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Extraction of pectin from pomelo *(Citrus maxima)* peels with the assistance of microwave and tartaric acid

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Abstract

Pectin is a component of the plant cell and can be found in the primary cell wall and the middle lamella of plant tissues. Currently, pectin is a very important raw material, especially in the food and the pharmaceutical industries. There are several ways to extract pectin from different materials and solvents. One way to synthesize pectin is from the extraction of pomelo peel and this research specifically studies the extraction using tartaric acid as the solvent and under microwave assistance. Some properties of the synthesized pectin were tested such as DE and viscosity. The pH value, the rate of pomelo peels/solvent (w/v), the level of irradiation power and the irradiation time affected the productivity of pectin. The results showed that at pH value of 1.5, rate of pomelo peel/solvent was 1/40 and the irradiation time during 9 minutes at 660W of microwave power. The yield of pectin obtained 23.83%, pure pectin was 80.88% and it was rated as a high methoxyl pectin (HMP) (DE = 92.75%) with a low viscosity. The study proved that the use of tartaric acid solvent and microwave increased the quality and yield of synthesized pectin; moreover, this method was also time and energy saving.

Introduction

Among other polysaccharides that are extracted from plant materials, pectin is an extremely complex polysaccharide and is widely used as a functional ingredient in food and pharmaceutical industries. In food technology, it is used as a thickening and gelling agent (May, 1990). Its medical uses are anti-diarrhea, detoxification and blood glucose reducer (Voragen *et al*., 1995). The worldwide annual consumption of pectin is approximately 45 000 tones every year, occupying the global market value of at least 400 million Euros (Savary *et al*., 2003). Pectin consists of D–galacturonic acid units and is classified into two groups: high methoxyl pectin (HMP) and low methoxyl pectin (LMP) that depend on the degree of esterification (DE). There are many sources of pectin in nature such as pomelo peel, apple pomace, citrus peel, sugar beets, dragon fruit peel, etc… Apart from the apple pomace and the citrus peel which are the most common commercial sources for producing pectin, other novel sources, including sugar beets and sunflower heads, have also been investigated (Joye and Luzio, 2000).

Pomelos *(Citrus Maxima)* belongs to *Citrus* group and *Rutaceace*. Pomelo is widely planted in Vietnam with different varieties (Quoc *et al*., 2014). The spongy white peel can account up to 30% of the total fruit weight and is a good source for pectin extraction (Methacanon *et al*., 2013). The extraction of pectin usually uses two kinds of solvents: inorganic acid solvent such as hydrochloric, sulfuric or nitric acid and organic acid solvent such as oxalic, tartaric or acetic acid. All of them utilize traditional heating. However, it is proved that the microwave extraction shortens the pectin extraction time, also reduces the solvent consumption and higher yields than the conventional method did (Seixas *et al*., 2014). Utilizing the microwave to aid the heating process and tartaric acid as solvent to extract pectin from orange peel (Liang *et al*., 2011) and passion fruit (Seixas *et al*., 2014). The extracted pectin from both studies were proved to have high yield, also time and energy saving. Recognizing the potential of microwave assistance and tartaric acid solvent in pectin extraction, this research focuses on pectin extraction using pomelo peels as raw material due to its abundant growth in Vietnam region. The following properties would be reported to rate the effectiveness of this synthesis: the pH value, the ratio of pomelo peel/solvent, the level of irradiation power and the extracted time.

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Material and Method

Materials

The ripe "Nam Roi" pomelo was chosen as the raw material in these experiments. It was harvested from Vinh Long province (Vietnam). The spongy white peels were removed using a paring knife and cut into small pieces, then they were blanched in steam at 90°C for 5 minutes (Guo *et al*., 2011). After blanching, the peels were dried at 50°C for about 10 hours (Moisture of dried peels were approximate 9%) and grounded into powder (Particles size was under 0.6 mm).

Isolation of pectin

Five gram of pomelo peels were acidified with 0.25% (v/v) tartaric acid solution with ratio of peels/ acid 1/70, 1/60, 1/50, 1/40 and 1/30 (Joye and Luzio, 2000) and acid pH 1, 1.5, 2, 2.5 and 3 (Zhou *et al*., 2010). It was then placed in the microwave for 3, 6, 9 and 12 minutes (Seixas *et al*., 2014) at the power 195, 379 and 660W (Quoc *et al*., 2014). After microwave heating, the mixture was cooled down to room temperature and filtered using filter fabric. Then 96% (v/v) ethanol solution was used to precipitate pectin (the pectin solution/alcohol ratio was 1/3) for 60 minutes at pH 3.5. The precipitated pectin was washed with 96% ethanol to remove the mono and disaccharides. At the end, the mixture was dried at 70°C for 4 hours and then stored in bags.

Determination of content of pectin

According to Mui (2001), crude pectin (0.15 g) was added in 250 ml flask, then adding 100 ml of 0.1 N NaOH. Crude pectin was soaked in NaOH solution for 7 hours, then added 50 ml of 1 N CH_3COOH and 50 ml $CaCl_2$ after 5 minutes and kept it in 1 hour. The solution was boiled for 5 minutes, filtered by filter paper and dried for 1 hour. Calcium pectate was washed with hot water until not having Cl^- ion in the solution. After washing, it was dried for about 2 hours at 105°C. The pure level of pectin was calculated according to the following formula below:

$$P = \frac{m \times 0.92 \times 100}{M}$$

P (%): the pure level of pectin
m (g): weight of calcium pectate
M (g): weight of crude pectin
0.92: pectins have 92% in volume of calcium pectate

The yield of pectin was determined by formula:

$$Y = \frac{a_0}{a} \times 100$$

Y (%): the yield of pectin
a_0 (g): the weight of pectin (a_0 = PxM)
a (g): the weight of dried pomelo peel

Determination of degree of esterification (DE)

This method was slightly modified from titrimetric method of Owens *et al*. (1952) and Pinheiro *et al*. (2008). Pectin (0.5 g) was added in 250 ml flask and dissolved in 5 ml ethanol, 1 g NaCl and some drops of phenolpthalein. Adding 100 ml of warm deionized water dissolved pectin. The solutions were titrated with 0.1 N NaOH and the result was recorded as V_1. Then 25 ml of 0.25 N NaOH was added in this solution which were stirred at room temperature for 30 minutes. After that, 25 ml of 0.25 N HCl was added and the solutions were shaken until the pink color disappeared. The solution was titrated again with 0.1 N NaOH and the final result was recorded as V2. The DE value was calculated according to the following formula below:

$$\%DE = \frac{V_2 \times 100}{V_1 + V_2}$$

Determination of viscosity of pectin

Determination of viscosity by Rheometer (Brookfield DV–III Ultra–USA), spindle "61" and speed 100 rpm.

Color evaluation

Color parameters were measured on pectin samples (powder form) and color was determined using by a Chroma Minota CR-410. Values were recorded as lightness (L*, ranging from 0 to 100, corresponding to black to white, respectively), chroma (C*, representing color intensity or saturation), and hue angle (h°, representing by degrees of the angle) (Cook, 2000).

Results and Discussion

Effect of pH on the extraction yield of pectin and DE value

The extraction of pectin is influenced by several variables such as temperature, extraction time, pH, solid/solvent ratio (May, 1990), irradiation time and irradiation power (Wang *et al*., 2007; Seixas *et al*., 2014). Figure1 shows that there are significant differences at $p_{value} = 0.05$ from the pH values. As the pH increases, the pectin yield decreases. The highest yield was 32.43% at pH = 1.5 but DE had the minimum value (76.39%). The lowest yield was 6.23% at pH = 3. The lower the pH values were the presence of H^+ ions increased, hence it would

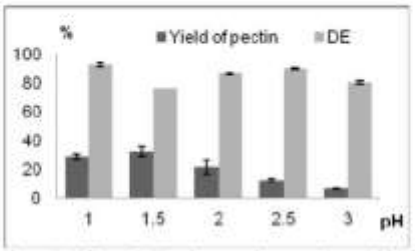

Figure 1. Effect of pH values on the extraction yield of pectin and DE value

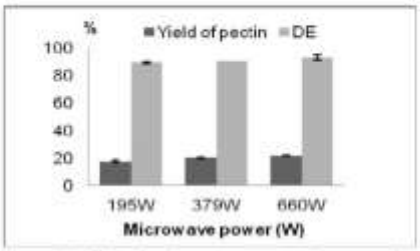

Figure 3. Effect of microwave power on the extraction yield of pectin DE value

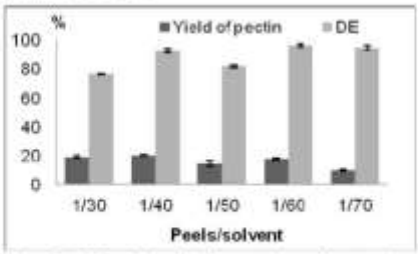

Figure 2. Effect of peels/solvent ratio on the extraction yield of pectin and DE value

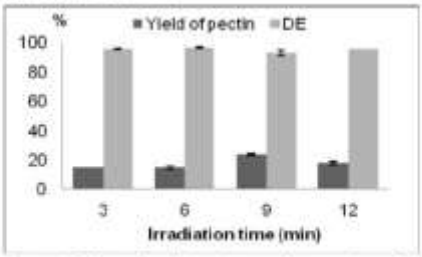

Figure 4. Effect of irradiation time on the extraction yield of pectin and DE value

increase the hydrolysis protopectin (Kertész, 1951). Besides, reducing pH value could promote the release of the pectin molecules from raw peel because the linkage of pectin with hemicellulose was separated (Rombouts and Thibault., 1996). Using pomelo, the content of pectin was higher than using from lemon, grapefruit, orange whose the content of pectin were respectively 28.7%, 22.9% and 19.3% with HCl solvent ratio of 1/60 at 80°C, for 1 hour and pH of 1.6 (Huang, 1973). The range of DE was greater than 50% (from 76.39 to 92.85%), so the type of pectin was HMP. Pomelo extracted pectin also had higher DE than lemon extracted one (DE = 64.15 ± 1.65%) with citric acid solvent (pH = 2), microwave operated at 628W at pH 2 and irradiation time of 9 minutes (Seixas et al., 2014). Thus, the optimum pH value at 1.5 was chosen for the next survey.

Effect of peels/solvent ratio on the extraction yield of pectin and DE value

There are significant differences at $p_{value} = 0.05$ from peels/solvent ratios (w/v). At the peels/solvent ratio of 1/40, the extraction yield obtained its highest value (20.44%) and the DE value was 92.75% (Figure 2). The use of large amounts of water would reduce the viscosity of the solution, so extraction process was more efficient. In addition, the high concentration gradient of solvent/peels can impulse the extraction speed (Coulson and Richardson, 1978). However, this ratio was quite low, the concentration of pectin in the extraction solution was also low. The water in solution increased, thus the alcohol volume for precipitating pectin also increase dramatically and this would not be effective economy (El-Nawawi and Shehata, 1987). The obtained results were higher compared the study conducted by Zhou *et al.* (2010). In their research, pectin was extracted from pomelo using tartaric acid as a solvent and conventional heating. The content pectin was 18.73% at pH of 1.5, 90°C, 60 minutes and the material/solvent ratio of 1/15.

Effect of microwave power on the extraction yield of pectin and DE value

Theoretically, the microwave irradiation disintegrates wall of cells and cut off the soft tissues in the cell. Thus, the cells decays quickly due to electromagnetic energy (Bagherian *et al.*, 2011). As the microwave capacity increases, the solution temperature also increases. At the moment, the dielectric constant of water decreased and it related to the pectin soluble in water (Hoshino *et al.*, 2009). Rising temperatures promoted the extraction because the diffusion coefficient would increase (Coulson *et al.*, 1978; Treybal, 1980).

There are significant differences at $p_{value} = 0.05$

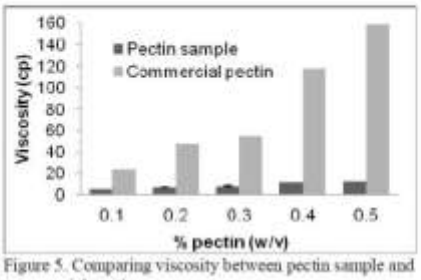

Figure 5. Comparing viscosity between pectin sample and commercial pectin

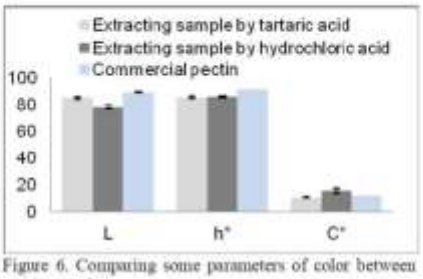

Figure 6. Comparing some parameters of color between pectin samples

from microwave powers, the highest pectin content was 21.66% at 660W and the lowest pectin content was 17.44% at 195W. DE values were approximate 89.23 - 92.75% (HMP) (Figure 3). Thus, the irradiation power at 660 W was the optimum value in this survey.

The yield achieved the highest value at the highest microwave power. It was consistent with the study by Bagherian *et al.* (2011), this results was higher than one from Liang *et al.* (2011) (18.73%), which combined tartaric acid and microwave to extracting pectin from orange peel at pH 2.0, 280W, irradiation time of 5 minutes and the material/solvent ratio of 1/10.

Effect of irradiation time on the extraction yield of pectin and DE value

There are significant differences at $p_{value} = 0.05$ from irradiation time, the highest pectin content was 23.83% at 9 minutes and the lowest pectin content was 14.72% at 6 minutes. DE values were approximate 92.75 - 96.7% (HMP) (Figure 4). Thus, the irradiation time at 9 minutes was the optimum value.

Extraction time was very important to extract pectin; if the extraction process was extended for long time, the pectin content can be destroyed by high temperatures (Vo and Luong, 2010). Besides, increasing the extraction time resulted pectic acid by product which was hydrolyzed by soluble pectin. Thus it reduced extraction yield of pectin. When extraction time was short, the links between pectin with other components such as cellulose, hemicellulose could not be severed, protopectin was also hydrolyzed to soluble form. It could influence the extraction efficiency (Kertest, 1951; Albersheim *et al.*, 1960).

Properties of pectin sample

Viscosity

Pectin solution is a non Newtonian fluid but it can become the Newtonian fluid at the low concentration (< 0.5%, w/v) (Voragen *et al.*, 1995). The pectin concentration and the viscosity value are directly proportional (Figure 5). The viscosity of sample was much lower than commercial pectin and this viscosity was similar with result of Quoc *et al.* (2014) (< 20cp), extracting pectin from pomelo peel used oxalic acid as a solvent and combined with microwave.

Color of pectin samples

There are significantly differences ($p_{value} < 0.05$) from L, h°and C° between the three kinds pectin (extracted pectin using tartaric acid, extracted pectin using hydrochloric acid, and commercial pectin). The L° value of received pectin (85.19) was higher than pectin extraction by HCl (78.13) (control sample) and lower than commercial pectin (89.37). Conversely, the C° value of received pectin was the lowest value (10.96) (Figure 6). Pectin product in study was bright white color similar the commercial pectin.

Conclusions

In this study, acid tartaric and microwave heating affected strongly to the pectin extraction and its properties. The optimum condition to extract pectin were at pH (1.5), rate of material/solvent (1/40), irradiation time (9 minutes), irradiation power (660W), the pure pectin peaked 23.83%. The obtained pectin was high methoxyl pectin (DE value was over 90%) and low viscosity. It can use in food industry, especially beverage and jam processing.

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Characterization and some Bioactivities of the Synthesized Citrus Pectin-ZnO Nanocomposites from Citron and Pomelo Fruits Peels

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Abstract

Pectin was extracted from the peels of citrus fruits (Citron: *Citrus medica* L. and Pomelo: *Citrus maxima* Merr.). In the extraction of fresh and dry pectin, acidic hydrolysis of the fresh or dry fruit peel samples was carried out followed by precipitation with ethanol. The yield percents of extracted pectins were 4.53 % (based on fresh peel) and 21.41 % (based on dried peel) from citron peels, and 3.03 % (based on fresh peel) and 9.18 % (based on dried peel) from pomelo peels. Extracted pectins were characterized by XRD, SEM, FT IR and TG-DTA analysis. The citrus pectin–ZnO nanocomposites were prepared by using co-precipitation method. Citron peel pectin-ZnO (CPPT-ZnO) nanocomposite (90.25 % yield) and pomelo peel pectin-ZnO (PPPT-ZnO) nanocomposite (64.95 % yield) were prepared by using zinc nitrate and 0.2 M sodium hydroxide solution at 28 ± 0.5 °C. The stirring time require for CPPT-ZnO was found to be 1.5 h and that required for PPPT-ZnO was 2h. The characteristics of the prepared citrus pectin-ZnO nanocomposites were studied by XRD, SEM, FT IR, TG-DTA, AAS and ED XRF (with C-H balance) spectroscopic methods. The crystallite sizes of CPPT-ZnO and PPPT-ZnO were 32.30 nm and 24.46 nm determined by XRD analysis.

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The morphological observation of the SEM results revealed that the sizes of ZnO in CPPT-ZnO and PPPT-ZnO were 70.59 nm and 61.55 nm, and were embedded in the pectin matrix. AAS analyses showed that the zinc ion concentrations in CPPT-ZnO and PPPT-ZnO prepared at 28 ± 0.5 °C were 3.88×10^5 ppm and 5.27×10^5 ppm. Both of the tested samples (CPPT-ZnO and PPPT-ZnO) were observed to show antimicrobial activity with inhibition zone diameters ranged between 15 mm to 20 mm against two tested microorganisms such as *Bacillus substilis* and *Staphylococcus aureus* and only CPPT-ZnO agansted *Escherichia coli* with inhibition zone diameters of 12 mm. Although both nanocomposites were active in tumor inhibitions, only the CPPT-ZnO was taken as positive in tumor inhibitions which shows inhibition percents 37.09 % (» 20%).

Keywords: extracted citrus pectins; alcohol precipitation method; pectin- ZnO nanocomposites; co-precipitation method; microbial inhibition; tumor inhibition.

1. Introduction

With the increase in production of processed fruit products, the amount of fruit wastes generated is increasing enormously. Large amount of these wastes poses the problem of disposal without causing environmental pollution. These wastes can be effectively disposed by manufacturing useful byproducts from them. A valuable byproduct that can be obtained from fruit wastes is pectin [1]. Pectin (derived from Greek meaning- "congealed, and curdled") is natural, non-toxic, and amorphous carbohydrate, present in cell wall of all plant tissues, which functions as an intercellular and intracellular cementing material. It was first isolated and described in 1825 by Henri Bracannot. It is commercially in form of white to light brown powder, mainly extracted from apple and citrus fruits. Fresh weight of plant material accomplishes 0.5–4.0 % of pectic substances [2]. Pectin is both inexpensive and abundantly available. Therefore, pectin is an excellent candidate for eco-friendly biodegradable applications. Pectin is commonly used in the food industry as a gelling and stabilizing agent. Pectin macromolecules are able to bind with some organic or inorganic substances via molecular interactions. So, pectin can be used to construct matrices to absorb desired materials and deliver them in a controlled manner [3]. Citrus pectin is great deal of promising research being conducted into the use of citrus pectin as a potential in fighting malignant diseases. Zinc oxide (ZnO), a safe source for Zn supplementation and it is commonly used to fortify foodstuff in the food industry. ZnO will decompose into Zn ions after consumption. Zinc is an essential nutrient in humans and animals for many physiological functions. Currently, inorganic -organic hybrid nanocomposite materials are of great interest because of their multifunctionality owing to a combination of different compounds incorporated. They are versatile platforms for biomedical applications and therapeutic intervention. Discussion about easy, simple, fast and low cost preparation and characterizations (XRD, SEM, FT IR, TG-DTA, ED XRF and AAS) of citrus pectin –ZnO nanocomposites and their vitro antimicrobial and antitumor activities were studied in this research work. There is an urgent need to develop new classes of anticancer agents.

1.1 The Selected Fruits for the Present Research Work

The selected fruits for this research work were (Citron) *Citrus medica* L. and (Pomelo) *Citrus maxima* Merr. [4]. Botanical aspect of selected fruits (Citron and Pomelo) were

- Family : Rutaceae
- Genus : Citrus
- Species : *C. medica* and *C. maxima*
- Botanical names : *Citrus medica* L. and *Citrus maxima* Merr.
- English names : Citron and Pomelo
- Myanmar names : Shouk and Kywegaw
- Parts used : Fruits, Leaves, Flower Stems, Barks and Roots (Figures 1)

(i) (ii)

Figure 1: Photographs of (i) citron (*Citrus medica* L.) and (ii) pomelo (*Citrus maxima* Merr.) trees

1.2 Aim and Objectives of the Present Work

The aim of the present study was to extract pectin from *Citrus medica* L. (Citron) and *Citrus maxima* Merr. (Pomelo) peels, to synthesize the extracted citrus pectin-ZnO nanocomposites, to characterize the synthesized citrus pectin-ZnO nanocomposites and to find vitro antimicrobial and antitumor activities of the synthesized extracted citrus pectin-ZnO nanocomposites in chemical and medicinal purposes.

To achieve this aim, the research was carried out according to the following objectives;

- Collecting and identifying of *Citrus medica* L. (Citron) and *Citrus maxima* Merr. (Pomelo) peels samples.
- Preparing the extraction of pectin from the peel samples by employing alcohol precipitation method.
- Identifying the extracted pectins by using some chemical methods such as alcohol test, sugar and citric

acid solution test, Fehling's solution test, basic lead acetate solution test and iodine solution test.

