# Isolation and Characterization of Pectin from Orange Peel (Citrus Sp.)

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**Abstract :** Oranges are one of the horticultural commodities that have a role as a source of nutrition. One of the ingredients in orange peel that can be used as an industrial ingredient is pectin. Extraction of pectin using HCl solution with a pH of 2. The pectin that has been dissolved is precipitated with 96% ethanol. The pectin sediment is washed with an alkaline solution until normal pH and then dried in an oven. The purpose of this research is to isolate and characterize pectin from orange peel. The results obtained showed that the pectin samples had a higher water content than standard (<12%), ash content in the standard range (<10%), equivalent weight above standard (600-800 mg), high methoxyl (>7.12%) , has lower galacturonic content than standard (≥65%), and belongs to high pectin ester (≥50%). The pectin quality standart used IPPA (International Pectin Producers Association).

## INTRODUCTION

Orange (Citrus sp.) is an annual plant originating from Asia, especially China. Since hundreds of years ago, this plant has existed in Indonesia, both as a wild plant and as a plant in the yard (Novriansyah, 2019). The edible part of the fruit flesh is called the endocarp (Hidayati et al., 2021). The endocarp is made up of segments called carpels or locules. Within these segments are thin-walled sacs of fruit juice. The endocarp is surrounded by a part of the orange called the peel. Citrus fruit peel consists of flavedo and albedo. Flavedo is the outer skin which is located at the bottom of the epidermal layer and contains chromoplasts and oil sacs, while the inner skin which is called albedo is a layer of foam tissue (Fauzie, 2021).

Several previous studies conducted by Latupeirissa et al., (2019), Perina et al., (2017), and Arimpi & Pandia (2019) reported the presence of pectin content in orange peels. Pectin comes from the Latin "pectos" which means thickener or which can make something hard or solid. Pectin is a structural heteropolysaccharide found in terrestrial plants. Pectin can be applied to various food fields such as gelling agents, stabilizers, and fat substitutes (Fadli, 2021)

Pectin or pectate compounds are complex polysaccharides with large molecular weights found in the middle lamella or intercellular spaces in higher plant tissues (Sirait & Eniyani, 2020). The joint bonds between polysaccharides and cellulose fibers form a strong network that acts as an adhesive between cells.

Fruits and vegetables contain lots of pectin. Pectin can naturally be found in most food-source plants, especially fruits and can be extracted from the flesh, flowers, and waste in the form of skins, seeds, and pomace (Usmiati et al., 2016; Lattupeirissa et al., 2019).

According to Latuperissa et al. (2019) separation of pectin from plant tissue is carried out by an extraction process which is a separation process from solid and liquid materials with the help of a solvent. The solvent

used must be able to extract the desired substance without dissolving other materials. Pectin from orange peel can be obtained through an extraction process with acid, base and water solvents. Pectin stabilization is produced by a layer of water through electrostatic bonds between negatively charged pectin molecules and positively charged water molecules. The addition of ethanol (a dehydrating agent) can reduce the stability of pectin dispersion and cause clumping due to the dehydrating effect which disrupts the balance of pectin with water (Fitria, 2013). This study aims to isolate and characterize pectin from orange peels.

## METHOD

### Tools

The tools used for the extraction and characterization of pecans are beaker glasses, measuring pipettes, dropping pipettes, statives & clamps, measuring cups, magnetic stirrers, hotplates, Whatman filter paper, muffle furnaces, desiccators, rotary evaporators, ovens, and FTIR.

### Materials

The materials used in this study include jackfruit peel, HCl, distilled water, ethanol, phenolphthalein.

### Pectin Isolation

In general, the method of isolating orange peel pectin begins with material preparation, material extraction, pectin deposition isolation, and pectin drying. Material preparation is done by drying and grinding the orange peel. Extraction of materials can be done using strong acids such as hydrochloric acid or acetic acid. Precipitation of pectin by using an alkaline solution such as ethanol. Pectin drying can be done using an oven with a temperature between 45-50°C

