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RESEARCH ARTICLE

# Optimisation of the extraction process of pectin polymer from red dragon skin (*Hylocereus polyrhizus*)

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## Keywords

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## Abstract

**Background:** The variation of pectin extracted from red dragon skin (*Hylocereus polyrhizus*) depends on the type of acid, temperature, and length of extraction time. **Objective:** This research was conducted to determine the characteristics of pectin produced from red dragon skin based on using citric, oxalic, and acetic acids at pH 4, extraction temperature of 60°C and 75°C, and duration of 120 minutes. **Method:** A total of six conditions for pectin extraction were performed and the obtained product was dried using the freeze-dryer. Furthermore, the optimisation included yield, moisture content (MC), equivalent weight (EW), methoxyl content (MeO), galacturonic acid content (GA), degree of esterification (DE), sugar and organic acid levels (SO), and Fourier Transform Infrared Spectroscopy (FT-IR) was performed. **Result:** The EW value of red dragon skin and commercial apple pectin were comparable but the MeO value was slightly different from those extracted with 75°C oxalic acid. Compared to commercial apple and orange skin, the GA value of red dragon pectin extracted with 75°C oxalic acid was not different. **Conclusion:** FT-IR spectra contained groups -OH, -CH, and C=O and were confirmed to have a GA structure. The best product was obtained by conducting extraction under the condition of 75°C for 120 minutes using oxalic acid.

## Introduction

Red dragon fruit skin (*Hylocereus polyrhizus*) is discarded as waste, contributing to environmental pollution. This waste is a source of pectin compounds with a relatively large yield above 10% (Ismail *et al.*, 2012). Based on previous research, pectin has the potential to be developed into polymers used for microparticles (Souza *et al.*, 2021). According to the degree of esterification (DE), this compound can be categorised into high and low methoxyl (MeO). The features obtained depend on the extraction conditions, specifically the type of acid, temperature, and extraction time (Abboud *et al.*, 2020)

Low or high MeO pectin with DE, galacturonic acid (GA) concentration, and equivalent weight (EW) can be generated by varying the acid and extraction

temperature (Hashim, 2018). In this context, greater DE and MeO levels cause pectin to gel quickly, increasing the synthesis of microparticle polymers. Variations in extraction conditions, namely temperature and extraction time, affect the quality characteristics of pectin. The DE value can reach optimum conditions with increased temperature and extraction time (Abboud *et al.*, 2020). In the presence of acid, the high temperature and prolonged extraction process may cause the methyl ester group to degrade and become carboxyl acids. A prolonged extraction process results in pectic acid-free of the methyl ester group's GA. Furthermore, the degradation of the pectin compound resulting from the depolymerisation mechanism of the GA pectin chain may lead to a decrease in DE (Colodel & Petkowicz, 2019).

Organic acids are used due to their lower toxic properties than mineral acids. These acids have a low level of acidity and do not degrade pectin to pectic acid (Cinkmanis *et al.*, 2020). In hydrolysing protopectin to create a pectin network, mineral acids hasten the release of H<sup>+</sup> ions. However, using mineral solid acids causes pectin to be degraded into pectic acid. Under acidic conditions, protopectin tends to be hydrolysed to pectinic acid or soluble pectin. Dissolving pectin can occur due to substituting protopectin divalent ions into hydrogen or breaking the bond between pectinic acid and cellulose (Guízar-Amezcuca *et al.*, 2022).

Citric acid is often used in pectin extraction, with the mechanism of hydrolysis of protopectin into a water-soluble form. This organic acid with non-corrosive properties is safe for pectin extraction to produce a good yield. The extraction at pH four with a temperature of 75°C for 120 minutes resulted in an average yield, sugar content, and DE of 8.10%, 2.3 mM, and 71%, respectively (Woo *et al.*, 2010). Oxalic acid can produce pectin with optimal characteristics from super red dragon skin (*Hylocereus costaricensis*). The best pectin was obtained at a temperature and concentration of 70°C and 0.05 N for 60 minutes with oxalic acid as a solvent. Acetic acid is also used to extract pectin from super red dragon skin. At a temperature of 60°C, a concentration of 0.05 N for 60 minutes produced a DE value of more than 50%. (Septiana & Puspa, 2017).

This research used organic solvents, citric, oxalic, and acetic acids, at pH four for 120 minutes at 60°C and 75°C. Pectin derived from red dragon skin is expected to have the same physical characteristics as commercial pectin.