- Characterizing the extracted pectins by modern spectroscopic methods such as FT IR, XRD, SEM and TG-DTA as well as comparing the reported data.
- Synthesizing the extracted pectin-ZnO nanocomposites by using coprecipitation method with zinc nitrate hexahydrate [Zn $(NO_3)_2$.$6H_2O$)].
- Characterizing the synthesized extracted pectin-ZnO nanocomposites by using coprecipitation method with zinc nitrate hexahydrate [$(Zn(NO_3)_2$ $6H_2O$)] by modern spectroscopic methods such as FT IR, XRD, SEM, TG-DTA, ED XRF and AAS spectroscopic methods as well as comparing the reported data.
- Screening the antimicrobial activity of the extracted Citron Peel Pectin-ZnO (CPPT-ZnO) and the extracted Pomelo Peel Pectin- ZnO (PPPT-ZnO) at 280.5 prepared with zinc nitrate hexahydrate [$Zn(NO_3)_2$.$6H_2O$)].
- Investigating of the antitumor activity of the extracted Citron Peel Pectin-ZnO (CPPT-ZnO) and extracted Pomelo Peel Pectin-ZnO (PPPT-ZnO) prepared at 28 0.5 with zinc nitrate hexahydrate [$Zn(NO_3)_2$.$6H_2O$).

2. Materials and Methods

2.1 Collection of the samples

Citron fruits were purchased from Thirimingalar Zay, Hlaing Township and pomelo fruit peels from Bogalay Zay, Botahtaung Township, Yangon Region, Myanmar, during the months of October and November, in the year of 2012. After collection, the scientific names of Citron and Pomelo were identified by the authorized botanist at Botany Department, University of Yangon.

2.2 Sample preparation for the extraction of dry matter basic pectin

The fresh collected samples (peels) were washed with water and were separately cut into pieces. The two sample pieces were then air-dried at shade or at room temperature. These two dried sample pieces were separately stored in the air- tight containers.

2.3 Procedure for extraction of pectins from citron and pomelo fruits peels

The albedos or white portion of the rinds of the fruits rich in pectin were cut into thin slices of about 2.5mm thick. The thick albedos of citron and pomelo were obtained by removing green or yellow skin and glandular tissues and they were also cut into thin slices. The sliced materials were then heated to 85 °C for about 10 minutes and those materials were kept for extraction of pectin. The dried citron and pomelo fruit peels prepared according to the procedure as shown in Section 2.2 were also subjected to extract pectin. About 100 g of the prepared material was washed thrice with water of $1^1/_2$ times the weight of the material and with the holding time for 10 minutes. The materials were then dewatered by pressing through a cotton bag. The pressed pulps were then boiled with $1^1/_2$ times its own volume of $^M/_{75}$hydrochloric acid at 100 °C for one hour. The extract was

separated by squeezing hot suspension through a bag of cloth. It was cooled immediately, allowed to settle and clarified by centrifuging. The pulp left over from the first extraction was extracted second time by boiling for an hour with equal volume of $^{M}/_{75}$ hydrochloric acid. The experiments were repeated until four successive extractions had been done and the residue was finally discarded. The individual clarified extract was concentrated at 50 °C under reduced pressure. The extraneous materials which separated out during concentration were removed. The solution was finally concentrated to a syrup by low pressure evaporating method. The syrup was poured in a thin stream with a vigorous stirring into alcohol to give a final concentration of 70 %. The precipitated pectin was allowed to remain overnight. The gelatinous pectin precipitate was separated by squeezing the material through a mull cloth. It was washed twice with alcohol and finally dehydrated with acetone. Pectin so obtained was dried at 80 °C. The dried pectin was then powdered [5].

2.4 Study on some chemical reactions of extracted pectins

The extracted pectins were tested with alcohol, sugar and citric acid, Fehling's solution, basic lead acetate solution and iodine solution.

2.5 Spectroscopic study of the extracted pectins

The extracted pectins were characterized by FT IR (in KBr on Perkin-Elmer), XRD [(scanning time 10.0/70.0/0.05/0.75 (sec), Target Cu (40 kV, 20 mA), Parabolic filte)], SEM analysis by a JSM 5610LV, (JEOL Ltd., Japan) and TG-DTA by using DTG-60H Detector, at temperature 30 to 600 °C with a scanning rate of 50 mL/min, under nitrogen atmosphere at the Universities' Research Centre (URC).

2.6 Procedure for synthesis of extracted citron and pomelo peels pectin-ZnO nanocomposites

A modification of the co-precipitation method by [3] was employed. In a tipical procedure, 0.25 g for citron peel pectin or 0.15 g for pomelo peel pectin, 1.2 g Zn $(NO_3)_2 .6H_2O$, and 40 mL of 0.2 M NaOH solution was added dropwise under constant stirring. The mixed solution was stirred for 1.5 h for citron peel pectin or 2 h for pomelo peel pectin with an overhead stirrer on a hot plate at 28 ± 0.5 °C. The reaction was allowed to settle down at room temperature (~30°C) for 24 h. Then, the obtained white precipitate was centrifuged at 10,000 rpm for 10 min and collected and washed with distilled water several times to remove the byproducts. After drying in vacuum at 30 °C for 4 h, the final product was obtained as white powder (Figure 2). Then, the composites were analysed by XRD method.

2.7 Characterization of Extracted Citrus Pectin-ZnO Nanocomposite

The pectin-ZnO nanocomposites prepared from extracted citrus fruit peels pectins were characterized by FT IR, XRD, SEM, ED XRF, TG-DTA and AAS analysis. Commercial pectin was used for comparison purpose.

2.8 Characterization of the synthesized extracted citrus pectin-ZnO nanocomposites

The synthesized extracted citrus pectin-ZnO nanocomposites were characterized by FT IR (in KBr on Perkin-

Elmer), XRD [(scanning time 10.0/70.0/0.05/0.75 (sec), Target Cu (40 kV, 20 mA), Parabolic filte)], SEM analysis by a JSM 5610LV, (JEOL Ltd., Japan), ED XRF (ED XRF-700 Spectrometer, Shimadzu, Japan) TG-DTA by using DTG-60H Detector, at temperature 30 to 600 °C with a scanning rate of 50 mL/min, under nitrogen atmosphere at the Universities' Research Centre (URC) and by AAS method using a Shimadzu AA-6300 Atomic Absorption Spectrophotometer at Amtt Co. Ltd., Yangon.

(a) (b) (c)

Figure 2: Photographs of CPPT-ZnO and PPPT-ZnO nanocomposites (a) during precipitation (b) before griding and (c) after griding

2.9 Screening of some Bioactivities

This section included two parts. The first part was concerned with antimicrobial activity test and the second part with antitumor activity test on extracted citrus pectin- ZnO nanocomposites.

2.10 Screening of antimicrobial activity

The antimicrobial activities of extracted citrus pectin - ZnO nanocomposites were determined against six strains of microorganisms such as *Bacillus subtilis, Bacillus pumilus, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Escherichia coli* by employing agar well diffusion method [6] at Fermentation Department, Central Research and Development Centre (CRDC), Ministry of Industry I, Yangon, Myanmar.

2.11 Procedure for screening of antimicrobial activity by agar well diffusion method

Meat extract (0.5 g), peptone (0.5 g) and sodium chloride (0.25 g) were mixed with distilled water in a volumatic flask and the solution made up to 100 mL with distilled water. The pH of this solution was adjusted at 7.2 by adding with 0. 1 M sodium hydroxide solution and 1.5 g of agar was added. The nutrient agar medium

was put into sterilized conical flask and plugged with cotton wool and then autoclaved at 121 °C for 15 minutes. After cool down to 40 °C , one drop of suspended strain was inoculated to the nutrient agar medium with the help of a sterilized disposable pipette near the burner. About 20 mL of medium was poured into the sterilized petri dishes and left 10-15 minutes in order to set the agar. After that the agar wells were made with a 10 mm sterilized cork bare and the wells were filled with 0.1mL of each sample to be tested and the plates were incubated at 27 °C for 24 hours. After incubation, the diameters of inhibition zones including 10 mm wells were measured, and the inhibition zones diameters were taken as the antimicrobial activities of the samples used.

2.12 Screening of antitumor activity

In this section, antitumor activity screening of citrus pectin-ZnO nanocomposites was carried out by using Potato Crown Gall (PCG) test (or) Potato Disc Assay (PDA) method [7] at Fermentation Department, Central Research and Development Centre (CRDC), Ministry of Industry I, Yangon, Myanmar.

2.13 Procedure for antitumor activity screening by potato crown gall test or potato disc assay method

Fresh, disease free potato tubers were obtained from local markets and were used within 48 hours of transfer to the laboratory. Tubers of moderate sizes were surface-sterilized by immersion in 50 % solution hypochlorite (Clorox) for 20 minutes. The ends were removed and soaked for 10 minutes more in Clorox. A core of the tissue was extracted from each tuber by using surface-sterilized (ethanol and flame) 1.5 cm wide cork borer. And, 2cm pieces were removed from each end and discarded and the remainder of the cylinder is cut into 0.5cm thick discs with a surface-sterilized cutter. The discs were then transferred to 1.5 % agar plates (1.5 g of Difco agar was dissolved in 100 mL of distilled water, autoclaved and 20mL poured into each petridish). Each plate contained four discs. This procedure was done in the clean bench in the sterile room. Accurately weighed 8 mg of sample was dissolved in 2 mL of dimethyl sulphoxide (DMSO); this solution was filter through Millipole filters (0.22 µm) into a sterile tube. 0.5mL of this solution was added to 1.5 mL of sterile distilled water and 2 mL of broth culture of *A. tumefaciens* strain (48 hours culture containing 3-5 × 10^9 cells/mL) were added aseptically. Controls were made in this way; 0.5 mL of DMSO and 1.5 mL of sterile distilled water were added to the tube containing 2 mL of broth culture of *A. tumefaciens* (from the same 48 hours culture). Using a sterile disposable pipette, 1 drop (0.05 mL) from these tubes was used to inoculate each potato disc, spreading it over the disc surface. The process of cutting the potatoes and incubation must be conducted within 30 minutes. The plates were sealed with tape to minimize moisture loss and incubated at room temperature for 12 days. After incubation, Lugol's solution (I_2-KI) was added and the tumors were counted with a microscope and compared with control. The results are derived from the number of tumors on test discs versus those on the control discs. Inhibition is expressed as follow,

$$\% \text{ inhibition} = 100 - \quad \times 100 \qquad (1)$$

A negative percentage and stimulation is expressed as a positive percentage. 20 % inhibition in two or more independent assays is considered as significant activity of a test sample [6, 8]. All materials (petridishes and potato discs used) were sterilized before clean-up or discarding. The photographs for the screening of antitumor

activity can be generally illustrated in Figures 3.

(a) (b) (c) (d)

Figure 3: Photographs for screening of antitumor activity by Potato Crown Gall (PCG) test

(a) Surface sterilization of potato in "Clorox"

(b) Removed both ends by sterilized cutter

(c) Removed 2 cm from each end of core, cut the remader of core and cut 0.5 cm potato disc

(d) Tested peri dishes after sealing with tape before incubating

3. Results and Discussion

3.1 Collection and Preparation of Citrus Fruit Peels

Citrus medica L., Citron fruits were purchased from Thirimingalar Zay, Hlaing Township and *Citrus maxima* Merr., pomelo fruit peels from Bogalay Zay, Botahtaung Township, Yangon Region, Myanmar, during the month of October and November, in the year of 2012. After collection, their scientific names were identified by an authorized botanist, Department of Botany, University of Yangon.

In addition, the collected fresh peels were washed with water to remove impurities and were separately cut into pieces. The two samples were then air-dried at shade or at room temperature to prevent some reaction of sunlight with organic constituents of the samples for one month, and separately stored in the air tight containers so that the samples were free from getting molds as well as other contamination and were ready to be used for the extraction of pectin.

3.2 Extraction Methods of Pectin

Alcohol precipitation method is a conventional method. But it has the advantage of easy recovery of waste

alcohol, much less in loss of alcohol and no requirement of expensive instruments. The alcohol precipitation method involving no use of machinery and equipment provides a very good means of extracting pectin domestically from waste vegetables and fruits. If by-products of various fruit processing plants are available as a cheapest raw material, pectin production could be profitably processed on commercial scale by this method [5]. Alcohol precipitation method was chosen for the extraction of citrus pectin based on fresh and dry peels samples, since it is very convenient and cost-effective.

3.3 Yield of Pectin Extracted by Alcohol Precipitation Method

The yield (%) (Appendix I) of pectins extractd from citron peels (4.53 % based on fresh matter and 21 % based on dry matter) and pomelo peels (3.04 % based on fresh matter and 9.18 % based on dry matter) were found to be close to that given in literature (0.5- 4 %, based on fresh matter and 20-30 % based on dry matter) [2,9]. It was also observed that the yield percentages of pectins were variable with different sample sources based on fresh and dry peels. Pectin extracted from dried citron peels (CPPT) gave the best yield (21.42 %) with more favorable colour. If the yield of pectin is greater than 10 %, based on dry matter then the source of pectin is considered possible for commercial use [10]. Since the yield percent of pomelo pectin (PPPT) (9.81 %) was slightly less than 10 %, only the citron pectin can be considered to be used as commercial pectin. The experiments were done under 1 atm pressure at 100 ^{0}C for 1 hour in each extraction. In general, to complete the extraction of pectin, the process was done up to 4th extraction.

3.4 Chemical confirmation of the extracted pectins

Both pectins (CPPT and PPPT) were found to give a white flocculent precipitate with ethanol, a firm jelly with sugar and citric acid solution, blue gel precipitate with Fehling's solution, white gelatinous precipitate with basic lead acetate, and yellow gel with iodine solution (Figure 4). Since, alcohol test of the extracted pectins gave white flocculent precipitate, and sugar and citric acid solution test gave a firm jelly, the extracted pectins had the jelly properties. Fehling s solution test of the extracted pectins gave blue jelly precipitate, so, the pectins are non-reducing sugar. Basic lead acetate test gave white gelatinous precipitate, so the extracted pectins are glycosides. Iodine solution test of both of the extracted pectins did not give deep blue colour indicating the non-starch polysaccharide. From these observations, it can be inferred that the chemical tests confirmed the pectin characteristics of the extracted CPPT and PPPT pectins.

Citron Peel Pectin	**Pomelo Peel Pectin**
1. Sugar and citric acid test	**1. Alcohol test**
2. Alcohol test	**2. Sugar and citric acid test**
3. Fehling's solution test	**3. Fehling's solution test**
4. Lead acetate solution test	**4. Lead acetate solution test**

5. Iodine solution test **5. Iodine solution test**

Figure 4: Photograph of results of some chemical reactions of the extracted pectins

3.5 Spectroscopic Study of the Extracted Pectins

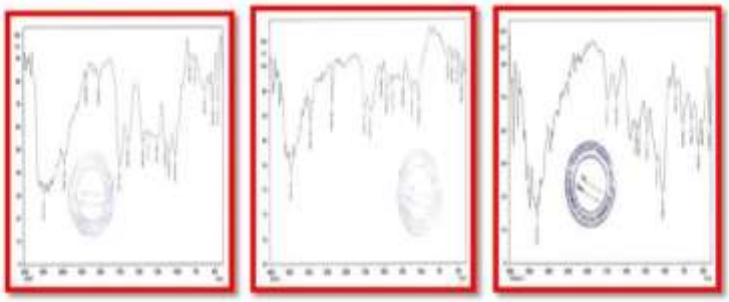
The characteristics of the extracted citrus pectins were studied by FT IR spectroscopic analysis, XRD analysis, SEM analysis and TG-DTA analysis.

The infrared spectra of the pectins extracted from citron peel (CPPT) and pomelo peel (PPPT) pectins are shown in Figure 5(a) and (b). The analysis of FT IR spectra revealed that the broader band of absorption between 2800-3479 cm^{-1} were due to O-H stretching vibration of alcoholic group and carboxylic acid group, 2931-2947 cm^{-1} were due to aliphatic C-H stretching vibration and whereas strong absorbance observed at1743-50cm^{-1}, 1635-74 cm^{-1} and 1442-50cm^{-1} were attributed to the ester carbonyl (C=O) groups, symmertric and asymmertric carboxyl ion stretching band (COO^-), respectively. Other bands responsible for C-H bending and O-H in plane bending of alcoholic vibration group was 1373cm^{-1} and for C-O stretching occurred at 1234-57cm^{-1}. The O-H bending vibration of acid group occurred at 920 cm^{-1} and O-H out of plane bending vibration of alcoholic group was at 740-56 cm^{-1}. The FT IR spectral data of both pectins are identical with that of the commercial pectin (Figure 5 (c)) and literature values [11].

In order to study the degree of crystallinity, a powder X- ray diffraction (XRD) analysis was performed on the citron peel pectin and pomelo peel pectin samples (Figure 6). Both XRD diffractograms showed the pectins are in amphous nature.

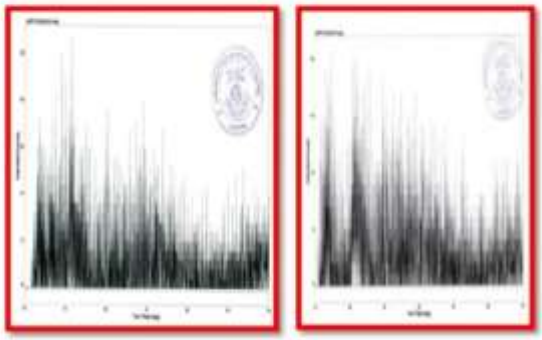
In order to study the surface morphologies, scanning electron microscopic (SEM) analysis was performed on the citron peel pectin and pomelo peel pectin samples. SEM micrograph of fibrous form of citron pectin showed non-smooth surface not orderly wave pattern (Figure 7(a) (i)) whereas pomelo pectin showed non- smooth surface in orderly fibrous structure (Figure 7 (a) (ii)). SEM micrograph of powder form of both of the extracted pectin showed non-homogeneous, non-smooth fibrous surface and irregular in shape (Figure 7 (b) (i) and (ii)).

TG-DTA thermogram profiles of both of the extracted pectin gave four weight loss and phenomena as shown in Figure 8. The first weight loss between 40 °C and 160 °C, was related to the moisture evaporation. The second weight loss between 160 °C and 270 °C, which shows the loss of chemical bonding of water molecule dehydration. The third weight loss was found between 270 °C and 412 °C and, it was induced by the thermal depolymerization of pectin chains and evaporation of the last water molecule. The last peak centered between 412 °C and 600 °C arised from the oxidation decomposition of pectin in the air. The peak nature and decomposition temperature of both of the pectins were similar to commercial pectin. The total weight loss for citron peel pectin (CPPT) was 95.40 %, for pomelo peel pectin was 91.27 % and for commercial pectin was 98.33 % [5]. The weight losses of the extracted pectins were lower than the commercial pectin.



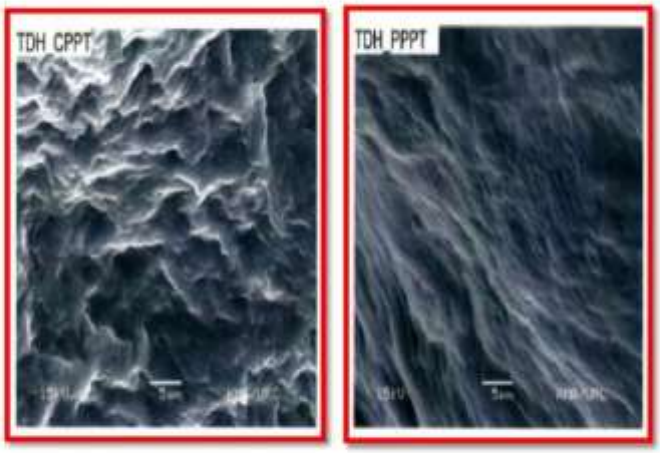
(a) (b) (c)

Figure 5: FT IR spectra of (a) extracted citron peel pectin (b) extracted pomelo peel pectin and (c) commercial citrus pectins



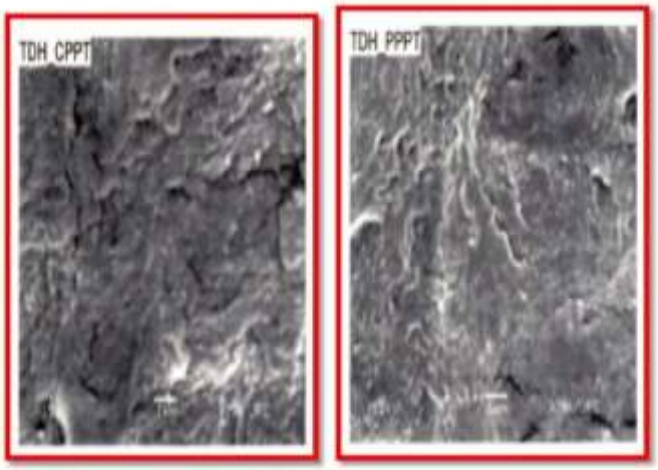
(i) (ii)

Figure 6: XRD diffractograms of extracted (i) citron and (ii) pomelo peel pectins



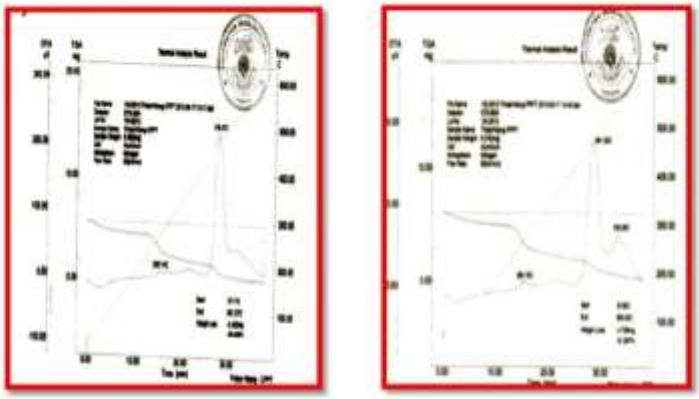
(i) (ii)

Figure 7 (a) : SEM micrographs of fibrous form of extracted (i) citron and (ii) pomelo peel pectins



(i) (ii)

Figure 7(b): SEM micrographs of powder form of extracted (i) citron and (ii) pomelo peel pectins



(i) (ii)

Figure 8: TG-DTA thermograms of extracted (i) citron and (ii) pomelo peel pectins

3.6 Study on Preparation of the Synthesized Citrus Pectin-ZnO Nanocomposites

The size of extracted Citron Peel Pectin- ZnO nanocomposite and Pomelo peel Pectin-ZnO nanocomposite synthesized at 28 ± 0.5 °C were determined. After the preparation of both composites, taking the XRD analysis and the average crystallite sizes were calculated by using Debye Scherrer formula. The lattice parameters were also obtained from the XRD analysis. The XRD diffractogram were shown in Figure 9. The percentage yields of the prepared extracted citron peel pectin- ZnO nanocomposite and pomelo peel Pectin-ZnO nanocomposite at 28± 0.5 °C were also calculated. The yield percents (Appendix II) of the composite obtained from citron pectin gave higher yield (90.52 %) than from pomelo pectin (64.95 %) at 28 ± 0.5 °C. But both the yield percents were >60 %, so both the yield percents of the composites demonstrate the successful preparation, providing potential benefits for industrial preparation of pectin-ZnO nanocomposites from an economic and environmental point of view.

