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# Characterization of Siam Orange Peel Pectin Using NADES and Pre-Treatment of Pressing Temperature

*Rizky Astricia Putri, Anwar Kasim and Fitriani Kasim*

*Department of Agricultural Industrial Technology, Faculty of Agricultural Technology, Andalas University, West Sumatra, Indonesia,*

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CORRESPONDING AUTHOR

*E-mail: anwarkasim@ae.unand.ac.id

**ABSTRACT**

Pectin has been widely used as a gelling agent, thickener, and stabilizer, especially in the food industry. Pectin is obtained by extracting plant parts such as orange peel. Solvents are needed in the pectin extraction process. Usually, inorganic solvents used, such as HCl and $H_2SO_4$, have negative impacts on researchers and the environment. In this study, NADES (Natural Deep Eutectic Solvent) was used with citric acid and sucrose, which are safer and more environmentally friendly. Pre-treatment by pressing was carried out to reduce the water content so that the orange peel could be changed into a simplex and ready to be extracted from its pectin. This study aims to see the effect of pre-treatment pressing temperature and the ratio of materials and volume of NADES on the characteristics of the pectin produced. The results showed that pre-treatment pressing temperature and the ratio of materials and volume of NADES had a significant effect ($p < 0.05$) on the equivalent number, methoxyl content, galacturonic acid content, degree of esterification, and water content.

## 1. INTRODUCTION

### *1.1. Research Background*

The production of Siamese oranges in Indonesia is entirely developed. In 2022, Siamese orange production in Indonesia was 2.551.999 tons. Meanwhile, Siamese orange production in West Sumatra province in 2022 was 117.494 tons. Following the increase in orange production, waste in the form of orange peels has also increased [1]. The proportion of orange peels reaching 35% of the total citrus fruit will also increase [2]. According to [3], the thickness of Siamese orange peel ranges from 1.8 mm to 2.5 mm. The most dominant component of orange peel is pectin, with a content of around 18-20% [4]

Pectin is obtained by extraction using organic and inorganic solvents such as hexane, ethyl acetate, ethanol, HCl, H2SO4, and others, which have adverse effects on both researchers and the environment because they are toxic, volatile, and flammable [5]. Currently, many studies are trying to develop the use of environmentally friendly Natural Deep Eutectic Solvent (NADES) as an alternative solvent in the extraction process. The NADES groups that can be used based on their constituent compounds are (1) Ionic liquids consisting of organic acids; (2) Neutral NADES, namely a mixture of polyalcohols; (3) Neutral NADES and a combination of acids, for example, a mixture of sucrose and citric acid; (4) Basic NADES consisting of primary and neutral compounds; (5) Amphoteric NADES, namely a mixture of amino acids, polyalcohols and acids [6]. There is an essential stage in the production of pectin, namely the preparation of raw materials, where pre-treatment is carried out to remove dirt, sugar compounds, and other dissolved solids. This process also aims to inactivate the pectin esterase enzyme, which can hydrolyze pectin into pectate [7].

There are several ways to prepare raw orange peel materials for pectin. The most common way is to reduce the water content by drying it directly in the sun or using the oven method. However, a pre-treatment method can decrease the water content by using a hot press. In addition to reducing the water content, hot presses can break down cell walls, making the extraction process more accessible. There are several common ways to prepare orange peel raw materials for making pectin.

### *1.2. Literature Review*

#### *1.2.1. Siamese orange*

Siamese oranges are one of Indonesia's most widely cultivated local citrus fruits. They are easily accessible to the public because they are evenly distributed throughout the country [8].

The proportion of orange peel is 35% of the total citrus fruit [2]. If orange production in 2022 is 2.551.999 tons [1], the potential for orange peel waste produced is estimated to reach 893.199 tons wet weight. The most dominant component of orange peel is pectin, with pectin content in dry orange peel ranging from 18-20% [4].