## Methods

### Design

The red dragon fruit used was the *Hylocereus polyrhizus* variety, which is allowed to age for two months after the initial appearance of flower buds. The fruit was sourced from dedicated farmers in Sleman, Yogyakarta, Indonesia. The prepared fruit segments were then subjected to a controlled drying process using a food dehydrator set at 50°C for four hours. After this drying phase, the resulting material was finely pulverised into a powdered form. A thorough examination was conducted to assess the MC of the obtained powder. Following this assessment, 25 g dry simplicia was weighed. The subsequent stage included six distinct treatments, each comprising a specific solvent. The solvent included 0.1 N oxalic acid, 0.1 N citric acid, and 1000 ml acetic acid at pH 4.00. The extraction process

was done using a hot plate magnetic stirrer, with temperatures set at 60°C and 75°C for 120 minutes. After the extraction process, the resulting filtrate was subjected to a filtration procedure.

Subsequently, the precipitation of pectin was executed by adding 96% alcohol to the filtrate, maintaining a precise ratio of 1:1 between the filtrate and the alcohol. This coagulation continued for 24 hours (Kurniawan & Adenia, 2022). The mixture was filtered to obtain wet pectin and dried using a Freeze Dryer at -40°C and pressure of 200 mTorr. Pectin yield, EW, MeO, GA, and DE tests were conducted using the previous methods regarding the provisions of the International Pectin Producers Association (IPPA) (Kurniawan & Adenia, 2022). The sugar and organic acid (SO) was quantified following the method stated in the Indonesian Pharmacopoeia VI. Meanwhile, FT-IR was carried out using the KBr pellets method.

### Yield

$$\text{Yield} = \frac{\text{Pectin weight obtained (g)}}{\text{Dragon fruit peel powder weight (g)}} \times 100\%$$

### Moisture content (MC)

A total of 0.5 g dry pectin was put into a moisture analyser, and the dryness level was checked at a temperature of 105°C for ten minutes.

### Equivalent weight (EW)

Approximately 0.25 g pectin was placed in a 250 ml Erlenmeyer flask, moistened with 2 ml of 95% ethanol before dissolving in 40 ml distilled water containing 1 g NaCl. Subsequently, the solution was dripped with five drops of phenolphthalein and titrated using 0.1 N NaOH until the colour change and titrated volume were recorded. The following formula determines EW:

$$\text{EW} = \frac{\text{Pectin weight (mg)}}{\text{NaOH titration volume (mL)} \times \text{Normality of NaOH}}$$

### Methoxyl content (MeO)

In the EW determination solution, 25 ml of 0.25 N NaOH was added, and the mixture was gently shaken before being left to stand for 30 minutes in a closed Erlenmeyer flask. Subsequently, 25 ml of 0.25 N HCl was introduced with six drops of phenolphthalein as an indicator. The solution was then titrated with 0.1 N NaOH until pink colouration was formed.

$$\text{MeO} = \frac{\text{Milliequivalents of NaOH} \times 31 \times 100}{\text{Pectin weight (mg)}}$$

\*The value 31 is obtained from the molecular weight of MeO in the form of CH<sub>3</sub>O.

**Galacturonic acid (GA)**

GA levels can be calculated from milliequivalents (meq) of NaOH obtained by determining EW and MeO. GA levels can be formulated as follows:

$$GA = \frac{176 \times 100}{z}$$

$$z = \frac{\text{Pectin weight (mg)}}{\text{meq of NaOH from EW} + \text{meq of NaOH from MeO}}$$

\*The number 176 is the lowest EW of pectic acid.

**Degree of esterification (DE)**

Calculation of DE from MeO and GA content produced is formulated as follows:

$$DE = \frac{176 \times \text{MeO}}{31 \times \text{GA}} \times 100$$

**Sugars and organic acids (SO)**

A total of 1 g pectin was introduced into a 500 mL flask. The flask was moistened with 3 ml to 5 ml ethanol P, and 100 ml water was added. The mixture was agitated and allowed to stand until complete dissolution was achieved. Subsequently, 100 ml ethanol P supplemented with 0.3 ml hydrochloric acid P was added, and the solution was rapidly subjected to filtration. Approximately 25 ml of resulting filtrate was transferred into a cup, and the contents were evaporated over a steam bath. The residue was dried under vacuum at 50°C for two hours and cooled before

determining the weight. The weight must not exceed 20 mg, and the levels of SO did not surpass 20 mg (Directorate General of Pharmaceuticals and Health Devices, 2020).