3.7 Comparison of Sizes, Lattic Parameters and Structures of the Synthesized Pectin-ZnO Nanocomposites

The comparison of lattic constants, structures and crystallite sizes of pectin-ZnO nanocomposites synthesized prepared at 28 ± 0.5 °C was studied by XRD analysis. The pectin-ZnO nanocomposites were also comparatively studied on their sizes, lattic constants and structures. The lattic parameters of the pectin-ZnO nanocomposites were analysed by XRD method and the average crystallite sizes were calculated by using Debye Scherrer formula. The size (Appendix III) of CPPT-ZnO and PPPT-ZnO obtained at 28 ± 0.5 °C were found to be 32.30 nm, and 24.46 nm. Since, the CPPT-ZnO and PPPT-ZnO nanocomposites obtained at 280.5 were also found to show lattice parameters (a = b = 3.42 and c = 4.89), (a = b = 3.40 and c= 5.35) respectively their structures were hexagonal which consistent with the reported results [3]. Consequently, both the composites at 280.5 gave

constant hexagonal structures.

3.8 Characterization of Pectin-ZnO Nanocomposites

The characteristics of pectin-ZnO nanocomposites obtained at 28 0.5 were studied by FT IR spectroscopic analysis, XRD analysis, SEM analysis, Crystallinity indexes, ED XRF analysis, TG-DTA analysis and AAS analysis. A typical FT IR spectrum pattern of the samples shows in the range of 400-4000 cm^{1}. The FT IR spectra of both of the extracted pectin-ZnO nanocomposites prepared at 280.5 showed characteristic peaks between 2500–3700 cm^{-1}, 2872–2962 cm^{-1}, 1550–1750 cm^{-1}, 1400–1650 cm^{-1} and 1000–1300 cm^{-1} corresponding, respectively, to –OH, -CH, C=O of ester and acid, and -COC- stretching of the galactouronic acid and an obvious absorption peak between 440–530 cm^{-1} can be found for the pectin-ZnO composites; this is a typical IR absorption peak of ZnO, originating from stretching mode of the Zn-O bond. It is found that peaks between 3317 $cm^{-1}$and 3550 cm^{-1} and at 1033 cm^{-1} are obviously weaker found in pectin-ZnO composites than that pectin. These observations confirmed the formation of composites between pectin and ZnO. The pectin peaks were not removed by washing the sample repeatedly, suggesting that interactions between pectin and ZnO are strong. The FT IR spectra of both of the pectin-ZnO composites were consistent with the FT IR spectrum of commercial citrus pectin (Figure 5 (c)) and also with literature values [11, 12]. The FT IR spectra were shown in Figures 9.

The X-ray diffraction patterns of extracted citrus pectin-ZnO composites obtained from high scale prepared at 280.5 showed all of the diffraction peaks can be readily indexed to a pure hexagonal structure. The intensities and positions of the peaks are in good agreement with the literature values. The nanoparticle size was calculated from the full width half maximum (FWHM) technique using Scherer's formula $D = K\lambda/(\beta \cos \theta)$, where K is Scherer constant (0.9), λ is the wavelength of Cu-Kα (1.54 ^{o}A) line, β is the full width of a peak at half of the maximum of the peak (FWHM) in the diffraction spectra (measured in radians), and θ is the Bragg s diffraction angle. The average crystallite sizes of nanoparticles prepared at 280.5 were respectively about 32.30 nm for citron peel pectin-ZnO (CPPT-ZnO), 24.46 nm for pomelo peel pectin-ZnO (PPPT-ZnO). The XRD diffractogram were shown in Figures 10.

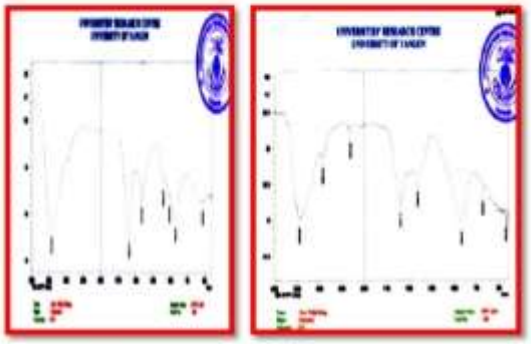
The SEM images of extracted pectin-ZnO composites prepared at 280.5 exhibited numerous spherical perturbances on the surface and embedded in the pectin matrix with a regular morphology and narrow size distribution. The average sizes of the composites prepared at 280.5 in the SEM image were found to be about 70.59 nm for citron peel pectin-ZnO (CPPT-ZnO), and 61.15 nm for pomelo peel pectin-ZnO (PPPT-ZnO) which are larger than that calculated by Debye-Scherrer formula from the XRD pattern. The SEM images were shown in Figures 11.

The crystallinity index was calculated by using the following crystallinity index equation:

$$I_{cry} = D_p\,(SEM)/D_{cry}\,(XRD)\ (I_{cry} \geq 1.00) \qquad (2)\ [13]$$

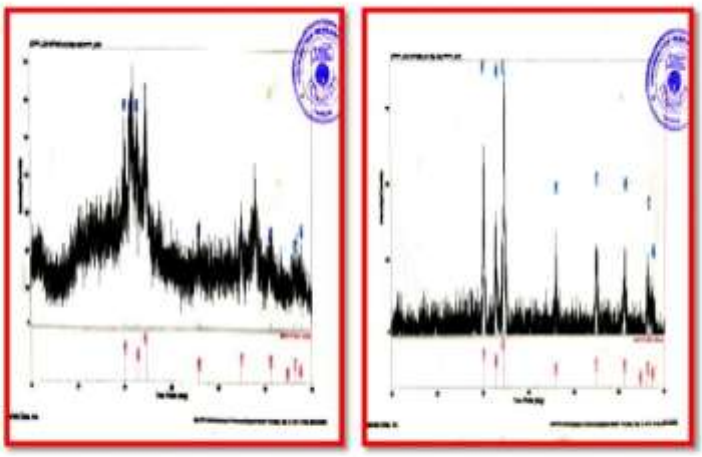
Where, I_{cry} is the crystallinity index; D_p is the particle size (obtained from SEM morphological analysis; D_{cry} is the particle size (calculated from the Scherrer equation). If I_{cry} value is close to 1, then it is assumed that the

crystallite size represents monocrystalline whereas a polycrystalline have a much larger crystallinity index. The crystallinity indexes of extracted citrus pectin-ZnO composites prepared at 280.5 were (2.18 and 2.50). Since both the crystallinity indexes were greater than 1, both of the nanocomposites have polycrystalline particle type. ED-XRF with C-H balance analysis revealed the presence of 99.946 % of ZnO, 0.044% of NiO and 0.010 % of C-OH compound in CPPT-ZnO and 99.867 % of ZnO, 0.079 % of Fe_2O_3, 0.044 % of NiO and 0.010% of C-OH compound in PPPT-ZnO nanocomposites prepared at 28 0.5. From these results, it was found that both pectin-ZnO composites prepared at 280.5 actually contain the highest percentage of ZnO. The spectra were shown in Figure 12. TG-DTA thermograms of the extracted pectin-ZnO nanocomposites prepared at 280.5 were illustrated in Figures 13. There are four steps concerning with the weight loss. The first weight loss was found between 40 °C and 160 °C, related to the moisture evaporation. The second weight loss was between 160 °C and 270 °C, which show the evaporation of crystallized water molecule. The third weight loss was between 270 °C and 412 °C, it is induced by the thermal depolymerization of pectin chains and evaporation of the last crystallized water molecule. The temperature for thermal depolymerization of pectin chains in pectin-ZnO nanocomposite is a little higher than that of pectin alone, revealing the depolymerization has been hindered to some degree. This may be due to the existence of strong interactions between pectin molecules and ZnO. The last peak centered between 412°C and 600 °C should arise from the oxidation decomposition of pectin in the air. The total weight loss for citron peel pectin-ZnO nanocomposite (CPPT-ZnO) is 47.70 % and for pomelo peel pectin is 45.99 %. These pectin-ZnO nanocomposites were prepared at 280.5. The contents of zinc ions in citron peel pectin-ZnO nanocomposite, and pomelo peel pectin-ZnO nanocomposite at 280.5 were respectively found to be 3.88 10^5 ppm and 5.27 10^5 ppm quantitatively determined by AAS method. From these results it was found that, the amounts of zinc ion in CPPT-ZnO composites prepared at 280.5 (3.88 10^5 ppm) was significantly lower than the Zn ion contents in PPPT-ZnO (5.27 10^5 ppm). From these observations, it may be inferred that CPPT-ZnO contained larger amount of pectin than PPT-ZnO.



(i) (ii)

Figure 9: FT IR spectra of CPPT-ZnO and PPPT-ZnO nanocomposites

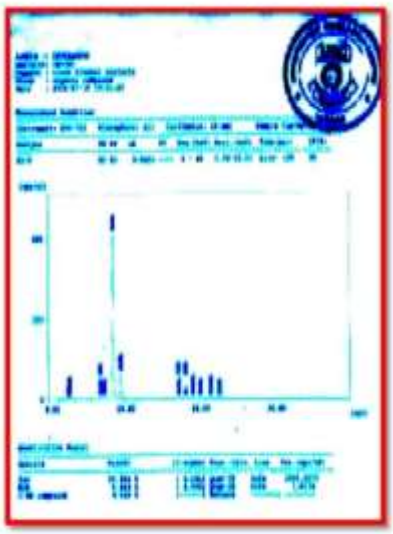


(i) (ii)

Figure 10: XRD diffractograms of (i) CPPT-ZnO and (ii) PPPT-ZnO nanocomposites

(i) (ii)

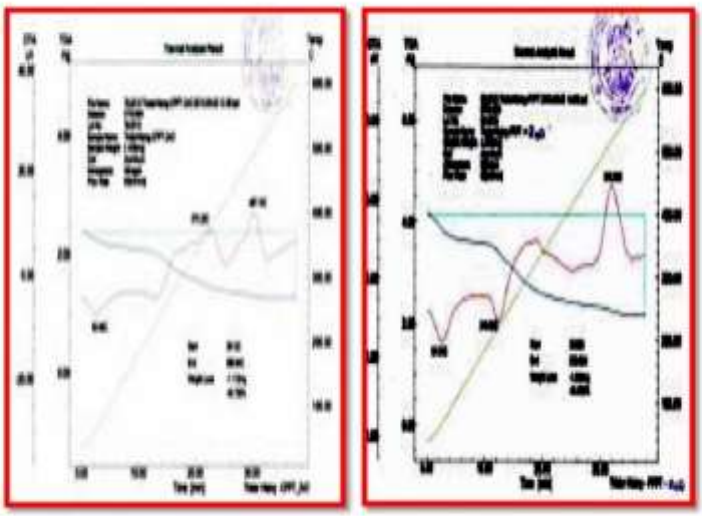
Figure 11: SEM micrographs of CPPT-ZnO and PPPT-ZnO nanocomposites with four scales and four magnifications (i) 10 µ, (ii) 2 µ, (iii) 1µ and (iv) 0.5 µ



(i)

(ii)

Figure 12: ED XRF with C-H balance spectra of (i) CPPT-ZnO and (ii) PPPT-ZnO nanocomposites



(I) (II)

Figure 13: TG-DTA thermograms of (i) CPPT-ZnO and (ii) PPPT-ZnO nanocomposites

3.9 Some Bioactivities of Citrus Pectin-ZnO Nanocomposites

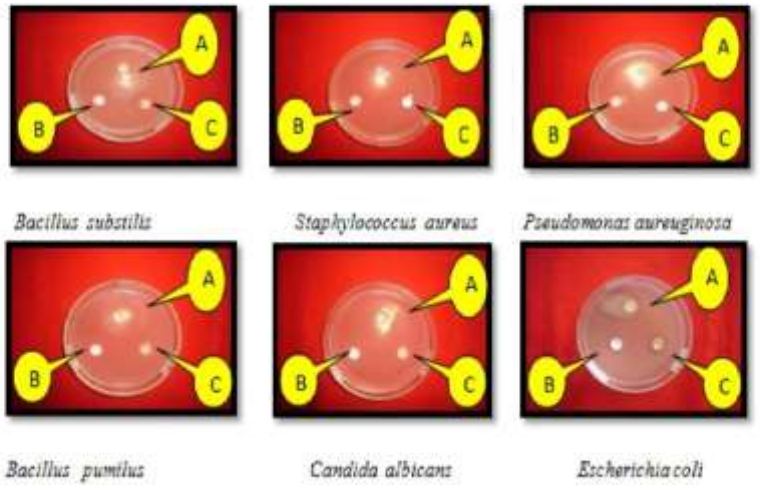
Some bioactivities such as antimicrobial and antitumor activities of citron peel pectin-ZnO and pomelo peel pectin-ZnO nanocomposites prepared at 28 ± 0.5 °C were investigated as described in Section 2.10 and 2.12 respectively.

3.10 Antimicrobial activity of citrus pectin-ZnO nanocomposites

An antimicrobial activity of citron peel pectin-ZnO nanocomposites and pomelo peel pectin-ZnO nanocomposites synthesized at 28 ± 0.5 °C was investigated by employing agar well diffusion method (Section 2.11). In this study, the samples were tested on six species of microorganisms such as *Bacillus substilis*, *Staphylococcus aureus*, *Pseudomonas aureuginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* species. The inhibition zone diameter shows the degree of the antimicrobial activity. The larger the inhibition zone diameter, the higher the antimicrobial activity.

The inhibition zones of the samples tested against six microorganisms tested are shown in Figure 14. According to the results, CPPT-ZnO prepared at 28 ± 0.5 °C exhibited antimicrobial activity against three species of microorganisms such as *Bacillus substilis*, *Staphylococcus aureus* and *Escherichia coli* with inhibition zone diameter 12 mm to 20 mm whereas PPPT-ZnO prepared at 28 ± 0.5 °C showed antimicrobial activity against

only two species of microorganisms such as *Bacillus substilis* and *Staphylococcus aureus* with the inhibition zone diameters 15 mm to 20 mm.



A = CPPT-ZnO

B = PPPT-ZnO

C = ZnO

Figure 14: Inhibition zones of citron peel pectin-ZnO (CPPT-ZnO) and pomelo peel pectin -ZnO (PPPT- ZnO) nanocomposites against six tested microorganisms

3.11 Antitumor activity of citrus pectin-ZnO nanocomposites

In this study, cultured to use in the Potato Crown Gall (PCG) test with two tested samples were citron peel pectin-ZnO and pomelo peel pectin-ZnO nanocomposites prepared at 28 ± 0.5 ℃.

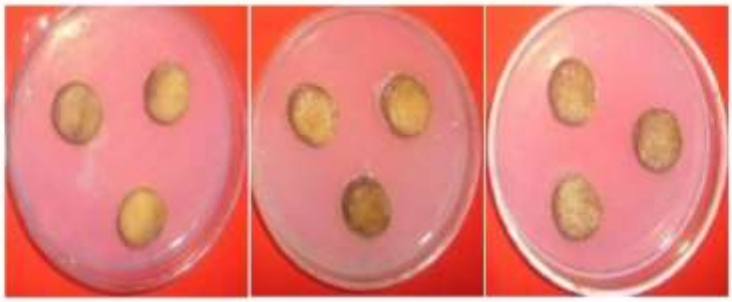
The antitumor activity of both of the citron peel pectin-ZnO and pomelo peel pectin-ZnO nanocomposites synthesized at 28 ± 0.5 ℃ was investigated by using PCG test (Figure 15). For inoculation of the potato disc, 48 hour broth cultures containing 5×10^{9}cells/mL were used.

The tested samples were dissolved in DMSO, diluted and mixed with the bacterial culture for inoculation. After preparing the inoculums, the bacterial suspension was inoculated on the cleaned and sterilized potato discs, and incubated for 12days, at room temperature. After that, the tumors were appeared on potato discs and checked by

staining the knob with Lugol's (I_2-KI) solution.

In the control, the formation of white knob on the blue background indicated the presence of tumor cells because there is no protein in tumor cells. The numbers of tumor were counted under microscope and after counting the tumors, the inhibition percent were calculated by using formula. From this experiment, it was found that the tumor inhibition percent (Appendix IV) was observed by testing with both of the samples.

Since tumor inhibition was taken as positive when the inhibition percent was 20, only one CPPT-ZnO prepared at 28 ± 0.5 °C showing tumor inhibition of 37.09 % was taken as positive in tumor inhibition. In this *in vitro* PCG assay, the concentration for each test sample used was of 25µg/disc.



(i) (ii) (iii)

Figure 15: Antitumor assay for (i) Control (ii) CPPT-ZnO and (iii) PPPT-ZnO by Potato Crown Gall (PCG) test

4. Conclusion

The present observations describe a successful preparation of novel citrus pectin-ZnO nanocomposites from fruits waste precursor.

Pectin extracted from citron peels (CPPT) gave the best yield (21.42%) based on dry sample. Since the yield percent of pomelo pectin (PPPT) (9.18%) was less than 10%, only the citron pectin can be considered to be used as commercial pectin.

In the preparation of extracted citrus pectin-ZnO nanocomposites, both of the nanocomposites were obtained more than 60 % in yields. Hence, this research can provide potential benefits for industrial production of pectin - ZnO nanocomposites from economic and environmental points of view.

Furthermore, outcomes of both of the tested samples were active in the tested activities. So, the total contributions for this research work were both the tested samples could be used in the treatment of the

respective disease such as antifungal activity, human skin infection, pneumonia, septic arthritis, peritonitis, mastitis, septicemia, inflammation, diarrhea, food poisoning, urinary tract infections according to the antimicrobial activity and also could be used in the treatment of tumors as well as in the protection of pre-cancer which is pre-malignant diseases according to the antitumor activity with high yields of the composites.

Acknowledgements

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Appendix.

Appendix. (I)

Calculation of Yield of Pectin

Yield of Pectin (%) = × 100 %

Appendix. (II)

Calculation of Percentage Yield of Pectin-ZnO Nanocomposites

For Extracted Citron Peel Pectin-ZnO Nanocomposites Synthesized with Zinc Nitrate at 280.5,

$Zn(NO_3)_2.6H_2O + 2NaOH \rightarrow ZnO + 2NaNO_3 + 7H_2O$

1mol 1mol

1mol of $Zn(NO_3)_2.6H_2O$ gives 1mol of ZnO

297.38 g of $Zn(NO_3)_2.6H_2O$ gives 81.38 g of ZnO

1.2g of $Zn(NO_3)_2.6H_2O$

= g

= 0.3283 g

Amount of Pectin = 0. 25 g

% yield == 100 %

% yield= × 100 %

= 90.52%

For Extracted Pomelo Peel Pectin-ZnO Nanocomposites Prepared with Zinc Nitrate at 280.5,

Amount of Pectin = 0.15 g

% yield = × 100%

= 64.95 %

Appendix. (III)

Calculation of Average Crystallite Sizes by Using Debye- Scherrer Formula

D =

Where, D = Average crystallite sizes

K = 0.9

= wavelength of the X-ray diffraction = 1.54 °A

= 0.154 nm

= FWHM of the observed peak = FWHM ×

= FWHM × (0.0175)

= angle of diffraction

Appendix. (IV)

Calculation of percent inhibition of CPPT-ZnO and PPPT-ZnO at 280.5

% inhibition = 100 - × 100

20, May, 2015

External Examiner's Assessment Report

1. Candidate - Thida Hlaing (ဌေးဝေးဝင်း)
2. Title - PREPARATION, CHARACTERIZATION AND SOME BIOACTIVITIES OF EXTRACTED CITRUS PECTIN-ZnO NANOCOMPOSITES FROM CITRON (*CITRUS MEDICA* L.) AND POMELO (*CITRUS MAXIMA MERR.*) FRUITS PEELS
3. Research Area - nanocomposites
4. Assessment
   (a) The candidate has collected data and also experiment work done to extract pectin from Citron (*CITRUS MEDICA* L.) and Pomelo (*CITRUS MAXIMA* MERR.) fruits' peels.
   (b) She has also determined some physicochemal properties of extracted pectin and prepared pectin-ZnO nanocomposites from different sources of citrus and pomelo fruits' peels.
   (c) She has studied the antimicrobial and antitumor bioactivities of the prepered pectin-ZnO nanocomposite.
   (d) I enjoy reading her thesis and it is well written and well presented. I am quite satisfied with her work and believe that her work can be a significant contribution for the aspect of the application of the pectin-ZnO nanocomposites.
5. Viva Voce Examination- Satisfactory
6. Recommendation- I am pleased to recommend Thida Hlaing should be awarded the Ph.D Degree in Chemistry for having done this important research.