Several previous studies have succeeded in isolating pectin from orange peels. Based on the method used by Latuperissa et al., (2019) the percentage of pectin content obtained was 82.82%. The pectin functional groups detected included the OH (alcohol), CH3 (aliphatic), C=O (ester), C=C (alkene), and C-O- (ether) functional groups. Characterization of pectin shows that pectin contains an equivalent weight of 2011.6 mg; methoxyl content of 1.17%; galacturonic acid levels of 41.64%; water content of 28.46%; ash content of 11.92%; and the degree of esterification of 15.95%. Sulihono et al., (2012) reported that the pectin that had been successfully isolated had a dry weight percent of 26.695%. The pectin is the best pectin than the pectin from other treatment variations. The pectin has a moisture content of 14.6%, an ash content of 3.4%; degree of esterification of 85.44%; pectin equivalent weight 2083.33; methoxyl content of 8.73%; and galacturonic acid levels of 58.08%.

The concentration of the base solution and the length of time during which the pectin is deposited have an effect on the condition of the pectin. Based on the method used by Aji et al., (2017) the best pectin yield was obtained from pectin which was precipitated with
0.25 N ethanol for 90 minutes. Arimpi et al., (2019) reported that the best pectin yield in his research was pectin precipitated with ethanol at a concentration of 95% for 20 hours

### Characterization

Characterization of orange peel pectin includes moisture content, ash content, galacturonic acid, equivalent weight, methoxyl content, and functional group analysis using FTIR.

### Water Content Test

1 gram of pectin sample was put into a crucible which had been weighed and put in an oven at 105°C for 1 hour. Then it is cooled in a desiccator and weighed until a constant weight is obtained (Pardede et al., 2013).

$$\% \text{ Water content} = \frac{W0 - W1}{W0} \times 100\%$$

Note:
W0 = Weight of Starting Substance
W1 = Constant Weight

**Ash Content**

1 gram of pectin was put into a constant weight crucible and put into the muffle furnace at 600°C for 3 hours. Then placed in a desiccator for one hour and then weighed until the weight is constant (Pardede et al., 2013).

$$\% \text{ Ash content} = \frac{\text{Weight of as}}{\text{Weight of pectin}} \text{ x } 100\%$$

**Determination of Pectin Equivalent Weight of Jackfruit Peel**

Determination of the pectin equivalent weight of jackfruit peel was carried out by acid-base titration. A 0.5 gram sample of pectin was added to 5 mL of 96% ethanol and dissolved in 100 mL of distilled water containing 1 gram of NaCl. The solution was titrated with 0.1 N NaOH using 1% phenolphthalein indicator. The titration is stopped when the color of the solution turns pink (pH 7.5) (Febriyanti et al., 2018)

$$\text{Equivalent weight} = \frac{\text{Weight of pectin (mg)}}{\text{V NaOH x N NaOH}}$$

**Determination of Methoxyl Pectin Levels in Jackfruit Peel**

The neutral solution of the equivalent weight determination was added to 25 mL of 0.25 N NaOH. The solution was homogenized and left for 30 minutes at room temperature in a closed state. Then 25 mL of 0.25 N HCl was added and titrated with 0.1 N NaOH using phenolptalein 1 indicator. The titration was stopped when the color of the solution turned pink (pH 7.5) (Febriyanti et al., 2018).

$$\% \text{ Methoxyl Content} = \frac{\text{V NaOH x 31 x N NaOH}}{\text{Weight of pectin (mg)}} \text{ x } 100\%$$

Note: 31 is the molecular weight of methoxyl (CH3O)

**Determination of Galacturonic Levels of Jackfruit Peel Pectin**

The level of galacturonic pectin in jackfruit peel is calculated based on the mEq (milliequivalent) value of NaOH obtained from the determination of BE and methoxyl content (Febriyanti et al., 2018).

$$\% \text{ Galacturonic} = \frac{176 \times 0{,}1z \times 100}{\text{Weight of samples (mg)}} \text{ x } \frac{31 \times 0{,}1y \times 100}{\text{Weight of samples (mg)}} \text{ X } 100\%$$

Note:

y = volume of NaOH from methoxyl determination

z = volume of NaOH from equivalent determination

176 = lowest equivalent weight of pectic acid

**Determination of Degree of Pectin Esterification of Jackfruit Peel**

Determination of the degree of esterification of jackfruit peel pectin is calculated based on the methoxyl content and galacturonic acid levels that have been obtained (Febriyanti et al., 2018).