**Fourier Transform Infrared Spectroscopy (FT-IR)**

The test used a transmission method by making KBr pellets. FT-IR vibration spectra showed the results of red dragon skin pectin from six treatment conditions (groups I-VI).

**Assessment**

Optimisation parameters were analysed statistically using the one-way ANOVA with a 95% confidence level ( $\alpha=0.05$ ), and the data were three times  $\pm$ SD replication.

**Results**

The results of six treatment conditions produced pectin powder with a bright colour, as shown in Figure 1. Optimisation results of the six extraction treatment conditions showed that the parameters met the requirements, as shown in Table I. In Table II, the FT-IR spectral data are presented. Figure 2 also showed that the pectins produced contained -OH, -CH, and C=O groups and were confirmed to have GA structure.

**Table I: Optimisation results of red dragon skin pectin (*Hylocereus polyrhizus*)**

Group	Yield (%)	Moisture content (%)	Equivalent weight (mg)	Methoxyl content (%)	Galacturonic acid content (%)	Degree of esterification (%)	Sugar and organic acid content (mg)
I	48.00	9.46	2144.48	6.70 $\pm$ 0.54	70.98 $\pm$ 5.36	53.58 $\pm$ 0.35	72.30 $\pm$ 2.45
II	48.04	11.32	2142.56	7.76 $\pm$ 0.55	76.97 $\pm$ 3.45	57.21 $\pm$ 2.09	78.43 $\pm$ 7.73
III	24.01	10.94	1720.16	5.35 $\pm$ 0.27	72.33 $\pm$ 3.86	41.97 $\pm$ 0.31	21.12 $\pm$ 8.39
IV	32.62	11.25	1813.21	5.61 $\pm$ 0.76	71.82 $\pm$ 1.81	45.60 $\pm$ 3.75	45.05 $\pm$ 1.91
V	27.26	10.31	2681.92	6.71 $\pm$ 0.53	67.55 $\pm$ 4.76	56.44 $\pm$ 3.88	32.77 $\pm$ 4.62
VI	37.52	11.31	2455.85	7.50 $\pm$ 0.26	74.37 $\pm$ 1.48	59.68 $\pm$ 1.62	52.37 $\pm$ 1.72
S1†	-	9.0	2928.09	7.87 $\pm$ 0.29	68.78 $\pm$ 0.56	66.26 $\pm$ 2.83	52.01 $\pm$ 2.88
S2‡	-	6.0	3075.87	8.49 $\pm$ 0.27	71.32 $\pm$ 0.90	67.63 $\pm$ 2.86	51.30 $\pm$ 2.03

†: Commercial pectin of apple; ‡: Commercial pectin of orange peel



**Figure 1: Dragon fruit skin pectin powder (*Hylocereus polyrhizus*)**

Table II: FT-IR spectra data

No.	Wave number (cm <sup>-1</sup> )								Functional groups
	I	II	III	IV	V	VI	S1	S2	
1	3467	3438	3453	3434	3448	3424	3438	3443	Stretching hydroxyl (OH)
2	2949	2934	2945	2934	2945	2949	2939	2949	Stretching -CH sp <sup>3</sup>
3	1750	1759	1755	1750	1755	1759	1759	1764	Stretching C=O both from carbonyl esters and carboxylates
4	1620	1629	1625	1615	1606	1611	1635	1620	Stretching C=O in ionised carboxylates (asymmetric)
5	1419	1428	1438	1428	1438	1433	1447	1443	Stretching C=O in ionised carboxylates (symmetrical)
6	1015	1011	1011	1011	1001	1001	1006	1011	CO ester