Dr. Aung Min
Rector
Yangon Institute of Education

Prof. Dr. Daw Hla Ngwe
Professor/ Head of Department
Department of Chemistry
University of Yangon

Lampiran 3. Jurnal 3

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Ekstraksi Pektin dari Limbah Kulit Jeruk (*Citrus sinensis*) dengan Metode Ekstraksi Gelombang Ultrasonik Menggunakan Pelarut Asam Klorida (HCl)

Extraction of Pectin from Orange Peel Waste (Citrus sinensis) by Ultrasonic Method Using Hydrochloric Acid (HCl) Solvent

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Abstrak
Pektin merupakan polisakarida komplek yang mengandung asam galakturonat yang dihubungkan oleh α-(1-4) glikodidik yang terdapat di dalam dinding sel tanaman. Senyawa pectin banyak digunakan di industri farmasi, makanan dan minuman. Percobaan ini bertujuan untuk mengekstraksi pektin dari limbah kulit jeruk dengan metode ekstraksi gelombang ultrasonik dan mengetahui pengaruh konsentrasi dari pelarut yang digunakan serta kecepatan pengadukan pada karakterisasi pektin yang dihasilkan. Penelitian ini dilakukan dengan metode gelombang ultrasonik menggunakan pelarut asam kemudian ditambahkan etanol kedalam filtrate untuk mengendapkan pektin setelah itu dilakukan proses terakhir yaitu pengeringan untuk mendapatkan pectin kering. Variabel tetap yang digunakan dalam percobaan ini yaitu massa sampel 25 gram, pelarut asam klorida (HCl), waktu ekstraksi 60 menit, suhu ekstraksi 60 °C, kecepatan gelombang ultrasonik 50 KHz dan waktu pengendapan 16 jam. Sedangkan variable berubah yaitu konsentrasi pelarut yaitu0,025 N; 0,05 N; 0,075 N dan kecepatan pengadukan 0 rpm; 50 rpm; 100 rpm; 150 rpm. Hasil percobaan menunjukkan bahwa rendemen hasil ekstraksi terbaik diperoleh pada konsentrasi 0,075 N yaitu 20,12 %; kecepatan pengadukan 150 rpm dengan kadar air 8,0 %, kadar abu 4,0 %, dan kadar metoksil 7,44 %.

Kata Kunci: *gelombang ultrasonik, pektin, kulit jeruk,. kadar metoksil*

Abstract
Pectin is complex polysaccharide contained D-galacturonic acid bonded by α -1,4 glucosidic in plant cell walls. Pectin widely used in pharmaceutical, food and beverage industries. This study evaluates the effect of solvent and stirring speed on pectin characteristic. The study utilizes ultrasonic wave and acid solvent at the presence of ethanol to yield pectin following by drying to obtain dried pectin. This study carries out using 25 grams orange peels, 10 %, chloride acid solvent, 60 minutes extraction time, temperature of 60 °C, ultrasonic wave speed 50 KHz and 16 hours settling time at various solvent concentrations 0.025 N; 0.05 N; 0.075 N and stirring speed 0 rpm; 50 rpm; 100 rpm; 150 rpm. The results showed that the highest yield of pectin extraction was obtained at concentration of 0.075 N with 20.12 %; stirring speed 150 rpm, water content 8.0 %; 4,0 % ash content, and 7,44 % methoxyl content.

Keywords: *ultrasonic wave, pectin, orange peels, methoxyl content*

Pendahuluan

Jeruk (*Citrus sinensis*) merupakan salah satu komoditas pada buah-buahan yang mempunyai peranan sangat penting dipasaran baik di dalam negeri dan dunia, dalam bentuk segar atau olahan. Di Indonesia, produksi jeruk telah menduduki posisi kedua teratas setelah pisang. Komponen pada tanaman jeruk yang siap dipanen terdiri atas 65 % buah yang dapat dimakan, 30 % kulit; 5 % biji [6].

Jumlah senyawa pektin yang berada di dalam kulit jeruk sebesar 29,84 %, sehingga kulit jeruk dapat digunakan sebagai bahan baku pembuatan pektin [11].

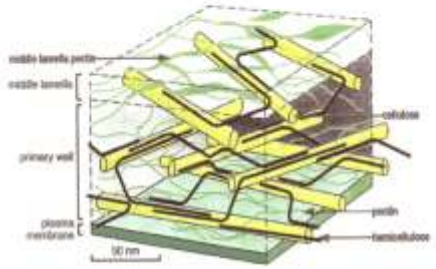
Kebutuhan pektin di Indonesia dari tahun ketahun mengalami peningkatan, pada tahun 2007 yaitu 183.050 kg/tahun s/d tahun 2013 yaitu 240.792 kg/tahun. Pada tahun 2020 diperkirakan kebutuhan pektin di Indonesia mencapai 1.320 ton/tahun, sehingga solusi untuk mengatasi kebutuhan pektin yang terus meningkat dengan memanfaatkan limbah kulit jeruk untuk diolah menjadi pektin dan dapat mengurangi pencemaran lingkungan [3].

Diterima : 03 September 2019 | Direvisi : 19 Juli 2019 | Disetujui : 30 September 2019

Berdasarkan kondisi ini maka penelitian ini bertujuan untuk mengekstrak pektin dari limbah kulit jeruk dengan metode gelombang ultrasonik dan melihat pengaruh konsentrasi dari pelarut asam klorida yang digunakan.

Teori

Pektin merupakan produk karbohidrat yang dimurnikan dari ekstraksi asam pada kulit buah. Senyawa pectin merupakan polimer dari asam D-galakturonat (turunan dari galaktosa) yang dihubungkan dengan ikatan beta-(1,4)-glukosida. Pada umumnya senyawa pektin diklasifikasikan menjadi tiga kelompok yaitu asam pektat, asam pektinat (pektin) dan protopektin. Protopektin banyak terdapat pada jaringan tanaman yang masih muda dan jumlahnya tergantung pada tingkat kematangan buah tersebut [12]. Pada Gambar 1 dapat dilihat struktur pektin yang ada pada tumbuhan.



Gambar 1. Struktur Pektin pada Tumbuhan

Pektin merupakan komponen utama pada lamella tengah yang terdapat pada tanaman, pectin berperan sebagai perekat dan menjaga stabilitas jaringan dan sel. Pada tabel 1 dapat dilihat nilai dari kandungan pektin yang baik berdasarkan standar mutu *International Pectin Producers* Association (IPPA).

Tabel 1. Standar Mutu Pektin Berdasarkan Standar Mutu *International Pectin Producers Association*[5]

Faktor Mutu	Kandungan
Kekuatan gel	Min 150 grade
Kandungan metoksil :	
• Pektin metoksil tinggi	> 7,12%
• Pektin bermetoksil rendah	2,5 - 7,12%
Kadar asam galakturonat	Min 35%
Susut pengeringan (kadar air)	Maks 12%
Kadar abu	Maks 10%
Kadar air	Maks 12%
Derajat esterifikasi untuk :	
• Pektin ester tinggi	Min 50%
• Pektin ester rendah	Maks 50%
Bilangan Asetil	0,15 – 0,45%
Berat Ekivalen	600 – 800 mg

Metodologi Penelitian

Bahan baku yang digunakan pada percobaan ini adalah kulit buah jeruk dan bahan kimia berupa asam klorida (HCl), air (H_2O), etanol (C_2H_5OH), peraknitrat ($AgNO_3$), natrium hidroksida (NaOH), natrium klorida (NaCl), dan *phenolptalein*. Tahap awal adalah persiapan bahan baku dimana kulit buah jeruk dikeringkan untuk mengurangi kandungan air, kemudian sampel dihaluskan menggunakan belender untuk mendapatkan serbuk kulit jeruk agar mudah diestrak untuk menghasilkan filtrate yang maksimal.

Percobaan ini dilakukan dengan variasi konsentrasi yaitu 0,025 N; 0,05 N ; 0,075 N dan kecepatan pengadukan 0 rpm ; 50 rpm ; 100 rpm ; 150 rpm. Rangkaian alat yang digunakan pada percobaan ini dapat dilihat pada gambar 2.

Gambar 2. Rangkaian Peralatan Ekstraksi

Air di tambahkan kedalam bahan baku sebanyak 30 kali dari banyaknya massa sampel yang digunakan dan diekstraksi didalam *ultrason- bath* dengan gelombang 50 KHz dan pengadukan. Campuran yang telah diekstrak disaring dengan menggunakan kertas saring untuk memisahkan filtrate dari ampasnya. Setelah filtrate terpisah dilakukan pengendapan dengan menambahkan etanol, pengendapan dilakukan selama 16 jam. Endapan pektin yang diperoleh kemudian dikeringkan dalam oven pada temperatur 40°C selama 8 jam. Pektin kering yang dihasilkan kemudian dianalisa kadar air, kadar abu, berat ekivalen, kadar asam galakturonat dan kadar metoksilnya. Kemudian hasil analisa dibandingkan dengan standar mutu pektin berdasarkan standar mutu *International Pectin Producers Association* (IPPA).

Hasil

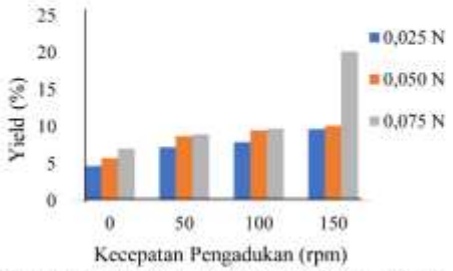
A. Pengaruh Konsentrasi Asam Klorida (HCl) Terhadap Perolehan *Yield* Pektin

Gambar 3 menunjukkan bahwa konsentrasi dan kecepatan pengadukan sangat berpengaruh terhadap rendemen pektin. Prinsip ekstraksi pektin adalah pengubahan protopektin yang tidak larut menjadi pektin yang dapat larut. Ekstraksi pektin ini dilakukan dengan cara hidrolisis asam maupun secara enzimatis.

Penggunaan asam klorida (HCl) sebagai pelarut dilakukan karena memiliki daya ekstrak yang tinggi dan ikatan valensi 1, sehingga tingkat keasaman

yang tidak terlalu tinggi. Tingkat keasaman yang tinggi tidak baik pada proses ekstraksi pektin, karena akan menyabahkan terdegradasinya pektin menjadi asam pektat dan membuat *yield* pektin yang semakin sedikit [10].

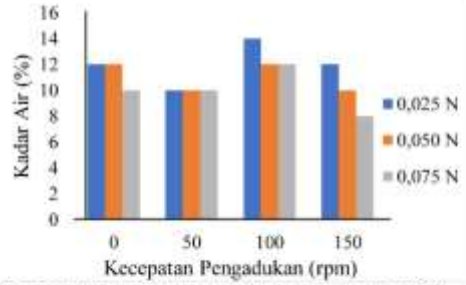
Jumlah pelarut yang digunakan untuk mengekstraksi juga sangat berpengaruh terhadap hasil *yield* pektin. Semakin banyak pelarut yang digunakan pada saat ekstraksi maka pektin yang terekstrak juga akan semakin banyak, karena pelarut dapat melarutkan hamper semua pektin yang terkandung di dalam kulit jeruk. Jumlah pelarut yang sedikit tidak dapat mengekstrak pectin secara optimal. Ekstraksi dilakukan selama pelarut yang digunakan belum jenuh. Pelarut yang telah jenuh tidak dapat mengekstraksi lagi dan kurang baik untuk melakukan ekstraksi karena gaya pendorong (*driving force*) semakin lama semakin kecil [7].



Gambar 3. Pengaruh Konsentrasi Asam Klorida (HCl) Terhadap Rendemen Pektin

B. Pengaruh Konsentrasi Asam Klorida (HCl) Terhadap Kadar Air Pektin

Kadar air bahan berpengaruh terhadap masa simpan. Kadar air yang tinggi menyebabkan kerentanan terhadap aktivitas mikroba. Dalam upaya memperpanjang masa simpan pektin, dilakukan pengeringan pada oven suhu 40 °C selama 8 jam. Pengeringan pada suhu rendah bertujuan meminimalkan degradasi pektin.

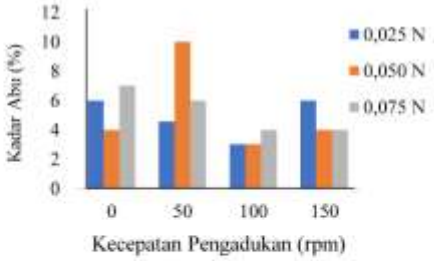


Gambar 4. Pengaruh KonsentrasiAsamKlorida (HCl) Kadar Air Pektin

Kadar air pektin yang dihasilkan berkisar antara 8-12%. Batas maksimum nilai kadar air yang diizinkan yaitu 12% [4]. Berdasarkan standar IPPA (*International Pectin Producers Association*), semua perlakuan masih memenuhi standar apabila kadar air pektin di bawah 12%. Kadar air yang dihasilkan dapat dipengaruhi oleh rendemen dari pektin. Semakin tinggi rendemen pektin, kadar air yang dihasilkan semakin tinggi [1]. Untuk melihat kadar air yang dihasilkan pada percobaan ini dapat dilihat pada gambar 4.

C. Pengaruh Konsentrasi Asam Klorida (HCl) Terhadap Kadar Abu Pektin

Kadar abu menunjukan bahwa masih ada komponen anorganik yang tertinggal didalam pektin. Semakin kecil kadar abu maka kemurnian pectin akan semakin baik. Pektin dengan mutu terbaik memiliki kadar abu 0% [5]. Perlakuan yang dilakukan dengan menggunakan asam dapat mengakibatkan terhidrolisis pectin dari ikatan kalsium dan magnesium. Peningkatan reaksi hidrolisis pada proto pectin mengakibatkan bertambah komponen Ca^{2+} dan Mg^{2+} di dalam larutan pengesktrak [9]. Hasil penelitian menunjukkan kadar abu pektin yang diperoleh berkisar antara 3% -10% yang sesuai dengan standar mutu kadar abu pektin yang ditetapkan IPPA (*International Pectin Producers Association*), yaitu maksimum 10%. Untuk melihat kadar abu yang dihasilkan pada percobaan ini dapat dilihat pada gambar 5.

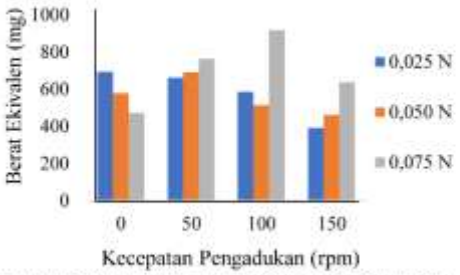


Gambar 5. Pengaruh Konsentrasi Asam Klorida (HCl) terhadap kadar abu pektin

D. Pengaruh Konsentrasi Asam Klorida Terhadap Berat Ekivalen Pektin

Berat ekivalen digunakan untuk menghitung kandungan dari asam *anhydrouronic* dan tingkat esterifikasi. Ditentukan dengan titrasi dengan natrium hidroksida dengan pH 7,5 baik indikator merah atau hitam [8].

Pada gambar 6 dapat dilihat adanya penurunan pada berat ekivalen, semakin tinggi kecepatan pengadukan dengan konsentrasi yang tinggi juga menghasilkan berat ekivalen yang semakin rendah. Pada percobaan ini berat ekivalen yang diperoleh dapat diterima, karena berdasarkan standar IPPA (*International Pectin Producers Association*) berat ekivalen adalah 600-800 mg.

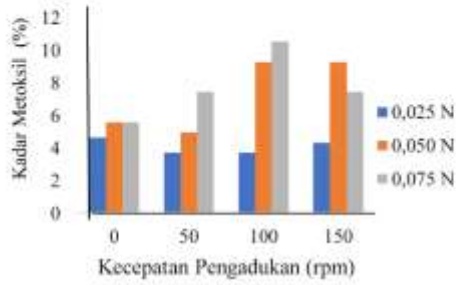


Gambar 6. Pengaruh Konsentrasi Asam Klorida (HCl) terhadap berat ekivalen pektin

E. Pengaruh Konsentrasi Asam Klorida (HCl) Terhadap Kadar Metoksil Pektin

Kadar metoksil dapat didefinisikan sebagai jumlah mol pada etanol yang terdapat didalam 100 mol asam galakturonat. Dimana kadar metoksil memiliki peranan yang sangat penting dalam menentukan sifat-sifat fungsional larutan pektin dan dapat mempengaruhi struktur dan tekstur dari gel pektin.

Kadar metoksil pectin hasil ekstraksi berkisar antara 3,72-10,54%. Menurut [9] Pektin dapat dikatakan bermetoksil tinggi apabila memiliki nilai kadar metoksil sama dengan atau lebih dari 7%, apabila kadar metoksil dibawah 7 % maka dapat dikatakan pectin tersebut bermetoksil rendah. Hal ini dikarenakan kadar asam galakturonat yang terdapat dalam kulit jeruk manis banyak. Semakin banyak kadar asam galakturonat yang termetoksil maka kadar metoksilnya semakin tinggi [7]. Untuk melihat kadar metoksil yang dihasilkan pada percobaan ini dapat dilihat pada gambar 7.



Gambar 7. Pengaruh Konsentrasi Asam Klorida (HCl) terhadap kadar metoksil pektin

Kesimpulan

Kesimpulan yang dapat diambil pada percobaan yang telah dilakukan yaitu :

1. Rendemen yang terbaik pada konsentrasi 0,075 N dan kecepatan pengadukan 150 rpm yaitu 20,12 %.
2. Kadar air yang tertinggi diperoleh sebesar 14% pada Konsentrasi 0,025N dan kecepatan pengadukan 100 rpm.
3. Kadar abu yang terbaik diperoleh sebesar 3% pada konsentrasi 0,025 N dan 0,050 N dengan kecepatan pengadukan 100 rpm.
4. Kadar metoksil tertinggi diperoleh sebesar 10,54% pada konsentrasi 0,075 N dan kecepatan pengadukan 100 rpm.
5. Kualitas pektin yang dihasilkan dengan pelarut asam klorida (HCl) ini telah memenuhi kriteria yang telah ditetapkan oleh IPPA (*International Pectin Producers Association*).

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Analisis Pektin Albedo Buah Jeruk Pamelo sebagai Adsorben Logam Berat Timbal (Pb), Kadmium (Cd) dan Tembaga (Cu)

(*Pectin Analysis of Pamelo Citrus Albedo* (*Citrus maxima* (Burm.f) Merr.) *as Adsorbent of Heavy Metal Plumbum (Pb), Cadmium (Cd) and Copper (Cu)*)

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ABSTRACT

Pamelo citrus is a fruit plant having the potential to be developed in Indonesia. One of the centers for producing large oranges or Pamelo citrus in South Sulawesi is Pangkep Regency. One of the components of the Pamelo citrus is pectin. Pectin is a polysaccharide compound with a high molecular weight which is widely found in plants and it can absorb metals because they contain carboxylic groups. This study aimed to analyze pectin from albedo pamelo citrus fruit as an adsorbent for heavy metal plumbum (Pb), cadmium (Cd) and copper (Cu). The method used was atomic absorption spectrophotometer with the equation analysis of heavy metal adsorption by pectin of pamelo citrus determined by Langmuir and Freundlich adsorption isothermal method. The results showed that pectin of albedo pamelo citrus following the isothermal of Freundlich adsorption which showed the adsorbent capacity of Pb, Cd and Cu with the KF values 0.561 mg/g, 0.010 mg/g and 0.066 mg/g, respectively. Meanwhile, n values were 0.823 for Pb, 0.321 for Cd and 1.121 for Cu, and in commercial pectin following the isothermal of Freundlich adsorption with the KF values of Pb, Cd and Cu were 0.650 mg/g, Cd 0.015 and Cu of 0.77 mg/g, respectively. Then the n values were 0.641 for Pb, 0.811 for Cd and 1.183 for Cu.

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ABSTRAK

Jeruk pamelo merupakan tanaman buah jeruk yang potensial untuk dikembangkan di Indonesia dan salah satu sentra produksi jeruk besar atau jeruk pamelo di Sulawesi Selatan adalah Kabupaten Pangkep. Salah satu kandungan buah jeruk pamelo adalah pektin. Pektin merupakan senyawa polisakarida dengan bobot molekul tinggi yang banyak terdapat pada tumbuhan dan pektin dapat menyerap logam karena mengandung gugus karboksilat. Penelitian ini bertujuan untuk menganalisis pektin dari buah jeruk pamelo sebagai adsorben logam berat timbal (Pb), kadmium (Cd) dan tembaga (Cu). Metode yang digunakan yaitu spektrofotometer serapan atom dengan analisis persamaan adsorpsi logam berat oleh pektin buah jeruk pamelo ditentukan dengan menggunakan isotermal adsorpsi Langmuir dan Freundlich. Dari hasil penelitian yang diperoleh pektin albedo buah jeruk pamelo mengikuti isotermal adsorpsi Freundlich yang menunjukkan kapasitas adsorben dengan nilai K_F logam berat Pb 0,561 mg/g, Cd 0,010 mg/g dan Cu 0,066 mg/g. sedangkan, nilai *n* logam berat Pb 0,823, Cd 0,321 dan Cu 1,121 dan pada pektin komersial mengikuti isotermal adsorpsi Freundlich dengan nilai K_F logam berat Pb0,650 mg/g, Cd 0,015 mg/g dan Cu 0,077 mg/g. Dan nilai *n* logam berat Pb 0,641, Cd 0,811 dan Cu 1,183.