#### *1.2.2. Pectin*

Pectin is a reversible colloid that can dissolve in water, precipitate, dry, and redissolve without changing physical properties. When water is added, pectin forms a paste before dissolving [9]. Pectin is found in plant parts as a structural element in tissue growth and the main component of the middle layer of plants. Pectin also acts as an adhesive and maintains tissue and cell stability. Pectin is a high molecular weight polysaccharide compound; pectin is used as a gelling agent and thickener in jelly production [10]. Pectin quality standards can be seen in Table 1 [11].

**Table 1**. Pectin Quality Standards (SNI 01-2238-1991)

| Pectin Quality | Contents |
|---|---|
| Methoxyl Content | |
| - High Methoxyl | >7.12% |
| - Low Methoxyl | 2.5-7.12% |
| Galacturonic acid levels | Min 35% |
| Drying Loss (Moisture Content) | Max 12% |
| Degree of Esterification | Max 10% |
| - High ester pectin | Min 50% |
| - Low Ester Pectin | Max 50% |
| Equivalent numbers | 600-800 mg/Eq |

#### *1.2.3. Pectin Extraction*

Extraction based on its type is divided into solid-liquid extraction, leaching, and liquid-liquid extraction. Solid-liquid extraction or leaching is the process of separating solutes from insoluble solids called inert. There are two main steps in the solid-liquid extraction process: contact between the solid and the solvent and the separation of the solution from the inert solid. Solvents used for the extraction process must be required to dissolve the solute in the inert solid [9]. One extraction method is microwave-assisted extraction (MAE). MAE is an extraction method that uses microwave radiation to selectively accelerate extraction by heating the solvent quickly and efficiently [12]. Microwave techniques provide tangible benefits by shortening extraction time and reducing energy consumption [13].

In the extraction process, a solvent is needed. Organic solvents commonly used to obtain pectin generally include HCl, $H_2SO_4$, and others that adversely affect researchers and the environment because they are toxic, volatile, and flammable [5]. So, there is a need for a solvent with a new concept of "green solvent" that provides minimal detrimental effects on researchers, the environment, and equipment. Solvents, such as recently discovered ionic liquids, are Natural Deep Euthetic Solvent (NADES) to extract biopolymers and various plant molecules [14].

### *1.3. Research Object*

This research aims to (1). This research aims to see the effect of pre-treatment pressing temperature and the ratio of ingredients and volume of NADES on the characteristics of pectin produced according to Indonesian National Standards (SNI)

## 2. MATERIALS AND METHODS

### *2.1. Research Location*

The research was conducted in the Laboratory of Agricultural Product Biochemistry and Food Nutrition, the Laboratory of Bioindustry Technology and Agro-Industry Environment, and the Instrument Laboratory of the Faculty of Agricultural Product Technology, Andalas University.

### *2.2. Materials and Tools*

The materials used are waste of Siamese orange peel obtained from squeezed orange juice traders around the Padang City Sport Centre, citric acid, sucrose, water, 96% ethanol, pp indicator, 0.1N NaOH, aluminium foil, calico cloth, and muslin cloth. The tools used are 1 set of hot hydraulic press, microwave oven (LG MS2322D), centrifuge (Hettich Max Universal 320 Cat: 1401), FTIR spectrophotometer (Shimadzu FTIR Tracer-100), hotplate, magnetic stirrer, oven, furnace, Erlenmeyer flask, beaker, funnel..

### *2.3. Research Design*

The design used in this study was a factorial Completely Randomized Design (CRD) with two factors, namely factor A is the pressing temperature pre-treatment consisting of 3 levels, namely (A1 = pressing at room temperature, A2 = pressing at a temperature of 50 ºC and A3 = pressing at a temperature of 75 ºC) and factor B is the ratio of materials and NADES volume (b / v) consisting of 3 levels, namely (B1 = 1: 5, B2 = 1: 10 and B3 = 1: 15). The data obtained were then analyzed statistically using analysis of variance (ANOVA). The results of the study of the data obtained if Fcount ≥ Ftable, then further testing was carried out with Duncan's New Multiple Range Test (DNMRT). The value is declared significant if ($p<0.05$) and not significant if the value is ($p>0.05$).