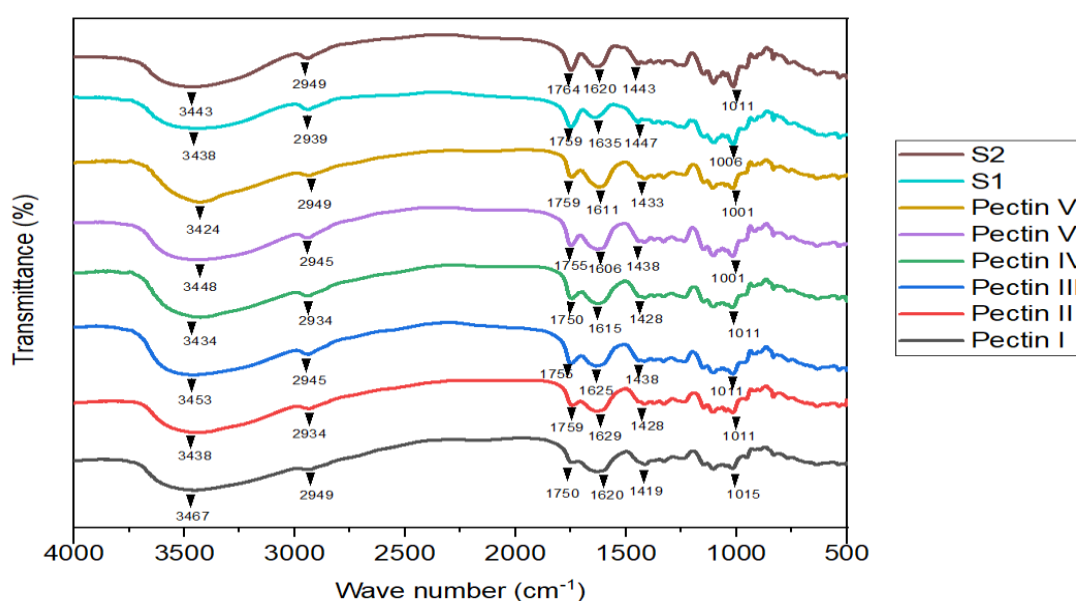


Figure 2: The infrared spectral profile

## Discussion

Oxalic acid ( $H_2C_2O_4$ ) is a bivalent weak acid that ionises by releasing two  $H^+$  ions to have a  $K_a$  value of two. Furthermore, it has a  $K_{a1}$  value of  $5.9 \times 10^{-2}$  and a  $K_{a2}$  of  $6.5 \times 10^{-5}$ . The ionisation of acetic acid ( $HC_2H_3O_2$ ) only produces  $H^+$  ions, which will form  $H_3O^+$  ions in water because acetic acid is monoprotic. The  $K_a$  value in acetic acid is  $1.8 \times 10^{-5}$ , while citric acid ( $H_3C_6H_5O_7$ ) is a type of polyprotic acid with three  $K_a$  values, namely  $7.1 \times 10^{-4}$ ,  $1.7 \times 10^{-5}$ , and  $3.9 \times 10^{-7}$  (Kasmiyatun & Jos, 2008). From these data values, oxalic acid has a higher acid strength than citric and acetic acids. Therefore, the yield value of pectin is higher than acetic and citric acid solvents. Due to the carboxylic functional group, citric and acetic acids are necessary organic molecules. The number of functional groups per molecule separates

the two substances. Acetic acid only has a carboxylic acid group, while citric acid has three, which is the main distinction (Kasmiyatun & Jos, 2008).

In this study, the yield value obtained at  $75^\circ C$  was higher than  $60^\circ C$  for the three types of acid used during extraction. This is because a higher extraction temperature causes the kinetic energy to increase. Therefore, more of the extracting solution diffuses into the simplicia cell tissue of dragon fruit skin to extract pectin (Cinkmanis *et al.*, 2020).

Pectin from  $75^\circ C$  extraction was darker due to the oxidised anthocyanin compounds in dragon fruit skin. Browning reactions, including caramelisation, Maillard, and browning, occurred due to reactions between protein and sugar and heat. The Maillard reaction is a complex three-step process involving peptide-free

amino groups and reducing sugar, forming Schiff bases, Amadori products, dehydration, and amino acid breakdown, resulting in polymeric compounds (Ioannou, 2016).

A certain amount of drying is conducted to extend the shelf life, and low temperatures are also used to reduce pectin degradation. MC analysis is obtained from the maximum allowable MC of pectin at most 12% (Codex Alimentarius, 2009). MC analysis yields data where the MC value is consistently below 12%, satisfying the specified requirements. The study revealed that free GA groups in EW, not esterified in the pectin chain, created a modest amount of pectin. Decreased temperature and shorter extraction times minimised depolymerisation, resulting in a greater EW. The results showed that citric acid extract had the smallest EW value, and temperature doesn't significantly affect EW value. (Mashau *et al.*, 2021). Based on the one-way ANOVA statistical test results, the value of  $\text{sig} = 0.000 < 0.05$ , meaning there was a significant difference between the groups. EW value in group V was 0.120 compared to pectin in group S1 with no significant difference.

The concentration of GA in a pectin extract plays an important role in determining its purity, functional characteristics, structural attributes, and textural qualities (Chan *et al.*, 2017). This parameter significantly influences the final gel texture and structure, a relationship further compounded by polysaccharides such as arabinose and galactose.