Kata kunci: Pektin, Jeruk pamelo, Timbal, Kadmium, Tembaga, Isotermal adsorpsi Langmuir dan Freundlich.

PENDAHULUAN

Bidang industri di Indonesia pada saat ini berkembang cukup pesat. Perkembangan dunia industri banyak memberikan dampak terhadap kehidupan manusia baik yang positif maupun negatif. Dampak negatif yang dihasilkan adalah peningkatan konsentrasi bahan-bahan pencemar yang mengganggu lingkungan. Hal ini dapat dilihat dengan semakin banyaknya industri yang memproduksi berbagai jenis kebutuhan manusia seperti industri kertas, tekstil, dan penyamakan kulit, dengan adanya pertambahan industri tersebut, maka semakin banyak pula hasil sampingan dan limbah yang akan mencemari lingkungan sekitar, salah satunya adalah logam berat. Limbah logam berat merupakan limbah yang bersifat racun dan berbahaya. Beberapa logam berat yang dapat mencemari lingkungan dan bersifat toksik adalah krom (Cr), perak (Ag), kadmium (Cd), timbal (Pb), seng (Zn), merkuri (Hg), tembaga (Cu), besi (Fe), molibdat (Mo), nikel (Ni), timah (Sn), kobalt (Co) dan unsur-unsur logam ringan seperti arsen (As), aluminium (Al), dan selenium (Purwaningsih, 2009).

Berbagai usaha dilakukan untuk menetralisir pencemaran lingkungan akibat dari logam berat, seperti pemanfaatan berbagai produk biomaterial sebagai penyerap logam. Pemanfaatan dari bahan material ini merupakan alternatif yang dapat dipilih karena memiliki biaya yang minimal dalam proses produksinya. Salah satu biomaterial yang dapat dimanfaatkan sebagai penyerap logam adalah pektin (Wong, *et al.*, 2008).

Jeruk pamelo merupakan tanaman buah yang potensial untuk dikembangkan di Indonesia dan salah satu sentra produksi jeruk besar atau jeruk pamelo di Sulawesi Selatan adalah Kabupaten Pangkep.Telah dilakukan isolasi pektin dengan cara ekstraksi dari buah jeruk pamelo asal Kabupaten Pangkep menggunakan asam klorida 0,2 N pH 2 pada suhu 80^0C menghasilkan pektin varietas daging merah 5,74 % dan varietas daging putih 5,73 % dengan karakterisasi pektin yang dihasilkan sesuai dengan standar yang telah ditetapkan dan spektrum hasil analisis FTIR menunjukkan kesesuaian struktur pektin hasil isolasi dengan pektin komersial yaitu terdapat vibrasi (-OH), ikatan ($-CH_3$), ikatan (-C-H), karboksil (-C=O), dan eter (-O-) (Aminah, 2017). Selain itu juga, Sulihono (2012) telah mengisolasi pektin dari buah jeruk pamelo dengan cara ekstraksi menggunakan asam klorida 0,2 N pada suhu 80^0C dan lama waktu ekstraksi 120 menit menghasilkan pektin sebesar 26,70 %. Dan Mery (2017) telah mengisolasi pektin dari kulit jeruk siam dengan

waktu sentuh optimum yang diperlukan biosorben kulit jeruk siam untuk menyerap timbal adalah pada waktu 60 menit dengan persentasi serapan 99,18% dan kapasitas serapan sebesar 4,959 mg/g. Kondisi optimum untuk timbal diperoleh pada pH 4,0 dengan persentasi serapan 97,48% dan kapasitas serapan sebesar 4,947 mg/g.

Pektin dapat menyerap logam karena mengandung gugus karboksilat. Gugus karboksilat dari pektin dapat bereaksi dengan ion logam berat untuk membentuk senyawa kompleks yang tidak larut dalam air dan dapat diekskresi melalui feses. Reaktivitas pektin terhadap ion logam berat sangat tergantung pada derajat esterifikasinya (Syah, 2010).

METODE PENELITIAN

Alat dan Bahan

Alat yang digunakan yaitu pengaduk magnetik (*magnetic stirrer),* Sentrifuge, Spektrofotometer Serapan Atom (SSA), timbangan analitik (*Ohaus Carat Series)*

Bahan yang digunakan yaitu albedo buah jeruk pamelo varietas daging merah pangkep, aquades, asam klorida (HCl), etanol 96%, larutan standar $Pb(NO_3)_2$ 1000 ppm (*Merck),* larutan standar $Cd(NO_3)_2$ 1000 ppm (*Merck)* dan larutan standar $Cu(NO_3)_2$ 1000 ppm (*Merck)* dan pektin komersial.

Pengolahan Sampel

Sampel buah jeruk pamelo (*Citrus maxima* (Burm.f) Merr.) yang diperoleh dari Kabupaten Pangkep dikupas dan diambil albedonya (lapisan kulit berwarna putih) lalu dibersihkan dan dipotong kecil-kecil kemudian dikeringkan menggunakan oven pada suhu 50°C hingga kering.Albedo yang telah kering dihaluskan dan diperoleh serbuk albedo buah jeruk pamelo (Huyen, 2014).

Isolasi Pektin dengan Metode Ekstraksi

Ekstraksi pektin dari albedo buah jeruk pamelo

Sebanyak 50 gram albedo yang telah diserbukkan ditambahkan aquadest sebanyak 1:20 (Syarifuddin, 2015) lalu diasamkan dengan HCl 0,2 N sampai campuran menjadi pH 2, kemudian dipanaskan pada suhu 80°C selama 120 menit (Sutioso, 2012). Selanjutnya dilakukan penyaringan dengan menggunakan kain saring (kain blacu) dan filtrat diambil, kemudian dilakukan pengentalan setengah volume semula dengan pemanasan suhu 80°C lalu didinginkan pada suhu kamar (Dewayani, 2014; Aminah, 2017).

Hasil ekstraksi

Filtrat yang telah didinginkan ditambahkan etanol 96% yang telah diasamkan dengan perbandingan volume antara etanol 96% dan filtrat 1:1dan diendapkan selama 12 jam (Sulihono dkk, 2012). Endapan pektin dipisahkan dengan penyaringan menggunakan kertas saring dan dicuci dengan etanol 96%. Endapan pektin kemudian dikeringkan dalam oven pada temperatur 40°C hingga 8 jam.Pektin yang telah kering, kemudian dihaluskan lalu ditimbang dan hitung kadar rendamennya (Hariyati, 2006 ;Aminah, 2017).

Analisis Pektin Buah Jeruk Pamelo sebagai Adsorben Logam Berat Timbal (Pb), Kadmium (Cd) dan Tembaga (Cu)

Pembuatan larutan standar logam berat Pb, Cd dan Cu

Timbal (Pb)

Larutan standar $(Pb(NO_3)_2)$ 1000 ppm diambil sebanyak 5 mL dimasukkan kedalam labu ukur 50 mL, ditambahkan aquades sampai batas tanda sehingga diperoleh larutan standar 100 ppm. Kemudian dibuat larutan standar dengan seri konsentrasi 1, 2, 3, 4 dan 5 ppm.

Kadmium (Cd)

Larutan standar $Cd(NO_3)_2$ 1000 ppm diambil sebanyak 5 mL dimasukkan kedalam labu ukur 50 mL, ditambahkan aquades sampai batas tanda sehingga diperoleh larutan standar 100 ppm. Kemudian dibuat larutan standar dengan seri konsentrasi 1, 2, 3, 4 dan 5 ppm.

Tembaga (Cu)

Larutan standar $Cu(NO_3)_2$ 1000 ppm diambil sebanyak 5 mL dimasukkan kedalam labu ukur 50 mL, ditambahkan aquades sampai batas tanda sehingga diperoleh larutan standar 100 ppm. Kemudian dibuat larutan standar dengan seri konsentrasi 1, 2, 3, 4 dan 5 ppm.

Pembuatan larutan uji logam berat Pb,Cd dan Cu

Larutan standar logam berat Pb, Cd dan Cu dengan konsentrasi 100 ppm masing-masing dibuat larutan standar dengan seri konsentrasi 5, 10 dan 15 ppm.

Uji penyerapan pektin komersial sebagai pembanding terhadap logam berat Pb, Cd danCu

Sebanyak 3 buah erlenmeyer disiapkan dan dimasukkan pektin komersial sebanyak 0,5 gram kedalam masing-masing erlenmeyer, kemudian ditambahkan 50 mL larutan logam berat Pb dengan konsentrasi 5, 10dan 15 ppm pada setiap erlenmeyer. Selanjutnya diaduk menggunakan pengaduk magnetik (*magnetic stirrer)* selama 2 jam. Larutan tersebut disentrifugasi dengan kecepatan 3000 rpm selama 5 menit, ambil bagian supernatanya dan ukur kadarlogam dengan menggunakan SSA. Dilakukan perlakuan yang sama untuk logam berat kadmium (Cd) dan tembaga (Cu) (Arlofa, 2015; Wayan, 2014).

Uji penyerapan pektin albedo buah jeruk pamelo terhadap logam berat Pb, Cd danCu

Sebanyak3 buah erlenmeyer disiapkan dan dimasukkan pektin hasil ekstraksi albedo buah jeruk pamelo sebanyak 0,5 gram kedalam masing-masing erlenmeyer, kemudian ditambahkan 50 mL larutanlogam beratPb dengan konsentrasi 5, 10dan 15 ppm pada setiap erlenmeyer. Selanjutnya diaduk menggunakan pengaduk magnetik (*magnetic stirrer)* selama 2 jam.Larutan tersebut disentrifugasi dengan kecepatan 3000 rpm selama 5 menit, ambil bagian supernatanya dan ukur kadarlogam dengan menggunakan SSA. Dilakukan perlakuan yang sama untuk logam berat Cd dan Cu (Arlofa, 2015; Wayan, 2014).

Analisis Data

Data yang diperoleh dari spektrofotometer serapan atom digunakan untuk menentukan konsentrasi logam berat yang tersisa setelah proses adsorpsi dengan menggunakan pektin yang dapat dijelaskan dengan dua persamaan isoterm yaitu isoterm Langmuir dan isotherm Freundlich. Isoterm Langmuir didasarkan pada kurva hubungan antara Ce terhadap Ce/(x/m) dan isoterm Freundlich didasarkan pada kurva hubungan antara log Ce terhadap log (x/m),

Dengan nilai persamaan sebagai berikut :

$$C_{adb} = C_o - C_e,$$

$$xm = (C_o - C_e) \times Vm$$

$$Q = \frac{Co-Ce}{Co} \times 100\%$$

Keterangan :

C_o = Konsentrasi logam sebelum adsorbsi (ppm)

C_e = Konsentrasi logam yang tersisa (ppm)

C_{adb} = Konsentrasi logam yang teradsorbsi (ppm)

x/m = Jumlah mol logam yang teradsorbsi oleh pektin

(mol)

Q = Presentasi adsorbsi (%)

V = Volume larutan (L)

m = Berat pektin (g).

HASIL DAN PEMBAHASAN

Pada penelitian ini telah dilakukan isolasi pektin dari albedo buah jeruk pamelo varietas daging merah asal Kab. Pangkep Sulawesi Selatan. Rendamen yang diperoleh sebesar 7,48 %. Hasil isolasi pektin dari buah jeruk pamelo dianalisis kapasitas daya serapnya sebagai adsorben terhadap logam berat timbal (Pb), kadmium (Cd) dan tembaga (Cu) dan pektin komersial sebagai pembanding. Proses adsorpsi logam berat yaitu pektin ditambahkan dalam larutan logam dengan konsentrasi yang berbeda kemudian di *stirrer* selama 2 jam. Filtrat yang dihasilkan diuji dengan menggunakan Spektrofotometer Serapan Atom (SSA).

Analisis kapasitas daya serap pektin sebagai adsorben logam berat Pb, Cd dan Cu. Pertama dilakukan pengukuran larutan standar masing-masing logam berat dengan seri konsentrasi 1, 2, 3, 4 dan 5 sehingga diperoleh nilai absorbansi yang hasilnya ditunjukkan pada Tabel 1.

Tabel 1. Absorbansi larutan standar logam Pb, Cd, dan Cu

Konsentrasi logam (ppm)	Absorbansi Logam		
	Pb	Cd	Cu
0	0,0007	0,0007	0,0001
1	0,0068	0,2950	0,0859
2	0,0203	0,5960	0,1630
3	0,0299	0,8547	0,2412
4	0,0431	1,1631	0,3134
5	0,0547	1,3725	0,3855

Absorbansi larutan standar logam berat timbal (Pb), kadmium (Cd) dan tembaga (Cu) dibuat kurva baku yaitu plot antara konsentrasi (ppm) dan absorbansi untuk menghasilkan persamaan linier y = bx + a. Dari persamaan linier larutan standar akan dihitung nilai absorbansi logam berat Pb, Cd dan Cu yang telah teradsorpsi oleh pektin, dimana akan dihasilkan konsentrasi logam yang tersisa (Nilai x atau C_e).

Setelah dilakukan pengukuran larutan standar logam berat, selanjutnya dilakukan pengukuran larutan uji kapasitas daya serap pektin sebagai adsorben terhadap logam berat Pb, Cd dan Cu dengan seri konsentrasi 5, 10 dan 15 ppm dan dihasilkan nilai absorbansi yang ditunjukkan pada Tabel 2 untuk pektin komersial sebagai pembanding dan Tabel 3 untuk pektin buah jeruk pamelo.

Tabel 2. Absorbansi logam berat Pb, Cd dan Cu yang telah diadsorpsi oleh pektin komersial sebagai pembanding.

Konsentrasi logam (ppm)	Absorbansi Logam		
	Pb	Cd	Cu
5	0,002	0,081	0,081
10	0,003	0,147	0,147
15	0,006	0,174	0,174

Tabel 3. Absorbansi logam berat Pb, Cd dan Cu yang telah diadsorpsi oleh pektin albedo buah jeruk pamelo.

Konsentrasi logam (ppm)	Absorbansi Logam		
	Pb	Cd	Cu
5	0,001	0,072	0,112
10	0,004	0,119	0,239
15	0,005	0,153	0,351

Proses adsorpsi logam Pb, Cd dan Cu oleh pektin albedo jeruk pamelo dan pektin komersial sebagai pembanding dapat dijelaskan dengan dua persamaan isoterm yaitu isoterm Langmuir dan Freundlich, yang digunakan untuk menjelaskan proses absorpsi pada permukaan zat padat. Isoterm Langmuir didasarkan pada kurva hubungan antara C_e terhadap $C_e/(x/m)$ dan isoterm Freundlich didasarkan pada kurva hubungan antara log C_e terhadap log (x/m) (Wayan, 2014). Hasil adsorpsi logam Pb, Cd dan Cu oleh pektin albedo jeruk pamelo dan pektin komersial dengan parameter-parameter isoterm Langmuir dan Freundlich ditunjukkan pada Tabel 7 dan Tabel 8. Dari parameter tersebut dapat ditentukan isoterm adsorpsi logam Pb, Cd dan Cu oleh pektin albedo buah jeruk pamelo dan pektin komersial dengan membandingkan koefisien korelasinya (R^2) untuk menghasilkan kapasitas adsorpsi suatu adsorben.

Untuk menentukan nilai kapasitas adsorpsi logam dapat ditentukan dengan nilai K_F yang menunjukkan kapasitas serapan suatu adsorben, semakin besar nilai K_F maka semakin besar pula kapasitas adsorben menyerap adsorbat dan nilai n menunjukkan derajat nonlinieritas antara konsentrasi larutan adsorpsi, yaitu mengukur penyimpangan linieritas adsorpsi dan biasanya digunakan untuk mengetahui tingkat kebenaran suatu adsorpsi dengan nilai n <1, maka dapat dipastikan bahwa adsorpsi ini merupakan proses kemisorpsi dan sebaliknya jika >1, dipastikan bahwa adsorpsi yang terjadi merupakan proses fisiosorpsi (Ozcan, 2005).

Tabel 4. Parameter-parameter isoterm Langmuir dan Freundlich logam berat oleh pektin komersial sebagai pembanding.

Logam Berat	C_o	C_e	C_{ads}	x/m	C_e/ (x/m)	LogC_e	Logx/m	Q (%)
Pb	5	0,363	4,637	0,115	3,156	-0,440	-0,939	92,74
	10	0,454	9,546	0,238	1,907	-0,342	-0,623	95,46
	15	0,727	14,273	0,368	1,975	-0,138	-0,434	95,15
Cd	5	2,737	2,263	0,056	48,875	0,437	-1,251	45,26
	10	5,725	4,275	0,106	54,009	0,757	-0,974	42,75
	15	6,937	8,063	0,201	34,512	0,841	-0,696	53,75
Cu	5	1,263	3,737	0,093	13,580	0,101	-1,031	74,74
	10	2,697	7,303	0,182	14,818	0,430	-0,739	73,03
	15	4,394	10,606	0,265	16,581	0,642	-0,576	70,70

Tabel 5. Parameter-parameter isoterm Langmuir dan Freundlich logam berat oleh pektin buah jeruk pamelo.

Logam Berat	C_o	C_e	C_{ads}	x/m	C_e/ (x/m)	log C_e	log x/m	Q (%)
Pb	5	0,272	4,728	0,118	2,305	-0,565	-0,928	94,56
	10	0,545	9,455	0,236	2,309	-0,263	-0,627	94,55
	15	0,636	14,364	0,359	1,771	-0,196	-0,444	95,76
Cd	5	2,337	2,663	0,066	35,409	0,368	-1,180	53,26
	10	4,462	5,538	0,138	32,333	0,649	-0,860	55,38
	15	5,987	9,013	0,225	26,608	0,777	-0,647	60,08
Cu	5	1,394	3,606	0,090	15,488	0,144	-1,045	72,12
	10	3,065	6,935	0,173	17,716	0,486	-0,761	69,35
	15	4,539	10,461	0,261	17,390	0,656	-0,583	69,74

Pada proses fisiosorpsi gaya yang mengikat adsorbat oleh adsorben adalah gaya *van der waals*. Molekul terikat sangat lemah dan energi yang dilepaskan pada adsorpsi fisika relatif rendah. Sedangkan pada proses adsorpsi kimia (kemisorpsi), interaksi adsorbat dengan adsorben melalui pembentukan ikatan kimia. Kemisorpsi terjadi diawali dengan adsorpsi fisik, yaitu partikel-partikel adsorbat mendekat ke permukaan *van der waals* atau melalui ikatan hidrogen. Kemudian diikuti oleh adsorpsi kimia yang terjadi setelah adsorpsi fisika. Dalam adsorpsi kimia partikel melekat pada permukaan dengan membentuk ikatan kimia (biasanya ikatan kovalen) dan cenderung mencari tempat yang memaksimumkan bilangan koordinasi dengan substrat. Mekanisme proses adsorpsi dapat digambarkan sebagai proses dimana molekul meninggalkan larutan dan menempel pada permukaan zat adsorben secara kimia dan fisika (Atkins, 1999).

Konsentrasi larutan juga berpengaruh terhadap adsorpsi. Semakin tinggi suatu zat terlarut, maka semakin banyak pula zat terlarut yang dapat diadsorpsi oleh adsorben. Berdasarkan koefisien korelasi (R^2) yang diperoleh dari isoterm Langmuir dan isoterm Freundlich menunjukkan bahwa adsorpsi yang lebih sesuai dengan adsorpsi logam timbal oleh pektin komersial adalah isotermal Freundlich dibandingkan dengan model isotermal Langmuir. Hal ini bisa dilihat dari nilai R^2 persamaan isotermal Freundlich yaitu 0,886 sedangkan untuk persamaan isotermal Langmuir adalah 0,432. Berdasarkan isotermal Freundlich nilai K_F diperoleh sebesar 0,650 mg/g dan nilai *n* sebesar 0,641.Untuk logam Cd menunjukkan bahwa adsorpsi yang lebih sesuai dengan adsorpsi logam Cd oleh pektin komersial adalah isotermal Freundlich dibandingkan dengan model isotermal Langmuir. Hal ini bisa dilihat dari nilai R^2 persamaan isotermal Freundlich yaitu 0,897 sedangkan untuk persamaan isotermal Langmuir

adalah 0,274. Berdasarkan isotermal Freundlich nilai K_F diperoleh sebesar 0,015 mg/g dan nilai *n* sebesar 0,811.Untuk logam Cu menunjukkan bahwa adsorpsi yang lebih sesuai dengan adsorpsi logam Cu oleh pektin komersial adalah isotermal Freundlich dibandingkan dengan model isotermal Langmuir. Hal ini bisa dilihat dari nilai R^2 persamaan isotermal Freundlich yaitu 0,998 sedangkan untuk persamaan isotermal Langmuir adalah 0,997. Berdasarkan isotermal Freundlich nilai K_f diperoleh sebesar 0,077 mg/g dan nilai *n* sebesar 1,183.