### *2.4. Research Procedures*

The peel of the Siamese orange obtained from the squeezed orange juice traders around the Padang City Sports Centre was cleaned with running water and then dried. Then, it was reduced in size, put into a ring press lined with calico cloth, and given a pressing pre-treatment with a pressing temperature (room temperature, 50ºC and 75ºC). The orange peel was dried again using a microwave oven with medium-low power for 2 minutes. The orange peel was then ground with a grinder and sieved with a 40-mesh sieve. NADES as a solvent was made by mixing 1 mol of citric acid with 4 mol of sucrose into 18 mol of water. The mixture was heated with a magnetic stirrer at a temperature of 70ºC until a clear liquid was formed, then this NADES was diluted with distilled water in a ratio of 1:1. Extraction of Siamese orange peel pectin was carried out using the microwave-assisted extraction method according to the treatment where the power used was medium-low for 15 minutes. The extracted orange peel is then separated between the filtrate and residue; the residue is added with 96% ethanol, as much as 2x the filtrate. Left for 18 hours, then filtered using muslin cloth. The gel obtained is pectin,

which must then be washed with 96% ethanol until the washing water becomes clear. The wet pectin is then dried in an oven at 75ºC for 5 hours. The resulting dry pectin is then ground using a mortar.

### *2.5. Analysis Method*

The analysis observed in this study includes an equivalent number [15], methoxyl content [15], galacturonic acid content [15], degree of esterification [16], and water content using the oven method.

## 3. RESULTS AND DISCUSSION

### *3.1. Extracted Pectin*

The physically extracted pectin from the peel of the Siamese orange has a rough texture with coarse grains and a brownish-yellow color, as Table 2 shows.

**Table 2**. Pectin Extraction Results

| Treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A1B1 | A1B2 | A1B3 | A2B1 | A2B2 | A2B3 | A3B1 | A3B2 | A3B3 |
|  |  |  |  |  |  |  |  |  |

Pectin was then analyzed for equivalent numbers, methoxyl content, galacturonic acid content, degree of esterification, and water content. Table 3 below shows the characterization of Siamese orange peel pectin.

**Table 3**. Characterization of Siam Orange Peel Pectin

| Treatment | Parameters | | | | |
|---|---|---|---|---|---|
| | Equivalent weight (mg/eq) ± SD | Methoxyl content (%) ± SD | Galacturonic acid (%) ± SD | Esterification degree (%) ± SD | Water content (%) ± SD |
| A1B1 | 1065.25 ± 4.52* | 8.85 ± 0.04* | 66.78 ± 0.29* | 75.26 ± 0.01* | 8.46 ± 0.02* |
| A1B2 | 925.74 ± 2.54* | 9.34 ± 0.05* | 72.06 ± 0.33* | 73.62 ± 0.05* | 7.74 ± 0.02* |
| A1B3 | 865.79 ± 3.59* | 9.83 ± 0.02* | 76.15 ± 0.14* | 73.30 ± 0.90* | 5.65 ± 0.02* |
| A2B1 | 854.87 ± 3.69* | 8.18 ± 0.02* | 67.02 ± 0.16* | 69.28 ± 0.08* | 6.28 ± 0.02* |
| **A2B2** | **754.58 ± 3.64*** | **8.87 ± 0.03*** | **73.69 ± 0.17*** | **68.35 ± 0.15*** | **5.85 ± 0.01*** |
| **A2B3** | **726.19 ± 2.08*** | **9.12 ± 0.02*** | **75.99 ± 0.17*** | **68.11 ± 0.08*** | **3.36 ± 0.01*** |
| **A3B1** | **664.8 ± 1.09*** | **7.32 ± 0.01*** | **68.06 ± 0.14*** | **61.10 ± 0.01*** | **4.67 ± 0.01*** |
| **A3B2** | **644.21 ± 3.20*** | **7.73 ± 0.02*** | **71.21 ± 0.24*** | **61.63 ± 0.08*** | **3.55 ± 0.04*** |
| A3B3 | 574.53 ± 1.91* | 8.24 ± 0.01* | 77.42 ± 0.12* | 60.43 ± 0.08* | 2.06 ± 0.02* |

