly different; but the yield in 80 minutes had a significant difference from that of 20 minutes.

In a short extracting time, the bonds between pectin with other components such as cellulose, and hemicellulose could not be severed. Protopectin was hydrolyzed to a soluble form. It could influence the extraction yield (Quoc et al., 2015). For a long time of extraction, the pectic acid would be formed due to the hydrolyzing soluble pectin. Thus it reduced the extraction yield of pectin.

Consequently, the experiment of 80-minute extraction was the optimal time to achieve the highest pectin production yield from passion fruit peel in the research.

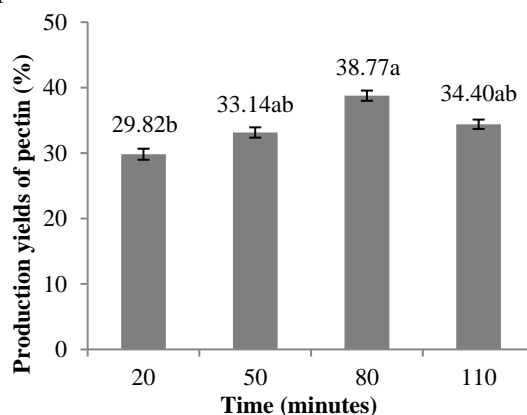


Figure 6. Effect of extraction time on pectin production yield

a, b Means with different superscripts differ significantly (p < 0.05). Data are presented as mean with n = 3 and standard deviation on top of each column.

3.6. Effect of extracted crude pectin from passion fruit peel on properties of mango jam

For estimating the gel-forming ability of pectin, the crude extracted pectin powder was applied in the production of mango jam. In addition, the commercial pectin powders were used for comparison. Mango jam samples were evaluated for colorimetric values and gel strength.

Table 5 shows the differences in colorimetric of the jam samples that had varying L*, a*, b* indexes. The colorimetric results discovered that mango jam exhibited the optimal index in commercial pectin. The differences in brightness and other color values were caused by the different types of pectin and concentrations used to alter the lightness, darkness, and color of mango jam.

Due to the purification and the bleaching process, the commercial pectin had ivory to milky white color and showed fewer impacts on the color of the jam product. On the other hand, the crude pectin extracted from passion fruit peel had not been bleached or purified and had a color ranging from pink-red to purple. As a result, mango jam turned

darker while applied with these types of pectin, especially for high used concentrations.

The gel strength of mango jam products with different types of pectin and concentrations were shown in Table 6.

Table 5. Colorimetric results of mango jam

Mango jam trials	Colorimetric values		
	L*	a*	b*
pectin 5% (A)	19.79 ± 2.01 ^b	2.71 ± 0.38 ^a	4.62 ± 0.60 ^b
pectin 5% + Calcium lactate 1% (B)	19.07 ± 1.55 ^b	2.46 ± 0.41 ^a	4.94 ± 0.68 ^b
pectin HMP 5% (C)	19.86 ± 2.11 ^b	1.34 ± 0.23 ^b	6.84 ± 1.54 ^a
pectin NH 2% (D)	22.82 ± 1.91 ^a	1.47 ± 0.14 ^b	7.85 ± 0.97 ^b

^{a, b} Means with different superscripts in a column differ significantly ($p < 0.05$). Data are presented as mean ± standard deviation with $n = 3$.

Table 6. The gel strength of mango jam products

Mango jam trials	Gel strength (g force)
pectin extracted 5%	22.47 ± 10.87 ^b
pectin extracted 5% + Calcium lactate 1%	38.53 ± 2.87 ^b
pectin HMP 5%	210.50 ± 13.61 ^b
pectin NH 2%	826.33 ± 268.56 ^a

^{a, b} Means with different superscripts in a column differ significantly ($p < 0.05$). Data are presented as mean ± standard deviation with $n = 3$.

According to the results, the sample had the highest hardness while applied with pectin NH at a concentration of 2%, followed by the mango jam using pectin HMP 5%. The trial used extracted pectin displayed a loose texture in comparison to its mixture with calcium lactate.

hardness of the products. Because of the high purity, commercial pectin powder showed high value of hardness than the extract from the passion fruit peel at a similar applying concentration.

The difference in gel strength of jam samples was strongly linked to the influence of types of pectin and concentrations used, resulting in varied

However, even though the gel-forming ability of the extracted pectin powder was weaker than commercial pectin in jam production, the crude extracted pectin expressed the applicability in the gel-forming of the testing product.

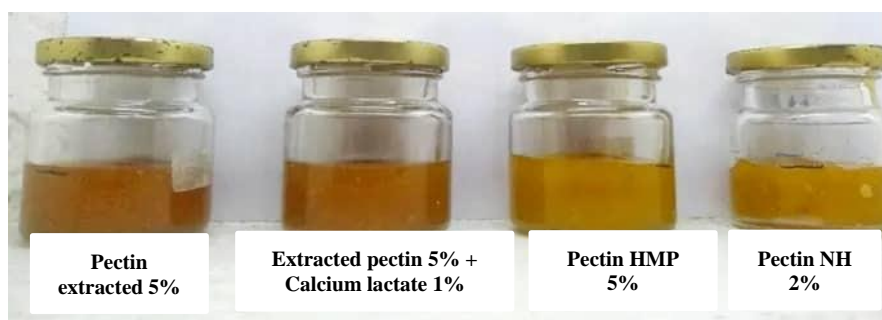


Figure 7. Samples of mango jam (A), (B), (C), and (D) in order from left to right



Figure 8. Crude pectin extracted, pectin NH, pectin HMP in order from left to right

4. CONCLUSIONS

The research revealed that passion fruit peel could be considered a great source of pectin and could be utilized for extraction. This study found that the topic's practical applicability to the processing of food products was significant. The amount of crude pectin extracted was influenced by the solvent type, the ratio of peel per solvents, and extraction conditions, but not by the stages of ripening. The extracting yield was recorded at the optimum point using citric acid at a ratio of 1:5, and extracted at

95°C for 80 minutes. Although the gel-forming of passion fruit pectin had weaker than commercial pectin, it could be applied for product coagulation. Therefore, the pectin in peel is more used, the benefit of the economy and environment is more gained.

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