Untuk pektin albedo buah jeruk pamelo menunjukkan bahwa adsorpsi yang lebih sesuai dengan adsorpsi logam Pb oleh pektin albedo buah jeruk pamelo adalah isotermal Freundlich dibandingkan dengan model isotermal Langmuir. Hal ini bisa dilihat dari nilai R^2 persamaan isotermal Freundlich yaitu 0,955 sedangkan untuk persamaan isotermal Langmuir adalah 0,474. Berdasarkan isotermal Freundlich nilai K_F diperoleh sebesar 0,561 mg/g dan nilai *n* sebesar 0,823. Untuk logam Cd menunjukkan bahwa adsorpsi yang lebih sesuai dengan adsorpsi logam kadmium oleh pektin albedo buah jeruk pamelo adalah isotermal Freundlich dibandingkan dengan model isotermal Langmuir. Hal ini bisa dilihat dari nilai R^2 persamaan isotermal Freundlich yaitu 0,978 sedangkan untuk persamaan isotermal Langmuir adalah 0,930. Berdasarkan isotermal Freundlich nilai K_F diperoleh sebesar 0,010 mg/g dan nilai *n* sebesar 0,321. Untuk logam Cu menunjukkan bahwa adsorpsi yang lebih sesuai dengan adsorpsi logam tembaga oleh pektin albedo buah jeruk pamelo adalah isotermal Freundlich dibandingkan dengan model isotermal Langmuir. Hal ini bisa dilihat dari nilai R^2 persamaan isotermal Freundlich yaitu 0,996 sedangkan untuk persamaan isotermal Langmuir adalah 0,659. Berdasarkan isotermal Freundlich nilai K_F diperoleh sebesar 0,066 mg/g dan nilai *n* sebesar 1,121.

Berdasarkan hasil nilai kapasitas adsorpsi terhadap logam berat timbal (Pb), kadmium (Cd) dan tembaga (Cu) yang didapatkan yaitu nilai K_F logam berat Pb pektin komersial lebih besar dibandingkan pektin albedo buah jeruk pamelo dan nilai $n<1$ maka proses adsorpsi termasuk proses kemisorpsi. Pada nilai K_f logam berat Cd pektin albedo buah jeruk pamelo lebih besar dibandingkan pektin komersial dan nilai $n<1$ maka proses adsorpsi termasuk proses kemisorpsi. Sedangkan nilai K_F logam berat Cu pektin albedo buah jeruk pamelo lebih besar dibandingkan pektin komersial dan nilai $n>1$ maka proses adsorpsi termasuk proses fisiosorpsi. Dan persentasi adsorpsi Q (%) untuk masing-masing konsentrasi logam berat Pb, Cd dan Cu menunjukkan persentasi adsorpsi logam yang tidak berbeda jauh dan semakin tinggi konsentrasi logam maka semakin besar persentasi adsorpsinya.

KESIMPULAN

Berdasarkan hasil penelitian yang telah dilakukan, maka dapat disimpulkan bahwa :

1. Pektin buah jeruk pamelo memiliki daya serap terhadap logam berat timbal (Pb), kadmium (Cd) dan tembaga (Cu).
2. Persentasi adsorpsi pektin albedo buah jeruk pamelo terhadap persentasi adsorpsi pektin komersial tidak berbeda jauh.
3. Pektin albedo buah jeruk pamelo mengikuti isotermal adsorpsi Freundlich dengan nilai K_F logam berat Pb 0,561 mg/g, Cd 0,010 mg/g dan Cu 0,066 mg/g dan nila*i n* logam berat Pb 0,823, Cd 0,321 dan Cu 1,121. Dan pektin komersial mengikuti isotermal adsorpsi Freundlich dengan nilai K_f logam berat Pb 0,650 mg/g, Cd 0,015 mg/g dan Cu 0,077mg/g dan nila*i n* logam berat Pb 0,641, Cd 0,811 dan Cu 1,183.

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Microwave Assisted Extraction (MAE) and Microwave-ultrasound Assisted Extraction (MUAE) of Pectin from Pomelo Peels

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***Abstract*—In the present study, microwave assisted extraction (MAE) and microwave-ultrasound assisted extraction (MUAE) were employed to recover pectin from pomelo peel. The effects of pH, irradiation time, microwave power, sonication time (only for MUAE) were investigated using Box–Behnken design (BBD) and the extraction condition was optimized. The highest validation experimental yield were 30.24±0.97% for MAE (irradiation time = 11.97 min) and 31.57±0.77% for MUAE (irradiation time = 10.11 min, sonication time = 17.72 min). The findings are agreeable with the predicted yield of 29.37% and 31.11% respectively for MAE and MUAE. It was observed that pH and microwave power have greater effect on extraction of pectin and the microwave irradiation time has slightly been reduced if ultrasound is incorporated. Considering the yield performance, shorter extraction time and less energy intensiveness, MAE is preferred to MUAE for the extraction of pectin from pomelo peel**

***Index Terms*—pectin extraction, ultrasound, microwave, optimization**

I. Introduction

Peels of the citrus family such as orange, lemon, lime and grapefruit and etc are potential source of pectin. Pomelo (*Citrus grandis* (L.) Osbeck) as the largest citrus fruits, is also targeted for pectin extraction. Pectin is an attractive biopolymer material [1] and has widespread applications in pharmaceutical, health, cosmetic, food, and feed industries owing to its good biocompatibility, non-toxicity, and biodegradability as well as high nutritional values such as mineral binding, prebiotic effect, cholesterol regulation, and anti-cancer action. Pectin is a family of heterogeneous polysaccharides with linear backbone comprised of repeating (1 → 4)-linked-α-D-galacturonic acid units [2].

Extraction of pectin is pivotal to biotechnology which involves separation of pectin from the plant matrix. It has been reported that, an ideal extraction method should be simple, safe, reproducible, inexpensive, provide high extraction rates, time saving, non-destructive on extraction compound and suitable for industrial application [3], [4]. Pectin extracted from citrus fruits peels could add value to the citrus processing industry if pectins can be extracted effectively by applying efficient extraction technologies. Many pectin extraction methods have been investigated with the use of acids in traditional heating extraction method. On the other hand, a number of up-to-date alternatives to traditional techniques have been proposed such as ultrasound assisted and microwave assisted extraction method to improve the yield performance, the process efficiency and the quality of the extracted compound [5]. Previous study on ultrasound-microwave assisted extraction (UMAE) of pomelo peel gave satisfactory pectin yield of 38% [6] which has inspired the present investigation on the feasibility of reversing the sequence of ultrasound and microwave techniques on pectin extraction. In this study, MAE and MUAE are optimized and their performances on pectin extraction are investigated. From the comparison study, the effect of ultrasound in the combined MUAE extraction system will be examined.

II. Materials and Methods

A. Materials

Pomelo (*Citrus grandis* (L.) Osbeck) fruit was supplied by Go Chin Pomelo Nature Park, Perak, Malaysia. The peels of the fruit were cut and washed thoroughly with fresh water followed by drying in a hot air oven (Memmert 600, Schwabach, Germany) at 60 °C until a constant weight is attained. The peel was powdered using a blender (Faber FBG 460, Kuala Lumpur, Malaysia) and sieved into 250 μm−400 μm. The dried peel powder was stored in dry condition using an air tight container prior to use. All solvents and chemicals used in this study were obtained from R&M (Selangor, Malaysia) and distilled water was used for all extraction and analytical processes.

B. Pectin Extraction Methods

In sole microwave assisted extraction (MAE), 10 g of dried pomelo powder was mixed with 290 mL distilled water and the pH (1.7−2.3) of the mixture solution was adjusted using citric acid. The microwave treatment of the mixture solution was carried out in a microwave oven (ME711K, Suwon, South Korea) and heated under different powers (350−650 W) and irradiation times (4−12 min). After the MAE extraction, the extract was

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filtered using centrifuge (Sigma 3-15P, Osterode am Harz, Germany) operated at 4000 rpm for 10 min. The supernatant was precipitated with 250 mL of 95% (*v*/*v*) ethanol and stored in dark condition at room temperature for 24 hours to allow pectin flotation. The pectin in the sample was subsequently separated by filtration and washed using 70% (*v*/*v*) ethanol twice and then dried in hot air oven at 65 °C until a constant weight was attained.

In the combined microwave-ultrasound assisted extraction (MUAE) on pomelo peel, similar aforementioned method was repeated for MAE before the microwave irradiated mixture solution was transferred to an ultrasonic bath (Branson 3800, Danbury, USA) for further extraction under sonication times (12−28 min). The extract from this combined techniques will subject to the same analysis procedure as previously described for MAE.

The percentage of dried pectin yield was determined using (1):

$$\text{Pectin Yield}(\%) = \frac{\text{weight of dried pectin}}{\text{weight of dried peel powder}} \times 100 \quad (1)$$

C. Optimization Study

Three levels Box-Behnken response surface design was employed as shown in Table I to investigate and optimize the effect of process variables on the pectin yield using MAE and MUAE. The variables for MAE were: pH (X_1: 1.7−2.3), microwave power (X_2: 350−650 W) and irradiation time (X_3: 4−12 min). The variables for MUAE were: pH (X_1: 1.7−2.3), irradiation time (X_2: 4−12 min), microwave power (X_3: 350−650 W) and sonication time (X_4: 12−28 min).

The statistical package Design Expert 6.0.6 (State-Ease Inc., Minneapolis, USA) was used to construct the experimental design, regression analysis and numerical optimization. The performance of the process generally can be described by the second-order polynomial equation and the generalized form of the equation is:

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum_i \sum_{j=2}^{k} \beta_{ij} X_i X_j \quad (2)$$

where Y represents the response variable, X_i and X_j are the independent variables affecting the response, and β_0, β_j, β_{jj}, and β_{ij} are the regression coefficients for intercept, linear term, quadratic term and interaction terms. The effects of process variables was analysed statistically by using analysis of variance (ANOVA) and the adequacy of the predicted optimum conditions was validated with the experimental results.

III. Results and Discussion

A. Optimization Study on the MAE and MUAE Extraction System

The pectin yield ranged from 10.48% to 29.02% for MAE and 10.59% to 30.24% for MUAE. The highest experimental yield was obtained when extraction conditions were pH of 1.7, microwave power of 650 W, irradiation time of 8 min and pH of 1.7, irradiation time of 8 min, microwave power of 650W, sonication time of 20 min.

Table II shows the analysis of variance (ANOVA) for MAE and MUAE yield of pectin with coefficient (R^2) of 0.990 and 0.917 respectively. The results indicated that the model used to fit response variables was significant ($p < 0.0001$) and adequate to represent the relationship between the responses and the independent variables.

Besides, Table II also shows that pH and microwave power exerted most effect on pectin yield for MAE and MUAE with $p < 0.0001$. The pectin yield increases significantly with decrease in pH and increase in microwave power. In MAE, an increase in irradiation time ($p < 0.01$) the pectin yield increased but not for MUAE as mild irradiation time ($p < 0.001$) was preferred.

Three-dimensional response surfaces for MAE and MUAE are shown in Fig. 1(a−c) & 1(d−i) respectively with the effects of the independent variables and their interaction on the yield of pectin. In term of yield, both MAE and MUAE preferred low pH and high microwave power within the range of investigation. MAE required longer irradiation time compare to MUAE, whereas MUAE at later prefer moderate sonication time. With regards to the total extraction time for optimized extraction condition, it is worth noting that microwave irradiation time in MUAE was shortened by mere 1.86 min as compare with the irradiation time in sole MAE but additional sonication time of 17.72 min was required. This could be explained by the effect of ultrasound on the plant surface which has enhanced the extraction performance. Sole MAE with long irradiation time may degrade pectin extracted. Hence, the combined MUAE might be an alternative for pectin extraction as microwave irradiation time would be reduced and the additional ultrasound extraction which does not involve heating will not cause thermal degradation of pectin.

The second-order polynomial equation for predicting pectin yield based on MAE and MUAE are expressed in terms of coded values as shown in Table III. An optimum pectin yield of 29.37% for MAE and 31.11% for MUAE were successfully predicted and the adequacy of the predicted optimum yield was validated. The experimental and the predicted results are very close within percentage error < 10%, indicating that the optimization was reliable.

Comparing between MAE and MUAE in term of pectin yield, there was only 1.74% increase using MUAE method. However, extra 15.86 min was needed which might not be feasible although MUAE might be an option to prevent thermal degradation of pectin as previously described.

TABLE I: Design Matrix of BBD and Pectin Extraction Yield Obtained from MAE and MUAE

	Microwave assisted extraction (MAE)		Microwave-ultrasound assisted extraction (MUAE)	
Run	Independent var.	Dependent var.	Independent var	Dependent var.

	x_1	(X_1)	x_2	(X_2)	x_3	(X_3)	Yield (%)	x_1	(X_3)	x_2	(X_2)	x_3	(X_3)	x_4	(X_4)	Yield (%)
1	0	(2.0)	-1	(350)	1	(12)	14.03	0	(2.0)	1	(12)	1	(650)	0	(20)	23.28
2	1	(2.3)	1	(650)	0	(8)	13.83	1	(2.3)	-1	(4)	0	(500)	0	(20)	11.90
3	1	(2.3)	-1	(350)	0	(8)	10.48	1	(2.3)	0	(8)	0	(500)	-1	(12)	13.46
4	1	(2.3)	0	(500)	1	(12)	13.39	0	(2.0)	1	(12)	0	(500)	-1	(12)	20.96
5	0	(2.0)	1	(650)	-1	(4)	19.24	-1	(1.7)	0	(8)	0	(500)	-1	(12)	24.14
6	0	(2.0)	0	(500)	0	(8)	15.67	1	(2.3)	0	(8)	0	(500)	1	(28)	13.02
7	-1	(1.7)	0	(500)	-1	(4)	21.12	0	(2.0)	0	(8)	0	(500)	0	(20)	21.91
8	0	(2.0)	0	(500)	0	(8)	15.11	0	(2.0)	0	(8)	0	(500)	0	(20)	22.05
9	0	(2.0)	0	(500)	0	(8)	13.78	1	(2.3)	1	(12)	0	(500)	0	(20)	14.62
10	0	(2.0)	1	(650)	1	(12)	20.22	0	(2.0)	0	(8)	-1	(350)	1	(28)	15.17
11	-1	(1.7)	-1	(350)	0	(8)	14.32	-1	(1.7)	0	(8)	1	(650)	0	(20)	30.24
12	-1	(1.7)	0	(500)	1	(12)	24.78	0	(2.0)	-1	(4)	0	(500)	-1	(12)	16.65
13	0	(2.0)	-1	(350)	-1	(4)	11.21	0	(2.0)	-1	(4)	0	(500)	1	(28)	10.59
14	1	(2.3)	0	(500)	-1	(4)	12.93	1	(2.3)	0	(8)	-1	(350)	0	(20)	16.65
15	-1	(1.7)	1	(650)	0	(8)	29.02	0	(2.0)	0	(8)	0	(500)	0	(20)	22.21
16	0	(2.0)	0	(500)	0	(8)	14.95	0	(2.0)	-1	(4)	1	(650)	0	(20)	19.45
17	0	(2.0)	0	(500)	0	(8)	15.45	-1	(1.7)	0	(8)	-1	(350)	0	(20)	20.18
18								0	(2.0)	1	(12)	-1	(350)	0	(20)	16.00
19								-1	(1.7)	0	(8)	0	(500)	1	(28)	25.26
20								1	(2.3)	0	(8)	1	(650)	0	(20)	15.59
21								0	(2.0)	0	(8)	1	(650)	1	(28)	21.19
22								-1	(1.7)	-1	(4)	0	(500)	0	(20)	16.41
23								0	(2.0)	1	(12)	0	(500)	1	(28)	19.98
24								0	(2.0)	0	(8)	0	(500)	0	(20)	19.33
25								-1	(1.7)	1	(12)	0	(500)	0	(20)	23.60
26								0	(2.0)	0	(8)	1	(650)	-1	(12)	23.00
27								0	(2.0)	0	(8)	-1	(350)	-1	(12)	13.73
28								0	(2.0)	-1	(4)	-1	(350)	0	(20)	14.91
29								0	(2.0)	0	(8)	0	(500)	0	(20)	21.22

TABLE II. ANALYSIS OF VARIANCE (ANOVA) FOR REGRESSION MODEL OF PECTIN YIELD OBTAINED FROM MAE AND MUAE

Microwave assisted extraction (MAE)						Microwave-ultrasound assisted extraction (MUAE)					
Source	SS	DF	MS	F	p	Source	SS	DF	MS	F	p
Model	382.054	9	42.45	73.58	< 0.0001	Model	545.833	14	38.99	11.01	< 0.0001
X_1-pH	186.342	1	186.34	323.00	< 0.0001	X_1-pH	248.339	1	248.34	70.12	< 0.0001
X_2-microwave power	130.169	1	130.17	225.64	< 0.0001	X_2-irradiation time	67.830	1	67.83	19.15	0.0006
X_3-irradiation time	7.841	1	7.84	13.59	0.0078	X_3-microwave power	108.661	1	108.66	30.68	< 0.0001
X_1^2	15.204	1	15.20	26.35	0.0013	X_4-sonication time	3.774	1	3.77	1.07	0.3194
X_2^2	0.002	1	0.00	0.00	0.9579	X_1^2	4.918	1	4.92	1.39	0.2583
X_3^2	5.693	1	5.69	9.87	0.0164	X_2^2	56.861	1	56.86	16.06	0.0013
X_{12}	32.206	1	32.21	55.83	0.0001	X_3^2	0.710	1	0.71	0.20	0.6613
X_{13}	2.560	1	2.56	4.44	0.0732	X_4^2	22.459	1	22.46	6.34	0.0246
X_{23}	0.846	1	0.85	1.47	0.2651	X_{12}	4.995	1	5.00	1.41	0.2547
Residual	4.038	7	0.58			X_{13}	30.914	1	30.91	8.73	0.0105
Lack of Fit	1.884	3	0.63	1.17	0.4263	X_{14}	0.608	1	0.61	0.17	0.6848

Pure Error	2.154	4	0.54	X_{23}	1.877	1	1.88	0.53	0.4786
Cor Total	386.092	16		X_{24}	6.452	1	6.45	1.82	0.1985
R^2	0.990			X_{34}	2.641	1	2.64	0.75	0.4024
Adj R^2	0.976			Residual	49.583	14	3.54		
				Lack of Fit	43.942	10	4.39	3.12	0.1423
				Pure Error	5.640	4	1.41		
				Cor Total	595.416	28			
				R^2	0.917				
				Adj R^2	0.833				

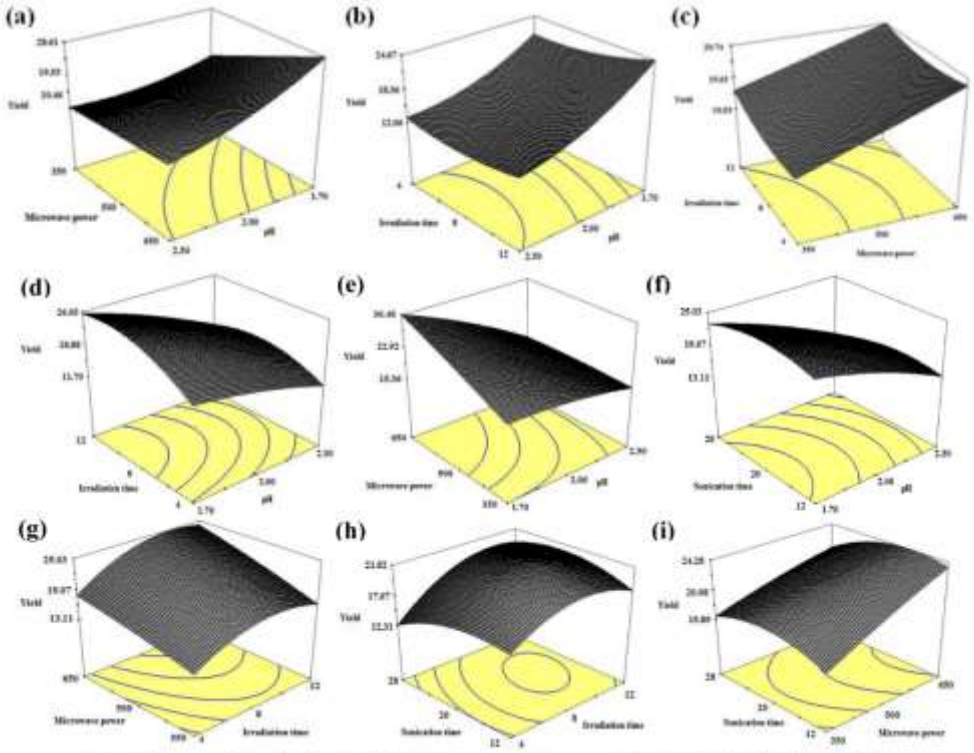

Figure 1. Response surface plots showing the effect of process variable on pectin yield, ((a)–(c)) MAE, ((d)–(i)) MUAE.

TABLE III. VALIDATION OF OPTIMUM EXTRACTION CONDITIONS

	Microwave assisted extraction (MAE)	Microwave-ultrasound assisted extraction (MUAE)
Optimum conditions	pH = 1.74, microwave power = 649.94 W, irradiation time = 11.97	pH = 1.73, irradiation time = 10.11 min, microwave power = 649.90 W, sonication time = 17.72 min
Equation Models	$Y = 14.99 - 4.83X_1 + 4.03X_2 + 0.99X_3 + 1.90X_1^2 + 0.02X_2^2 + 1.16X_3^2 - 2.84X_{1\ 2} - 0.80X_{1\ 3} - 0.46X_{2\ 3}$	$Y = 21.34 - 4.55X_1 + 2.38X_2 + 3.01X_3 - 0.56X_4 - 0.87X_1^2 - 2.96X_2^2 - 0.33X_3^2 - 1.86X_4^2 - 1.12X_{1\ 2} - 2.78X_{1\ 3} - 0.39X_{1\ 4} + 0.69X_{2\ 3} + 1.27X_{2\ 4} - 0.81X_{3\ 4}$
Predicted yield (%)	29.37	31.11
Experimental yield (%)	30.24±0.97	31.57±0.77
Percentage error (%)	2.88	1.47

IV. Conclusions

Optimum pectin yield of 29.37% for MAE and 31.11% for MUAE were obtained from pomelo peel extraction. In both the extraction techniques employed, pH and microwave power demonstrated highest impact on pectin yield. A slight increase in pectin yield using MUAE requires an additional 15.86 min making the combined extraction techniques not particularly suitable for pectin extraction.