Notes: *: significantly different ($p<0.05$)
Treatments in bold fulfill SNI

Based on Table 3, the average equivalent weight of pectin obtained in this study ranged between 574.53 mg/eq and 1065.25 mg/eq. The analysis showed that the highest equivalent weight was treated with A1B1 (pressing temperature at room temperature and difference in material and volume ratio NADES 1:5) of 1065.25 mg/eq. The treatment that produced the lowest equivalent weight was treatment A3B3 (pressing temperature 75°C and difference in material and volume ratio NADES 1:15) of 574.53 mg/eq.

The higher the press temperature, the lower the equivalent weight produced because the higher the temperature will result in de-esterification and depolymerization of pectin. De-esterification will release methoxyl groups, while depolymerization will break the pectin from its polymer chain. Both reactions will reduce the number of functional groups, contributing to calculating the equivalent number. In addition, the greater the difference in the ratio of ingredients and volume of NADES used, the lower the equivalent weight will be. This is because a larger solvent volume allows the pectin to be better dispersed. However, better dispersion can also increase pectin depolymerization, breaking the polymer chain into smaller molecules. These smaller pectin molecules have fewer active groups per unit mass, which can lower the equivalent number [17].

The methoxyl content obtained in this study ranged from 7.32% - 9.83%. The highest methoxyl content was produced in the A1B3 treatment (pressing temperature at room temperature and the difference in the ratio of materials and NADES volume 1:15) at 9.83%. The lowest methoxyl content was produced in the A3B1 treatment (pressing temperature 75°C and the difference in the ratio of materials and NADES volume 1:5) at 7.32%. Based on the results of this study, the higher the pressing temperature, the lower the methoxyl content. An increase in pressing temperature can cause a de-esterification reaction, which is a process in which the methoxyl group (-OCH3) on the galacturonic acid chain is released due to the breaking of the ester bond. Degradation of ester bonds in pectin can occur because the methoxyl group is susceptible to thermal degradation so that it can cause a decrease in methoxyl content [18]. Conversely, the greater the ratio of ingredients and the volume of NADES used,

the higher the methoxyl content produced. It is because, with the increasing volume of NADES, pectin can be dispersed better so that more pectin molecules containing methoxyl are dissolved in the solution. In addition, a larger volume of solvent can reduce the risk of degradation during extraction. Thermal or chemical degradation of pectin can cause a decrease in methoxyl content. With more solvent, the risk of degradation due to heat or extreme pH can be minimized to retain more methoxyl [19].

Based on Table 3 above, the different pressing temperatures and solvent volumes significantly affect the levels of galacturonic acid in pectin obtained. The average levels of galacturonic acid in pectin obtained in this study ranged between 66.78%-77.42 %. The treatment that produced the highest galacturonic acid content was the A3B3 treatment (pressing temperature 75°C and the difference in the ratio of materials and NADES volume 1:15) of 77.42%. The treatment that produced the lowest galacturonic acid content was the A1B1 treatment (pressing temperature room temperature and the difference in the ratio of materials and NADES volume 1:5) of 66.78%. High temperatures can accelerate the breakdown of pectin chains through hydrolysis reactions. Hydrolysis of pectin is the release of galacturonic acid from its polymer structure. Thus, the higher the temperature, the more galacturonic acid will be released into simpler galacturonic acid units [20]. In addition, the greater the ratio of material and volume of NADES used, the more galacturonic acid that is bound in pectin will also be extracted as the volume of the solvent increases because the excess solvent can help separate pectin more thoroughly from the source material, thereby increasing the galacturonic acid content in the extraction results [21].