$$\% \text{ Degree of esterification} = \frac{\% \text{ methoxyl x } 176}{\% \text{ galacturonict x } 31} \text{ x } 100\%$$

**Functional Groups Identification**

Jackfruit peel pectin samples were characterized by FTIR spectrophotometer to determine the functional groups of the compounds contained

## RESULT AND DISCUSSION

**Water Content**

The results obtained were that the pectin samples had a water content of 13%. The water content is higher than the standard set by IPPA (2002), which is a maximum of 12%. The high water content in the pectin produced can be affected by drying which is not optimal and also the storage conditions of pectin prior to the water content test. Storage in a damp place and a container that is not airtight will cause pectin to be exposed to outside air, so that the pectin becomes moist again. According to Fitria, (2013), the moisture content of pectin is affected by the degree of drying. If the degree of drying is low, what is seen is that the yield weight is greater than it really is.

The water content is also directly proportional to the ethanol concentration and soaking time. Ethanol can dehydrate pectin thereby disrupting the stability of its colloidal solution and as a result pectin will coagulate and during precipitation a replacement of water molecules by dissolved molecules occurs which results in wider contact between the pectin chains resulting in a complex network of polysaccharide molecules. Alcohol has a low molecular weight so it mixes perfectly with water through hydrogen bonds thereby reducing the number of ions or water molecules around pectin (Arimpi & Pandia, 2019).

The moisture content of the material greatly affects the shelf life of the material. Water content that is too high in the material causes the material's susceptibility to microbial activity (Martiyanti, 2017). Product with low water content are relatively more stable in long-term storage than product with high water content (Irawan & Prihanto, 2018). Drying is carried out at low temperatures to minimize pectin degradation (Hanum et al., 2012). As the settling time used increases, the resulting water content increases (Nantika, 2022).

**Ash Content**

The ash content indicates whether or not there are inorganic components left in the pectin after burning (Arimpi and Pandia, 2019). The gray ash content is the result of complete combustion using a combustion temperature of 550 to 600 ℃. Burning at higher temperatures can result in the loss of alkali and carbon dioxide content from carbonate compounds (Purnama & Tambun, 2015). Ash is defined as an inorganic material obtained from the residue or residue of burning organic matter (Nurviani et al., 2014). The mineral content contained in the material can be known from the ash content which also affects the level of pectin purity. The higher the level of pectin purity, the lower the ash content in pectin (Roikah et al., 2016).

The results of the analysis of ash content in this study amounted to 7.74%. The standard ash content set by IPPA (2002) is a maximum of 10%, so the ash content in this study has met the standards. Ash content testing was carried out to determine the residual levels of inorganic materials contained in the sample (Sulihono et al., 2012). Inorganic components can be in the form of calcium and magnesium hydrolyzed with protopectin. Ash content affects the level of pectin purity. The lower the ash content, the better the purity of the pectin (Picauly & Tetelepta, 2020). The ash content decreased with increasing concentration of ethanol as a precipitating agent. The decrease in ash content can also be caused by heating in acid with a long extraction time which will increase the hydrolysis reaction of protopectin which will turn into pectin and will precipitate a lot (Picauly and Tetelepta, 2020).

**Galacturonic Acid**

Polygalacturonic acid is the basic framework for pectin compounds that describe the purity of pectin (Munir et al., 2018). Galacturonic levels and pectin molecular charge play an important role in determining the functional properties of pectin solutions and affect the structure and texture of the pectin gel formed (Tuhuloula et al., 2013). The higher the galacturonic content, the higher the quality of the pectin (Chasanah et al., 2019).

The level of galacturonic is one of the parameters that determines the quality of pectin. Galacturonic acid consists of carboxyl groups which have the ability to bond with Mg2+ or Ca2+ ions so that polymer bonds can attach to one another. This causes a sticky feeling on the skin (Roikah et al., 2017). Pectin molecules are not straight, but coiled with fewer hydrogen bonds than in straight polymers such as cellulose. This is caused by the conformation of the chain, the polar position of the hydroxyl groups C2 and C3, there is no attraction between this hydroxyl group and the methyl group and the charge caused by the dissociated carboxyl group (Nantika, 2022).