Factors like extraction process, solvent selection, raw material source, and pectin quality directly affect GA production, with increased levels due to bond dissolution with hemicellulose (Gawkowska *et al.*, 2018). The pectin chain contains GA and other compounds such as D-galactose, L-arabinose, L-rhamnose, and other types of sugar. Additional substances can affect pectin chain composition, affecting GA concentration. Based on the one-way ANOVA statistical test results for GA, the value of  $\text{sig} = 0.062 > 0.05$ , meaning there was no significant difference between groups.

The functional characteristics of pectin are influenced by MeO content, which can be classified into low and high MeO pectin. Two extraction conditions, acetic and oxalic acid at 75°C, resulted in pectin with a MeO value greater than 7.12%, forming the high MeO pectin group. The results obtained from the determination of MeO are shown in Table I. The effects of increasing concentration, temperature, and time were seen in the high levels of MeO. This was because continually esterified free carboxyl groups were present (Pereira *et al.*, 2016). Based on the one-way ANOVA statistical test results for MeO, the value of  $\text{sig} = 0.000 < 0.05$ ,

meaning there was a significant difference between groups. The MeO value of group II had a significance value of  $> 0.05$  compared to groups S1, S2, and VI. Group VI also has a significance value of  $> 0.05$  compared to the MeO value of group S1.

The amount of carboxylic acid esterified from D-GA depends on DE. There are two categories for DE, namely high and low MeO pectin, defined as more and less than 50%. A lengthy extraction period converts the pectin into pectic acid to release GA from the methyl ester group. This methyl ester shows DE or the existence of an unesterified carboxylic acid (Bee Lin & Yek Cze, 2018).

The extraction temperature significantly impacted the DE value at 75°C, which was higher than at 60°C due to the optimal temperature range for acid extraction. High temperature and extended extraction time can break down pectin's methyl ester group into carboxyl acids, while excessive extraction time results in pectic acid. The concentrations of protopectin, pectin, and pectic acid vary depending on fruit maturity (Guízar-Amezcuca *et al.*, 2022). Based on the one-way ANOVA statistical results for DE, the value of  $\text{sig} = 0.000 < 0.05$ , meaning there was a significant difference between the groups.

The study found that higher temperatures, such as 75°C, resulted in higher levels of dissolved SO in all pectin sample groups I-VI, suggesting that the hydrolysis process is stronger. Based on the one-way ANOVA statistical test results for SO, the value of  $\text{sig} = 0.000 < 0.05$ , meaning there is a significant difference between groups.

The test results confirmed that the pectins produced had -OH, -CH, and C=O groups, forming the GA structure. Wave numbers 3425.30-3455.78 showed the vibration of the OH in a wide stretching position due to hydrogen bond interactions, and the absorbance value was small in proportion to the content of the extracted compound. Furthermore, there is also the possibility of widening due to trace water (Zaidel *et al.*, 2017). Vibrations at wave numbers 2936.19-2939.37 showed stretching -CH sp<sup>3</sup>. A shift in the absorption value and widening of the band occurred because the structure had conformational variations in the pyranose ring. Wave numbers 1741.73-1748 showed C=O stretching from the carbonyl ester and the carboxylate. The peaks at 1745 and 1635 were almost the same in absorption height, and the carbonyl ester and carboxylate amount was balanced. The ratio between the two peaks can vary depending on the type of pectin. The FT-IR spectra in Figure 2 show C-O stretching vibrations in the wave number region of 900-1300 cm<sup>-1</sup>. Pectin in six treatment conditions and two commercial samples had stretching vibrations at this number of locks. The FT-IR spectra of the Maillard reaction included the stretching

frequency of the enaminol group, Schiff base imine production, unique C=O vibrations, and -C=N vibration at 1660 cm<sup>-1</sup> (Resende & Franca, 2023).

## Conclusion

When compared with the pectins from commercial apple and orange skins, the GA value of the pectin extracted with 75°C oxalic acid was not significantly different. There was no discernible difference when comparing the SO value of pectin extracted using acetic and citric acids at 75°C to commercial pectin from apple and orange skins. The produced pectin had -OH, -CH, and C=O groups, and the GA structure was verified based on the results of the FT-IR test.

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## Conflict of Interest

The authors declare no conflict of interest.

## Author contributions

DMH contributed to the concept and design research, as well as research supervision. MFK contributed to the drafting script. DS participated in the research design, research, planning, and supervising. The final manuscript has been approved by all authors.

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