Based on Table 3, The pressing temperature and the different ratios of materials and volumes of NADES significantly affected the degree of pectin esterification obtained. The degree of pectin esterification obtained in this study ranged from 60.43%-75.26 %. In this study, the treatment that produced the highest degree of esterification was the A1B1 treatment (pressing temperature at room temperature and the difference in the ratio of materials and volume of NADES 1:5) of 75.26%. The treatment that produced the lowest degree of esterification was A3B3 (pressing temperature 75°C and the difference in the ratio of materials and volume of 1:15) of 60.43%). At higher pressing temperatures, there will be a decrease in the degree of esterification caused by the degradation of pectin compounds due to the depolymerization mechanism of the pectin galacturonic chain [20]. Higher temperatures tend to accelerate chemical reactions, including hydrolysis reactions in pectin. As the temperature increases, the ester bond between the carboxyl group of galacturonic acid and the methoxyl group becomes less stable and breaks down more quickly. This hydrolysis process will release the methoxyl group, decreasing the pectin's esterification. Therefore, an increase in temperature decreases the degree of esterification as more carboxyl groups are not esterified. In addition, the greater the difference in the ratio of ingredients and solvent volume, the lower the degree of esterification. A large volume of solvent provides more medium for hydrolysis reaction, which can accelerate the breaking of ester bonds in pectin. As a result, more ester bonds are broken, and the degree of esterification decreases. However, these ester bonds do not always come from methoxyl groups. Ester groups other than methoxyl, such as acyl or alkyl, can be hydrolyzed or broken down, while the methoxyl group remains bound. Thus, when non-methoxyl ester groups are de-esterified, the degree of esterification will decrease because the total number of ester groups is reduced. However, the methoxyl content may increase because this disconnection does not affect the existing methoxyl groups [22].

Based on Table 3 above, the percentage of water content produced in this study ranged from 2.06% to 8.46%. Based on the study's results, the treatment that gave the highest water content was treatment A1B1 (pressing temperature at room temperature and the difference in the ratio of materials and NADES volume 1:5) of 8.46%. The treatment that gave the lowest water content was treatment A3B3 (pressing temperature 75°C and the difference in the ratio of materials and NADES volume 1:15) of 2.06%. The higher the pressing temperature, the lower the water content of siamese orange peel pectin. The higher the temperature, the more water can be evaporated from the pectin, resulting in a low pectin moisture content [23] . The higher the difference in the ratio of ingredients and the volume of NADES, the lower the water content obtained. It is because the large volume of NADES can hydrolyse the pectin polymer so that the molecular chain becomes shorter. When the pectin polymer chain is broken, the water groups in the material will come out into free water, which causes the water content in the material to decrease [24].

## 4. CONCLUSION

The pre-treatment pressing temperature and the ratio of material and volume of NADES have a significant effect ($p<0.05$) on the equivalent number, methoxyl content, galacturonic acid content, degree of esterification, and water content of Siam orange peel pectin. Treatments that meet SNI for pectin are A2B2 (pre-treatment of pressing temperature 50ºC and NADES material to volume ratio 1:10), A2B3 (pre-treatment of pressing temperature 50ºC and NADES material to volume ratio 1:15), A3B1 (pre-treatment of pressing temperature 75ºC and NADES material to volume ratio 1:5) and A3B2 (pre-treatment of pressing temperature 75ºC and NADES material to volume ratio 1:10). The resulting weight equivalents (mg/eq) are 754.58 (A2B2), 726.19 (A2B3), 664.8 (A3B1), and 644.21 (A3B2). The resulting methoxyl content (%) are 8.87 (A2B2), 9.12 (A2B3), 7.32 (A3B1), and 7.73 (A3B2). The galacturonic acid content (%) are 73.69 (A2B2), 75.99 (A2B3), 68.06 (A3B1), and 71.21 (A3B2). Esterification degree (%) are 68.35 (A2B2), 68.11 (A2B3), 61.10 (A3B1), and 61.63 (A3B2). Water content (%) are 5.85 (A2B2), 3.36 (A2B3), 4.67 (A3B1), and 3.55 (A3B2).

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