The galacturonic content indicates the purity of pectin against other neutral organic matter, namely polysaccharides such as arabinnose, galactose and other sugars. The galacturonic content indicates the purity of the

pectin and is recommended not to be less than 65%.

Estimation of galacturonic acid content is very important to determine the purity and degree of esterification, as well as to evaluate the physical properties of pectin (Picauly and Tetelepta, 2020).

According to Nurviani et al (2014) the level of galacturonic acid is directly proportional to the quality of pectin. Based on research conducted by Arimpi & Pandia (2019), the higher the ethanol concentration and the longer the precipitation, the higher the galacturonic acid. This is because ethanol is polar so that it can precipitate more pectin and the longer the deposition, the hydrolysis reaction of protopectin to pectin will occur, the basic component of which is D-galacturonic acid. The galacturonic acid yield in this study was 9.488%. These results are still below the IPPA standard (2002) which states that the level of galacturonic acid pectin is at least 65%..

Previous research conducted by Kurniawan and Adenia (2022) showed high levels of galacturonic acid in pectin. This can be caused because with high concentrations pectin can turn into pectic acid so that the methyl ester group is reduced. The glycosidic bond of the pectin methyl ester tends to be hydrolyzed to galacturonic acid. Pectin will become pectic acid where galacturonic acid will be free from methyl esters (Kesuma et al., 2018). In addition, temperature and extraction time also affect the value of the degree of esterification. The higher the temperature and extraction time, the degree of esterification can reach optimum conditions, but there is also a decrease in the degree of esterification caused by the degradation of pectin compounds due to the depolymerization mechanism of the pectin galacturonic chains (Liew et al., 2014). In addition, pectin can also be degraded due to deesterification by increasing temperature and time.

**Equivalent Weight**

The content of free galacturonic acid groups that are not esterified in the pectin molecular chain is also called the equivalent weight (Aziz et al., 2018). The yield equivalent weight is determined based on the saponification reaction of the carboxyl group by NaOH where the equivalent weight will be inversely proportional to the volume of NaOH used to react with the carboxyl group. Pure pectic acid is a pectic substance which is entirely composed of polygalacturonic acid which is free from methyl ester groups or does not undergo esterification. The lower the pectin content, the lower the equivalent weight (Nantika, 2012).

The equivalent weight yield for pectin is 16,666.67 mg. The equivalent weight of pectin based on IPPA standards (2002) is in the range of 600-800 mg. The pectin produced in this study has an equivalent weight that does not meet the existing standards. The results obtained are still lower than the research conducted by Arimpi & Pandia (2019) and Latupeirissa et al., (2019). The equivalent weight of pectin depends on the type of plant, the quality of the raw material, the extraction method and the treatment in the extraction process (Febriyanti et al., 2018). The equivalent weight is inversely proportional to the degree of esterification between galacturonic acid and ethanol. The higher the degree of esterification, the lower the pectin equivalent weight.

Some things that can affect the equivalent weight value are the titration process and the nature of the extracted pectin itself. The molecular weight depends on the type of plant, the quality of the raw material, the extraction method, and the treatment in the extraction process. The higher the concentration, the higher the equivalent weight because high concentrations can cause the polymerization of pectin to be longer so that free acids are reduced (Nazaruddin, 2011). While the comparison of other data shows the opposite, namely the lower the pH, the lower the equivalent weight. The equivalent weight will decrease with the higher the extraction temperature and the longer the extraction time (Injilaudin et al., 2015).

**Methoxyl Levels**

Methoxyl content is the number of moles of ethanol contained in 100 moles of

galacturonic acid (Hanum et al., 2012). Methoxyl pectin levels have a very important role in determining the functional properties of pectin solutions and can affect the structure and texture of pectin gels (Arimpi and Pandia, 2019). Methoxyl levels affect the ability to form a good gel. The greater the methoxyl content, the greater the ability to form gels (Prasetyowati et al., 2009). Pectin can be called high methoxyl if it has a value of methoxyl content equal to or more than 7% whereas if methoxyl content is below 7% it can be said that the pectin has low methoxyl.