Acknowledgement

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Lampiran 6. Jurnal 6

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PEMBUATAN PEKTIN DARI LIMBAH KULIT JERUK (*Citrus sinensis*) DENGAN METODE EKSTRAKSI GELOMBANG ULTRASONIK MENGGUNAKAN PELARUT ASAM SULFAT (H_2SO_4)

PECTIN PRODUCTION FROM ORANGE PEEL (Citrus sinensis) WITH ULTRASONIC WAVES EXTRACTION METHOD USING SULFURIC ACID (H_2SO_4)

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Abstrak
Pektin dapat dimanfaatkan dalam berbagai bidang industri pembuatan jeli, selai, pembentuk gel, pengental, penstabil dan pengemulsi. Pada penelitian ini akan dilakukan ekstraksi pektin dengan bahan baku kulit jeruk (*Citrus sinensis*) menggunakan bantuan gelombang ultrasonik dan pelarut asam sulfit (H_2SO_4) yang bertujuan untuk mengetahui pengaruh variasi waktu pengendapan dan konsentrasi etanol sebagai bahan pengendap filtrat pektin terhadap karakteristik pektin yang dihasilkan. Penelitian dilakukan dengan waktu ekstraksi 30 menit dengan temperatur ekstraksi 70 ^{0}C menggunakan pelarut H_2SO_4. Variasi yang dilakukan adalah waktu pengendapan selama 14 jam, 16 jam 18 jam dan 20 jam dan konsentrasi etanol 75%, 85% dan 95%. Karakteristik pektin yang dihasilkan dengan karakteristik berturut-turut: rendemen berkisar antara 16,44%-22,44%; kadar air 4,0%-9,45%; kadar abu 2,34%-5,54%; berat ekivalen 510,20%-865,07 mg; kadar metoksil 7,35%-10,79%; dan kadar galakturonat 63,71%-95,74%. Waktu pengendapan 18 jam dengan etanol 95% menghasilkan rendemen terbanyak dengan karakteristik pektin terbaik yang telah memenuhi standar IPPA (*International Pectin Producers Association*).

Kata Kunci: *ekstraksi, gelombang ultrasonik, kulit jeruk, pektin.*

Abstract
Pectin can be utilized in various industries of making jelly, jam, gelling, thickener, stabilizer and emulsifier. In this study the extraction of pectin from orange peel (*Citrus sinensis*) using ultrasonic waves and sulfuric acid (H_2SO_4) was conducted to determine the effect of variation in settling time and concentration of the alcohol as the pectin filtrate precipitating material on the characteristics of the pectin produced. The study was carried out with 30 minutes extraction time, extraction temperature of 70 °C using H_2SO_4. Variations carried out were settling time for 14 hours, 16 hours, 18 hours and 20 hours, and concentration of the alcohol ; 65%, 75%, 85% and 95%. Characteristics of pectin produced with successive characteristics: yield ranged from 16.44%-22.44%; moisture content from 4.0-9.45%; ash content of 2.34%-5.54%; equivalent weight of 510.20-865.07 mg; methoxyl content of 7.35%-10.79%; and galacturonic content of 63.71%-95.74%. The 18-hour settling time with 95% ethanol produced the most yield with the best pectin characteristics that met IPPA (International Pectin Producers Association) standards.

Keywords: *Extraction, ultrasonic waves, orange peel, pectin.*

Pendahuluan

Jeruk manis (*Citrus sinensis*) adalah buah yang paling umum ditanam di dunia. Pohon jeruk ditanam di iklim tropis dan subtropis [14]. Kulit dari buah jeruk yang baru saja dipanen mengandung sekitar 70% air, 6-8% gula, dan asam organik dalam jumlah kecil [18] selain itu juga mengandung 30% pektin dalam basis kering [12].

Kebutuhan pektin di Indonesia semakin berkembang dengan bertambahnya industri-industri makanan [11]. Pektin adalah substansi alami yang terdapat pada sebagian besar tanaman pangan. Pektin dalam jaringan tanaman terdapat sebagai protopektin yang tidak larut dalam air. Oleh karena itu dilakukan hidrolisis protopektin menjadi pektin yang larut dalam air menggunakan pelarut asam dalam ekstraksi pektin. H_2SO_4 merupakan pelarut yang kuat menghidrolisis protopektin dan merupakan pelarut yang baik untuk banyak reaksi [3]. Sayah, et al., (2016) melaporkan hasil ekstraksi pektin dari kulit jeruk dengan membandingkan penggunaan jenis pelarut yaitu H_2SO_4 dan asam

sitrat menunjukan rendeman sebesar 33,63% dan 29,93% [13].

Pemilihan ekstraksi metode konvensional dan penggunaan suhu tinggi menyebabkan kualitas pektin menurun. Oleh karena itu perlu dilakukan upaya modifikasi proses ekstraksi untuk memperoleh rendemen yang lebih banyak lagi dengan memanfaatkan gelombang ultrasonik pada proses esktraksi pektin [1]. Teknik ultrasonik telah dipelajari untuk meningkatkan ekstraksi senyawa target dari sumber tanaman [7].

Meninjau penelitian yang telah dilakukan oleh Adhiksana yang membandingkan hasil rendemen pektin dari kulit pisang dengan metode konvensional danmetode ultrasonik. Rendemen diperoleh sebesar 25,59% (metode ultrasonik) dan 18,3 % (metode konvensional) [1]. Oleh karena itu, kontribusi utama dari pekerjaan ini adalah pemanfaatan gelombang ultrasonik untuk meningkatkan efisiensi ekstraksi, mengurangi waktu dan suhu pemrosesan [2].

Sifat fisik yang terpenting dari pektin adalah dapat membentuk gel dengan keberadaan asam dan gula. Karakteristik pektin yang diekstrak diharapkan sama seperti pektin komersial yang harus memenuhi standar mutu *International Pectin Producers Association (IPPA)* dan *Food Chemical Codex*. Faktor-faktor yang berpengaruh pada proses ekstraksi adalah perlakuan pendahuluan bahan sebelum ekstraksi, ukuran partikel, jenis pelarut, waktu, suhu dan proses pemisahan pelarut [20]. Berdasarkan penelitian Chua, et al., (2018) bahwa kondisi optimum ekstraksi pektin dari kulit buah naga menggunakan bantuan gelombang ultrasonik diperoleh pada suhu 71,8 °C dan waktu ekstraksi selama 25 menit [6].

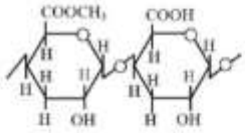
Pengolahan pektin dipengaruhi oleh sifat fisik dan cara ekstraksi, salah satunya adalah bahan pengendap dan lama pengendapan. Meninjau hasil penelitian Lumbantoruan, et al., (2014) bahwa interaksi antara konsentrasi pengendap dan lama pengendapan pada pektin yang dihasilkan dari ekstraksi kulit durian memberikan pengaruh yang berbeda sangat nyata terhadap rendemen, kandungan metoksil dan kadar galakturonat dan berbeda tidak nyata terhadap kadar air, kadar abu dan berat ekivalen [8]. Sehingga perlu dilakukan penelitian untuk mengetahui pengaruh waktu pengendapan dan konsentrasi bahan pengendap menggunakan etanol pada ekstraksi pektin dari kulit jeruk.

Berdasarkan studi pustaka yang telah dilakukan, sudah cukup banyak penelitian yang membahas tentang pektin. Ekstraksi pektin dari kulit jeruk dipilih karena ketersediaan bahannya yang melimpah dengan menggunakan metode ultrasonik.

Teori

Pektin adalah substansi alami yang terdapat pada sebagian besar tanaman pangan. Selain sebagai elemen struktural pada pertumbuhan jaringan dan komponen utama dari lamella tengah pada tanaman, pektin juga berperan sebagai perekat dan menjaga stabilitas jaringan dan sel. Pektin merupakan senyawa polisakarida dengan bobot molekul tinggi, pektin digunakan sebagai pembentuk gel dan pengental dalam pembuatan *jelly*, marmalade, makanan rendah kalori dan dalam bidang farmasi digunakan untuk obat diare [4].

Kelarutan pektin berbeda-beda, sesuai dengan kadar metoksilnya. Pektin dengan kadar metoksil tinggi larut dalam air dingin, pektin dengan kadar metoksil rendah larut dalam larutan alkali atau oksalat. Pektin tak larut dalam aseton dan alkohol. Kandungan pektin di dalam tanaman berbeda-beda tergantung pada sumber dan metode ekstraksi yang dilakukan [5]. Struktur kimia pektin dapat dilihat pada gambar 1 berikut [12].



Gambar 1. Struktur Kimia Pektin dengan Sejumlah Variabel Gugus Metil Ester

Berikut adalah standar mutu pektin berdasarkan standar mutu *International Pectin Producers Association* (2003) [15].

Tabel 1. Standar Mutu Pektin

Faktor Mutu	Kandungan
Kandungan metoksil :	
• Pektin metoksil tinggi	> 7,12%
• Pektin bermetoksil rendah	2,5% - 7,12%
Kadar asam galakturonat	> 65%
Kadar abu	< 10%
Kadar air	< 12%
Derajat esterifikasi untuk :	> 50%
• Pektin ester tinggi	< 50%
• Pektin ester rendah	600 – 800 mg
Berat Ekivalen	

Penggunaan ultrasonik pada proses ektraksi senyawa organik yang ada dalam tanaman dan biji-bijian dengan menggunakan pelarut organik

dapat berlangsung lebih cepat. Dinding sel dari bahan dipecah dengan getaran ultrasonik sehingga kandungan yang ada dadalamnya dapat keluar dengan mudah. Gelombang ultrasonik adalah gelombang bunyi dengan frekuensi yang lebih besar dari 20 KHz.

Cara kerja metode ultrasonik dalam mengekstraksi adalah sebagai berikut : gelombang ultrasonik terbentuk dari pembangkitan ultrason secara lokal dari kavitasi mikro pada sekeliling bahan yang akan diekstraksi sehingga terjadi pemanasan pada bahan tersebut, yang pada akhirnya akan melepaskan senyawa ekstrak. Terdapat efek ganda yang dihasilkan, yaitu pengacauan dinding sel sehingga membebaskan kandungan senyawa yang ada di dalamnya dan pemanasan lokal pada cairan dan meningkatkan difusi ekstrak. Energi kinetik dialewatkan ke seluruh bagian cairan, diikuti dengan munculnya gelembung kavitasi pada dinding atau permukaan sehingga meningkatkan transfer massa antara permukaan padat cair [1].

Metodologi Penelitian

Bahan Baku dan Peralatan

Bahan yang digunakan dalam penelitian ini adalah kulit jeruk, H_2SO_4, HCl, etanol 96% (C_2H_6O), *aquadest* (H_2O), NaCl, NaOH dan indikator *Phenolphtalein*.

Alat yang digunakan dalam penelitian ini adalah *ultrasound bath* Elmasonic S 300 H, *erlenmeyer*, *beaker glass*, termometer, pH meter, neraca analitik, statif dan klem, buret, gela sukur, corong gelas, batang pengaduk, *aluminium foil*, oven, cawan porselen, *furnace*, desikator, pipet tetes dan kertas saring.

Prosedur Pembuatan Pektin

1) Persiapan Bahan Baku

Kulit jeruk dicuci untuk menghilangkan kotoran kemudian dipotong dengan ukuran ± 0,5cm untuk memudahkan proses penghancuran bahan menggunakan blender. Kulit jeruk dikeringkan di dalam oven dengan suhu 60°C untuk mengurangi kandungan airnya, kemudian dihancurkan lagi hingga menjadi serbuk.

2) Ekstraksi Pektin dari Kulit Jeruk

Serbuk kulit jeruk yang dihasilkan dimasukkan ke dalam *erlenmeyer* sebanyak 25 gram, kemudian ditambahkan larutan H_2SO_4 0,050 N sebanyak 750 ml. Campuran kemudian dipanaskan di dalam alat *Ultrasound Bath* yang diisi air pada suhu 70°C, selama 30 menit dengan frekuensi 37 kHz. Perhitungan waktu ekstraksi dimulai saat tercapainya kondisi operasi percobaan. Selanjutnya dilakukan penyaringan dengan kertas saring dan filtrat diambil.

3) Pengendapan Pektin

Filtrat hasil ekstraksi yang telah dingin diendapkan menggunakan etanol dengan variasi konsentrasi 75%, 85% dan 95% dengan perbandingan volume 1:1 kemudian didiamkan dengan variasi waktu 14 jam, 16 jam, 18 jam dan 20 jam. Endapan pektin yang terbentuk dipisahkan dari filtratnya menggunakan kertas saring.

4) Pencucian Pektin

Endapan pektin yang terbentuk dicuci dengan etanol 96% sambil dilakukan pengadukan. Pemisahan endapan pektin dengan etanol 96% bekas pencucian dilakukan menggunakan kertas saring. Hal ini dilakukan beberapa kali hingga pektin bebas asam (tanda tidak lagi bereaksi dengan asam adalah ketika air bekas pencucian pektin berwarna merah bila ditetesi *phenolftalein*) [20].

5) Pengeringan Pektin

Pektin basah hasil pengendapan yang telah dicuci dan bebas asam selanjutnya dikeringkan dalam oven pada temperatur 50°C hingga berat konstan. Gel pektin yang telah kering kemudian ditimbang dan beratnya dicatat.

Hasil dan Pembahasan

Ekstraksi Pektin

Pada penelitian ini ekstraksi pektin dari kulit jeruk dengan memanfaatkan gelombang ultrasonik berfrekuensi 37 kHz dari Elmasonic S 300 H pada suhu 70°C, waktu ekstraksi 30 menit dan menggunakan pelarut H_2SO_4 0,05N.

Pada metode konvensional energi panas akan bergerak dari arah luar ke dalam bahan ekstrak. Sedangkan dengan ultrasonik, memanfaatkan peristiwa kavitasi dalam proses sehingga bisa mempercepat proses ektraksi sehingga waktu ektraksi lebih singkat. Secara prinsip gelombang ultrasonik terbentuk dari pembangkitan ultrasonik secara lokal dan kavitasi mikro pada sekeliling bahan yang akan diekstraksi sehingga terjadi pemanasan pada bahan tersebut, yang mengakibatkan terlepasnya senyawa ekstrak [1].

Seperti halnya pektin yang merupakan serat larut, yang terdapat di dinding sel tumbuhan, kavitasi dan gangguan sel yang disebabkan oleh gelombang *ultrasound* dapat meningkatkan transfer massa dari matriks padat ke pelarut meningkatkan ekstraksi pektin [7].

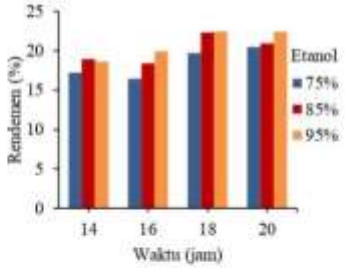
Pengaruh Waktu Pengendapan dan Konsentrasi Etanol pada Karakteristik Pektin Hasil Ekstraksi

Penelitian ini menjelaskan karakteristik pektin hasil ekstraksi dari kulit jeruk pada perlakuan perlakuan pengendapan selama 14

jam, 16 jam, 18 jam dan 20 jam menggunakan etanol sebesar 75%, 85% dan 95%.

Rendemen (*Yield*)

Rendemen pektin yang dihasilkan dari ektraksi kulit jeruk berkisar antara 16,44%-22,44%. Gambar 2 menyajikan persentasi rendemen pektin yang dihasilkan.



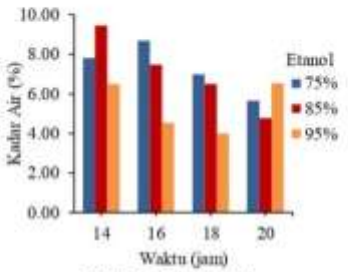
Gambar 2. Persentase Rendemen (*Yield*)

Gambar 2 menunjukkan pada perlakuan lama waktu pengendapan dapat dilihat pada penggunaan etanol 95% rendemen meningkat dengan semakin lama waktu pengendapan. Pada perlakuan perubahan konsentrasi etanol dapat dilihat pada waktu pengadapan 16 jam, 18 jam dan 20 jam, rendemen meningkat dengan semakin meningkat konsentrasi etanol yang digunakan.

Etanol di dalam larutan pektin akan bersifat sebagai pendehidroksi sehingga keseimbangan antara pektin dengan air akan terganggu dan pektin akan mengendap karena alkohol berbobot molekul rendah sehingga akan bercampur sempurna dengan air melalui ikatan hidrogen sehingga mengurangi jumlah ion atau molekul air disekeliling pektin sehingga pektin akan mengendap.

Kadar Air

Hubungan waktu pengendapan dan konsentrasi etanol yang digunakan terhadap kadar air dapat dilihat pada gambar 3.



Gambar 3. Kadar Air

Dari hasil pengujian kadar air pektin dari kulit jeruk terlihat bahwa kadar air akan semakin menurun seiring dengan kenaikan waktu pengendapan dan konsentrasi etanol yang digunakan.

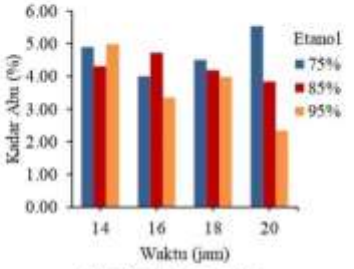
Kadar air pektin tertinggi diperoleh pada waktu pengendapan 14 jam dengan konsentrasi etanol 85% yaitu sebesar 9,45%. Kadar air pektin terendah diperoleh pada waktu pengendapan 18 jam dengan konsentrasi etanol 95% yaitu sebesar 4,0%. Hal ini disebabkan penambahan etanol dapat mendehidrasi pektin sehingga mengganggu stabilitas larutan koloidalnya dan akibatnya pektin akan terkoagulasi dan selama pengendapan terjadi penggantian molekul air oleh molekul terlarut yang mengakibatkan kontak yang lebih luas antara rantai-rantai pektin yang menghasilkan jaringan kompleks molekul polisakarida.

Alkohol berbobot molekul rendah sehingga akan bercampur sempurna dengan air melalui ikatan hidrogen sehingga mengurangi jumlah ion atau molekul air disekeliling pektin.

Kadar air dari pektin hasil ekstraksi pada penelitian ini berkisar antara 4,0%-9,45%. Syarat kadar air maksimum untuk pektin kering menurut IPPA (2003) yaitu maksimal 12%, dengan demikian kadar air pektin hasil penelitian ini memenuhi syarat mutu yang ditetapkan [15].

Kadar Abu

Kadar abu menunjukkan masih ada atau tidaknya komponen anorganik yang tertinggal di dalam pektin setelah pembakaran. Hubungan waktu pengendapan dan konsentrasi etanol yang digunakan terhadap kadar abu dapat dilihat pada gambar 4.



Gambar 4. Kadar Abu

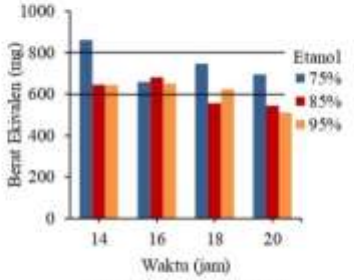
Kadar abu pektin tertinggi diperoleh pada waktu pengendapan 20 jam dengan konsentrasi etanol 75% yaitu sebesar 5,54%. Kadar abu pektin terendah diperoleh pada waktu pengendapan 20 jam dengan konsentrasi etanol 95% yaitu sebesar 2,34%. Pengaruh waktu pengendapan dapat dilihat pada gambar 4, dengan konsentrasi etanol 85% yang menunjukkan kecenderungan kadar abu mengalami penurunan dengan meningkatnya waktu pengendapan. Kadar abu juga mengalami penurunan dengan meningkatnya konsentrasi etanol sebagai bahan pengendap yang dapat dilihat pada pengendapan selama 18 jam dan 20 jam.

Komponen anorganik dapat berupa kalsium dan magnesium yang terhidrolisis bersama protopektin. Kadar abu berpengaruh pada tingkat kemurnian pektin. Semakin kecil kadar abu, maka kemurnian pektin akan semakin baik [3]. Syarat kadar abu maksimum untuk pektin kering menurut IPPA (2003) yaitu maksimal 10%, dengan demikian kadar abu pektin hasil penelitian ini memenuhi syarat mutu yang ditetapkan [15].

Berat Ekivalen

Berat ekivalen merupakan ukuran terhadap kandungan gugus asam galakturonat bebas (tidak teresterifikasi) dalam rantai molekul pektin. Asam pektat murni merupakan zat pektat yang seluruhnya tersusun atas asam poligalakturonat yang bebas dari gugus metil ester atau tidak mengalami esterifikasi. Asam pektat murni memiliki berat ekivalen 176. Tingginya derajat esterifikasi antara asam galakturonat dengan metanol mengakibatkan semakin rendahnya jumlah asam galakturonat bebas yang berarti semakin tingginya berat ekivalen [17].

Gambar 5 menunjukkan bahwa berat ekuivalen pektin hasil ekstraksi semakin menurun dengan meningkatnya waktu pengendapan pektin dan konsentrasi etanol yang ditambahkan.