In this study, the methoxyl content obtained was 8.432% which was categorized as a pectin with high methoxyl content (> 7.12%) and met IPPA standards (2002). The methoxyl content was influenced by two factors, namely the concentration of ethanol during pectin deposition and the duration of pectin deposition. In a study conducted by Arimpi & Pandia (2019) the highest methoxyl levels were found in samples with the highest ethanol concentration variations (95%) with the longest immersion variation (20 hours). This is due to the process of demethylation and deesterification (hydrolysis of ester groups) in pectin which can increase the levels of methoxyl produced. The more oxygen dissolved in the solution will speed up the reaction, thus if the deposition is long it will result in a demethylation process. The demethylation process will move the extracted methyl groups resulting in many methyl groups being released (Lumbantoruan et al., 2014).

**FTIR Analysis**

Analysis using the Fourier Transform Infrared (FTIR) is an analysis aimed at identifying the functional groups present in the sample. The results of the FTIR absorption spectrum test in this study can be seen in Figure 1 which shows wave absorptions as a qualitative parameter of the functional group groups in pectin.

Highly methoxyl pectin forms a gel in the presence of sugars or acids. Meanwhile, low-methoxyl pectin can form gels in the presence of polyvalent cations such as calcium (Ardiansyah et al., 2014). Based on research conducted by Chasanah, (2019), methoxyl levels will increase as the concentration of ethanol as a precipitating agent increases. This is due to the process of demethylation and deesterification (hydrolysis of ester groups) in pectin which can increase the levels of methoxyl produced. The more oxygen dissolved in the solution will speed up the reaction, thus a long deposition will result in a demethylation process and will move the extracted methyl groups resulting in the release of many methyl groups (Arimpi and Pandia, 2019).

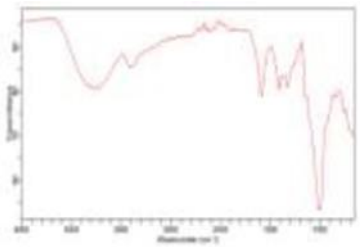

Figure 1. FTIR Results of Orange Peel Pectin

Table 1 shows the results of the absorption of FTIR waves in jackfruit skin pectin samples. This study uses wave numbers in the range of 4000-1000 cm-1. In general, the wave number area of 1000-2000 cm-1 is where the main pectin group is. Absorption at a wavelength of 3250.23859 cm-1 is the basis for indicating the presence of (-OH) groups in pectin. The stretching vibration of CH3 at the wavelength of 2899.86884 cm-1 is an indicator of the presence of methoxyl groups. Absorption at wave number 1587.84592 cm-1 for extracted pectin indicated the presence of a carboxyl group (C=O). Absorption at wave number 1416.38838 indicates the presence of a group (- O-) in jackfruit skin pectin (Nantika, 2022).

Table 1: FTIR Absorption Result of Jackfruit Skin Pectin

| Peak Number | Wavenumber ($Cm^{-1}$) | Intensity |
|---|---|---|
| 1 | 1013,83589 | 53,37083 |
| 2 | 1416,38838 | 80,58345 |
| 3 | 1587,84592 | 79,01083 |

| | | |
|---|---|---|
| 4 | 1878,57827 | 93,55517 |
| 5 | 2899,86884 | 85,50968 |
| 6 | 3250,23859 | 80,52972 |

The characterization that has been carried out shows that the orange peel pectin in this study is still not in accordance with the standards set by IPPA (2002). In line with several parameters such as moisture content, ash content, galacturonic acid, and equivalent weight which are not in accordance with IPPA standards (2002). Water content is 1% higher than standard. The temperature and drying time can be increased so that the pectin water content is reduced. The pectin ash content of orange peel is still higher than the standard, so it is necessary to reduce the acidic pH during the extraction process. Galacturonic acid is still below standard because there are non-uronic compounds which are also extracted (Antika & Kurniawati, 2017). The lower the acid pH and the longer the extraction temperature during extraction, the galacturonic acid level will increase. The equivalent weight is affected by the temperature during extraction. The higher the temperature, the lower the equivalent weight (Fitria, 2013).

## CONCLUSION

From the discussion that has been described, it can be concluded that orange peel contains pectin. The pectin isolated from orange peel in this study did not meet the pectin standard set by the International Pectin Producers Association. In line with several parameters such as moisture content, ash content, galacturonic acid, and equivalent weight which are not in accordance with the standards set by IPPA (2002).

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