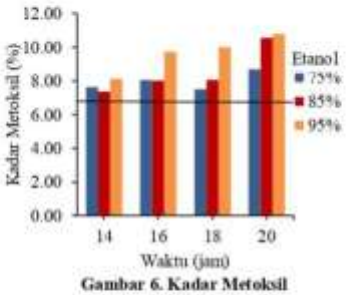
Gambar 5. Berat Ekivalen

Etanol umumnya dalam proses sintesa pektin sebagai larutan penggumpal dan pencuci agar dapat dipisahkan antara pektin dan pelarutnya serta larutan tambahan untuk menaikkan pH dari pektin yang asam karena pelarutnya [17]. Harga berat ekivalen ditentukan berdasarkan reaksi penyabunan gugus karboksil oleh NaOH. Banyaknya volume NaOH yang digunakan untuk bereaksi dengan gugus karboksil berbanding terbalik dengan nilai berat ekivalen. Semakin besar volume NaOH maka akan semakin kecil nilai berat ekivalen. Semakin kecil berat ekivalen artinya kandungan metoksil pektin semakin tinggi.

Berat ekivalen yang dihasilkan pada penelitian ini adalah 510,20-862,07 mg. Data standar mutu IPPA (2003) dimana berat ekivalen pektin berkisar antara 600-800 [15]. Hasil penelitian ini masih ada yang tidak memenuhi standar mutu yaitu pada waktu pengendapan 14 jam dengan konsentrasi etanol 75% dengan nilai berat ekivalen melebihi standar mutu, sedangkan pada perlakuan pengendapan 18 jam konsentrasi 85% dan pada waktu 20 jam dengan konsentrasi 85% dan 95% menghasilkan berat ekivalen yang nilainya di bawah standar mutu pektin.

Kadar Metoksil

Kadar metoksil didefinisikan sebagai jumlah mol etanol yang terdapat di dalam 100 mol asam galakturonat. Kadar metoksil pektin ini memiliki peranan yang sangat penting dalam menentukan sifat fungsional larutan pektin dan dapat mempengaruhi struktur dan tekstur dari gel pektin. Kadar metoksil pektin yang didapat dari hasil penelitian ini sekitar 7,35%-10,79% yang dapat dilihat pada gambar 6 berikut.



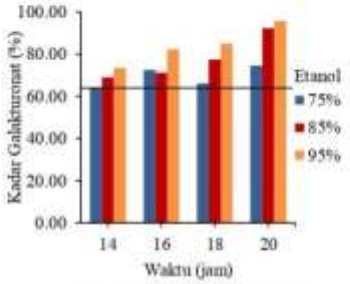
Gambar 6. Kadar Metoksil

Kadar metoksil mengalami peningkatan dengan meningkat nya waktu pengendapan serta kadar metoksil juga mengalami peningkatan dengan meningkatnya konsentrasi etanol sebagai bahan pengendap yang dapat dilihat pada pengendapan selama 18 jam dan 20 jam. Hal ini dikarenakan proses demetilasi dan deesterifikasi (hidrolisa gugus ester) pada pektin yang dapat meningkatkan kadar metoksil yang dihasilkan. Semakin banyak oksigen yang terlarut dalam larutan akan mempercepat reaksi, dengan demikian bila pengendapan yang lama akan mengakibatkan proses demetilasi. Proses demetilasi akan memindahkan gugus metil yang terekstraksi sehingga mengakibatkan banyak gugus metil yang dilepaskan [8].

Berdasarkan penelitian yang telah dilakukan, pektin yang dihasilkan termasuk pada pektin bermetoksil tinggi karena nilai kadar metoksil pektin memenuhi standar IPPA (2003) yaitu >7,12% untuk jenis pektin bermetoksil tinggi [15]. Dengan demikian pektin hasil ekstraksi dapat membentuk gel pada rentang pH=1 hingga 3,5 dan dengan penambahan gula 55%-85% [9].

Kadar Galakturonat

Salah satu yang menentukan mutu pektin adalah kadar galakturonat. Semakin tinggi kadar galakturonat maka mutu pektin semakin tinggi pula [16]. Hubungan waktu pengendapan dan konsentrasi etanol yang digunakan terhadap kadar galakturonat dapat dilihat pada gambar 7. Kadar galakturonat yang diperoleh berkisar antara 63,71%-95,74%. Kadar galakturonat pektin tertinggi diperoleh pada waktu pengendapan 20 jam dengan konsentrasi etanol 95% sedangkan kadar galakturonat pektin terendah diperoleh pada waktu pengendapan 14 jam dengan konsentrasi etanol 75%.



Gambar 7. Kadar Galakturonat

Pengaruh waktu pengendapan dapat dilihat pada gambar 7 dengan konsentrasi etanol 85% dan 95% yang menunjukkan kecenderungan kadar galakturonat mengalami peningkatan dengan meningkatnya waktu pengendapan. Kadar galakturonat juga mengalami peningkatan dengan meningkatnya konsentrasi etanol sebagai bahan pengendap yang dapat dilihat pada pengendapan selama 14 jam, 18 jam dan 20 jam. Hal ini disebabkan karena etanol bersifat polar sehingga dapat mengendapkan lebih banyak pektin dan semakin lama pengendapan maka akan terjadi reaksi hidrolisis protopektin menjadi pektin yang komponen dasarnya adalah asam D-galakturonat [9]. Syarat kadar galakturonat untuk pektin kering menurut IPPA (2003) yaitu minimum 65% [15], dengan demikian kadar galakturonat pektin hasil penelitian ini memenuhi syarat mutu yang ditetapkan kecuali pada waktu pengendapan selama 14 jam dengan etanol 75%.

Kesimpulan

Kesimpulan pada penelitian ini adalah perlakuan pengendapan pektin memberikan pengaruh pada rendemen dan karakteristik dari pektin yang telah diekstraksi. Semakin lama waktu pengendapan dan semakin besar konsentrasi etanol yang digunakan rendemen pektin semakin meningkat, sama halnya dengan karakteristik pektin yang berupa kadar metoksil dan kadar galakturonat pektin hasil ekstraksi juga meningkat, sedangkan karakteristik seperti kadar air, kadar abu dan berat ekivalen menunjukkan kecenderungan mengalami penurunan dengan meningkatnya lama waktu pengendapan dan konsentrasi etanol.

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Article

Evaluation of Pectin Extraction Conditions and Polyphenol Profile from *Citrus x lantifolia* Waste: Potential Application as Functional Ingredients

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Abstract: The citrus by-products pectin and polyphenols were obtained from *Citrus x lantifolia* residues. The use of acid type, solute-solvent ratio, temperature, and extraction time on pectin yield recovery was evaluated using a factorial design 3^4; pectin physicochemical characterization, polyphenol profile, and antioxidant activity were also determined. Results indicated a total polyphenol content of 3.92 ± 0.06 mg Galic Acid Equivalents (GAE)/g of citrus waste flour in dry basis (DB), with antioxidant activity of 74%. The presence of neohesperidin (0.96 ± 0.09 mg/g of citrus flour DB), hesperidin (0.27 ± 0.0 mg/g of citrus flour DB), and ellagic acid (0.18 ± 0.03 mg/g of citrus flour DB) as major polyphenols was observed. All of the factors evaluated in pectin recovery presented significant effects ($p < 0.05$), nevertheless the acid type and solute-solvent ratio showed the greatest effect. The highest yield of pectin recovery (36%) was obtained at 90 °C for 90 min, at a ratio of 1:80 (w/v) using citric acid. The evaluation of pectin used as a food ingredient in cookies elaboration, resulted in a reduction of 10% of fat material without significant texture differences ($p < 0.05$). The pectin extraction conditions and characterization from these residues allowed us to determine the future applications of these materials for use in several commercial applications.

Keywords: citrus polyphenols; citrus pectin; *Citrus x lantifolia*; pectin extraction conditions

1. Introduction

The citrus industry in Mexico represents an important economic and social activity. Worldwide, Mexico occupies the fifth place in citrus production; particularly, lemons represent the second most commonly produced citrus fruit in this country, where *Citrus x lantifolia* represents 50% of the citrus crop cultivated in Mexico [1]. Most of the citrus production is intended for fresh consumption; the rest is industrially transformed for the elaboration of juices, pulps, and fruit concentrates. Nevertheless, around 45% of the fruit is wasted; thus as production increases, the generation of solid and liquid waste also increases, which represents significant amounts of by-products not fully industrially exploited [2]. Nowadays, in order to reduce the environmental impact caused by waste materials from food industry, alternatives to obtain added value products that could be exploited in different areas are necessary; as an example, the extraction of biological molecules from citrus residues is an important part of an integrated system that could finish in bioethanol production with the residues free from compounds as

polyphenol and pectins, that can be used to favor fermentation process. Specifically, lemon peels are rich in polyphenolic compounds such as phenolic acids and flavonoids, which have been reported to be responsible for a variety of important biological effects [3]. Some of the biological properties reported by polyphenolic compounds are the reduction of cholesterol and blood sugar levels, anti-cancer effects, blood pressure lowering, anti-inflammatory properties, antimicrobial effects, antioxidant capacity, neuroprotection and cardioprotection, all of which are also of great interest for many industrial sectors [3–5]. Pectin, an acidic hydrocolloid widely used as a food ingredient for its gelling properties, is also an important biomolecule of high industrial interest found in citrus waste; it is considered to be a metabolite of biotechnological interest due to its stabilizing properties. Additionally, due to its high water content and easily adjustable physical properties [6], research regarding its potential uses in medical applications has emerged, for instance, nasal and oral drug delivery [7,8], cancer-target drug and gene delivery [9,10], and tissue engineering and wound healing [10]. Most of the commercial pectin is extracted from apple and citrus peels by the use of chloride acid, nevertheless, the trend of consumers looking to find products obtained in a more environmentally friendly way, searching for the lowest chemical residues generation as well as integrated citric waste utilization, led to the study of new extraction methods in order to maintain or improve upon recovery yields [2]. Furthermore, the prolonged commercial success of pectin has shown the importance of using fruit by-products as raw materials to utilize for production [11]. On the other hand, the use of pectin as an ingredient in bakery products has been suggested as an effective fat replacer [12]; pectin from *Yuja* (*Citrus junos*) pomace has been evaluated as an effective fat replacement (up to 10%) in cakes without volume losses. For that reason, the main objectives of this work were: (i) to determine the polyphenol profile from *Citrus x lantifolia* flour residues, (ii) to evaluate the principal factors affecting pectin extraction (solvent type, time, temperature, and solute/solvent radio), (iii) to analyze the physicochemical characteristics of the pectin extracted and compare it to a commercial one, and (iv) to evaluate the pectin obtained as a functional ingredient in a bakery product.

2. Materials and Methods

2.1. Biological Materials and Reagents

The raw material (Persian lime *Citrus x latifolia* peel, bagasse, and seed) was collected at the Akil Juicer from Union de Ejidos Citricultores, Akil, Yucatan. After juice and essential oils were obtained by mechanical methods (scraping), samples were transported to the research center (CIATEJ) and stored at −4 °C until further analysis. Folin-Ciocalteu's phenol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), analytical standards of gallic acid, caffeic acid, ellagic acid, naringin, hesperidin, neohesperidin, morin, quercetin, genistein, kaempferol, methanol and acetonitrile (chromatographic grade), and commercial citrus pectin were purchased in Sigma Aldrich (San Luis, MI, USA). Ultra-pure water was prepared in a Milli-Q water filtration system (Millipore, Bedford, MA, USA).

2.2. Flour Waste Preparation and Characterization

Waste products were oven dried at 65 °C for 48 h before polyphenol extraction. Moisture content was performed according to NMX-F-428-1982 [13], pH and acidity percentage were determined according to the Association of Official Agricultural Chemists (AOAC) method. Color was measured using a MiniScan Ez colorimeter, and L, a, and b parameters were obtained.

2.3. Polyphenol Extraction, Total Polyphenol Quantification (TPC), and Antioxidant Activity

Polyphenol extraction was performed according to the cryogenic methanolic extraction reported in MX/a/2012/014554 patent solicitude and by Sánchez-Contreras et al. [14]. Residual waste after polyphenol extraction was used for pectin recovery. The TPC was determined by Folin-Ciocalteu's phenol method [15], the absorbance was measured in a spectrophotometer (Thermo Scientific, Biomate 3S, Madison, WI, USA) at 760 nm. Estimation of TPC was carried out using gallic

acid as a standard and the results were expressed in mg of gallic acid equivalent per gram of citrus waste flour in dry weight (mg GAE/g DW). Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method [16], the absorbance was measured in a spectrophotometer (Thermo Scientific, Biomate 3S, Madison, WI, USA) at 517 nm. The DPPH scavenging activity was evaluated based on the percentage of DPPH radical scavenged according to Equation (1):

$$S_{DPPH} = S_b - (S_c - S_s)/S_b \times 100 \quad (1)$$

where S_{DPPH} is DPPH radical scavenging activity expressed as a percentage, S_b is the A517 nm of blank treatment, *Sc* is the A517 nm of sample solution, and *Ss* is the A517 nm of the background of sample.

2.4. Polyphenol Identification and Quantification by HPLC

A mixture of polyphenol standards were considered, containing: gallic acid, ellagic acid, caffeic acid, naringin, hesperidin, neohesperidin, morin, quercetin, genistein, and kaempferol. The retention time of each of the standards was taken as criterion to identify the polyphenol contents in the samples analyzed. To calculate the retention time of each standard, flavonoids were injected individually and the average of 15 individual determinations were taken as retention time value. To quantify the polyphenols identified in the samples, calibration curves of the standards at different concentrations (1, 5, 20, 50, and 100 ppm) were performed; each concentration was injected in triplicate in order to calculate the time of average retention of each of the flavonoids in the mixture. A Finnigan Surveyor Autosampler Plus Equipment was used, with Finnigan Surveyor PDA Plus Detector, the column used was a Phenomenex, 00F-4435-E0, Gemini 5 µ, C 18 110 A, 150 mm × 4.60 mm, 5 microns. The injection volume was 25 µL and the mobile phases used were A:HPLC water with formic acid (0.1%) solvent B: Acetonitrile with formic acid (0.1%), flow rate of 1 mL/min and gradient method starting with a minute at 90% of mobile phase (MP) A, then 40 min at 74% of MP A, 30 min at 35% of MP A, 5 min at 100% of MP B, and lastly, 5 min at 90% of MP A was performed to equilibrate the system. The determination time was 80 min at λ = 290 nm and λ = 350 nm detection.

2.5. Pectin Extraction

A factorial design 3^4 was used to evaluate the independent variables: acid type (hydrochloric, acetic and citric acid), solute-solvent ratio (1:30, 1:50, 1:80), temperature (60, 75, and 90 °C), and time extraction (30, 60, and 90 min) on the pectin yield recovery. Acids were adjusted to pH 2.2. The different acid solutions were placed in flasks of 100 or 250 mL according to the evaluated solute-solvent ratio, then flasks were heating individually to obtain the temperature proposed in the factorial design, and 2 g of citrus waste flour free of polyphenols was immersed into the solutions and kept in agitation during the periods of time evaluated. The resulted extracts were cool at ambient temperature and centrifuged at 5300 rpm for 15 min at 4 °C. The supernatants were further used for pectin recuperation, using ethanol (96% *v*/*v*) at 1:2 (*v*/*v*) ratio. The response variable of pectin yield was calculated according to the methods previously used by Baltazar et al. [2] (see Equation (2)), where g of recuperated pectin in DB indicates the weight in grams of the product recuperated after ethanol precipitation and oven drying for 24 h at 45 °C. The g of initial flour waste in D, represents the weight in grams of the raw material (*Citrus x latifolia* residues) used in the form of flour.

$$Pectin\ YIELD = \left(\frac{g \text{ of recuperated pectin in DB}}{g \text{ of initial flour waste in DB}} \right) \times 100 \quad (2)$$

2.6. Pectin Physicochemical Characterization

Three different pectin recuperation conditions were evaluated: two stages of acid hydrolysis extraction and alcohol precipitation (A), three stages of acid hydrolysis extraction, alcohol precipitation, and pectin washing (B), two stages of acid hydrolysis extraction with pH adjustment (pH = 6.5) before alcohol precipitation (C), then the resulting pectins were physicochemically characterized. Free Acidity

(FA) and Equivalent Weight (EW) were calculated according to [17], FA was expressed as meq of free carboxyl/g and EW was calculated with sample weight (mg) divided by the meq of the NaOH used for titration. Methoxy Content, Esterification Degree (ED), and Uronic Acid (UA) were calculated according to [18], Methoxy percentage was reported using Equation (3), ED was calculated using Equation (4), and UA was calculated according Equation (5). Identification and pectin conformation were determined according to [19] in order to obtain pectin gels.

$$Methoxy\ (\%) = \frac{\text{meq. of } NaOH \times MW \text{ of methoxy} \times 100}{sample\ weight\ (mg)} \quad (3)$$

$$ED\ (\%) = \frac{meq.\ of\ NaOH\ (methoxy\ content)}{meq.\ NaOH\ (free\ acidity) + meq.\ NaOH\ (methoxy\ content)} \times 100 \quad (4)$$

$$UA\ (\%) = \frac{meq.\ NaOH\ (free\ acidity) + meq.\ NaOH\ (methoxy\ content)}{sample\ weight\ (mg)} \times 176 \times 100 \quad (5)$$

2.7. Cookie Elaboration, Water Activity (a_W), Water Content (%), Physical and Textural Determinations

Cookies were prepared according to [20] with a slight modification, using wheat flour (22%), sugar (32%), vegetal fat (22%), egg powder (10%), coconut (7%), ammonium bicarbonate (0.5%), sodium bicarbonate (0.5%), raisins (6%), and water, then three percentages of the pectin previously obtained were evaluated for fat substitution (2.5%, 7%, and 10%). Water activity and water content (%) were measured using an a_W equipment Novasina AG (Lachen, Switzerland) and a thermobalance OHAUS MB45-2A0 (Switzerland), respectively. Physical characteristics of the cookies in terms of diameter (mm), thickness (mm), and spread ratio (diameter/thickness) were determined as the average of three measurements, using an electronic digital caliper (Truper) (Jilotepec, Mexico) [21]. Texture analyses were performed as maximal straight (textural hardness) using an EZ-SX Shimadzu Corporation, Japón equipment. Textural hardness was quantified using a three flexion points test. The results were expressed as the average of four measurements [21].

2.8. Statistical Analysis

Analysis of Variance (ANOVA) and Tukey comparison test ($p < 0.05$) of the results were determined using the software Statgraphics® Centurion, version XVI (Manugistic, Inc., Rockville, MD, USA). All the results analyzed by comparison test were the average of three independent determinations.

3. Results

3.1. Flour Citrus Waste Characterization

Previous to polyphenol and pectin extraction the Persian lime *Citrus x latifolia* waste was oven dried at 65 °C for 48 h; this procedure was performed in order to reduce water content of the residues to favor material preservation. The results of the physicochemical characterization of the flour obtained after the drying process are shown in Table 1. The moisture content was around 12%; this value favors preservation of the material during storage, avoiding microbial and enzymatic degradation. The pH obtained was 3.38 lower than the value obtained in the raw material (data not shown), the total titratable acidity obtained was 7.43%, reported as citric acid meq due to the greatest presence of this acid in lemon [22]. The color determination could provide information about possible sugar caramelization during the drying process, expressed as a brawn color, or degradation of the material with a low luminosity value [22]. As it can be observed in Table 1, color parameter results were related to a yellow color and luminosity was higher than 60%, indicating a well preserved flour.

Table 1. Physicochemical characterization of the Persian lime *Citrus x latifolia* flour waste.

Parameter		Determination
Moisture content %		12.12 ± 0.007
pH		3.38 ± 0.000
Titratable acidity % (citric acid meq)		7.43 ± 0.288
Color	Luminosity (L)	62.63 ± 0.08
	Parameter a	6.65 ± 0.04
	Parameter b	23.28 ± 0.070

3.2. TPC, Antioxidant Activity and Polyphenol Profile

As is shown in Table 2, the TPC of citrus waste was 3.92 ± 0.06 mg of GAE/g of citrus waste flour (DB), and an antioxidant activity of 73.2% ± 4.2% of $DPPH^+$ radical inhibition was observed. The major compound identified was neohesperidin with a concentration of 0.969 ± 0.099 mg/g of waste flour in DB, followed by hesperidin, ellagic acid, caffeic acid, morin, and, in lower concentrations, gallic acid, quercetin, kaempferol, and genistein (Table 2).

Table 2. Total polyphenol content, antioxidant activity, polyphenol identification, and concentration determination from citrus waste flour residues.

Type of Analysis	Determination	mg/g of Waste Flour in Dry Basis
Spectrophotometer analysis	Total Polyphenol content	3.92 ± 0.06
	Antioxidant activity (DPPH + Radical inhibition)	73.2% ± 4.2%
HPLC * analysis	Gallic acid content	0.074 ± 0.003
	Caffeic acid content	0.1560 ± 0.007
	Ellagic acid content	0.186 ± 0.0292
	Naringin content	0.003 ± 0.0001
	Hesperidin content	0.278 ± 0.011
	Neohesperidin	0.969 ± 0.099
	Morin	0.134 ± 0.004
	Quercetin	0.058 ± 0.0001
	Genistein	0.00045 ± 0.0001
	Kaempferol	0.015 ± 0.0001

* High pressure liquid chromatography; Values are expressed as mean ± standard deviation.

3.3. Pectin Extraction

Based on the factorial experimental design 3^4 the results of the different parameters evaluated on the yield of pectin extraction conditions are shown in Figure 1. Higher pectin yield values were observed at 90 °C with solute:solvent ratio of 1:80 with the three acids evaluated. Extractions with citric acid resulted in the best pectin yield compared to the others; the maximal pectin yield attained was 36% using citric acid. The multifactorial analysis of variance (ANOVA) indicated that the interaction of acid type and temperature as well as the four independently evaluated factors presented significant effect ($p < 0.05$) on the pectin extraction yield. According to the Pareto analysis (Figure 2), a major effect was related to acid type being higher with citric acid; solute:solvent ratio also presented a high effect, followed by temperature and time. For all of the factors evaluated, pectin extraction yield increased when the higher levels were evaluated.

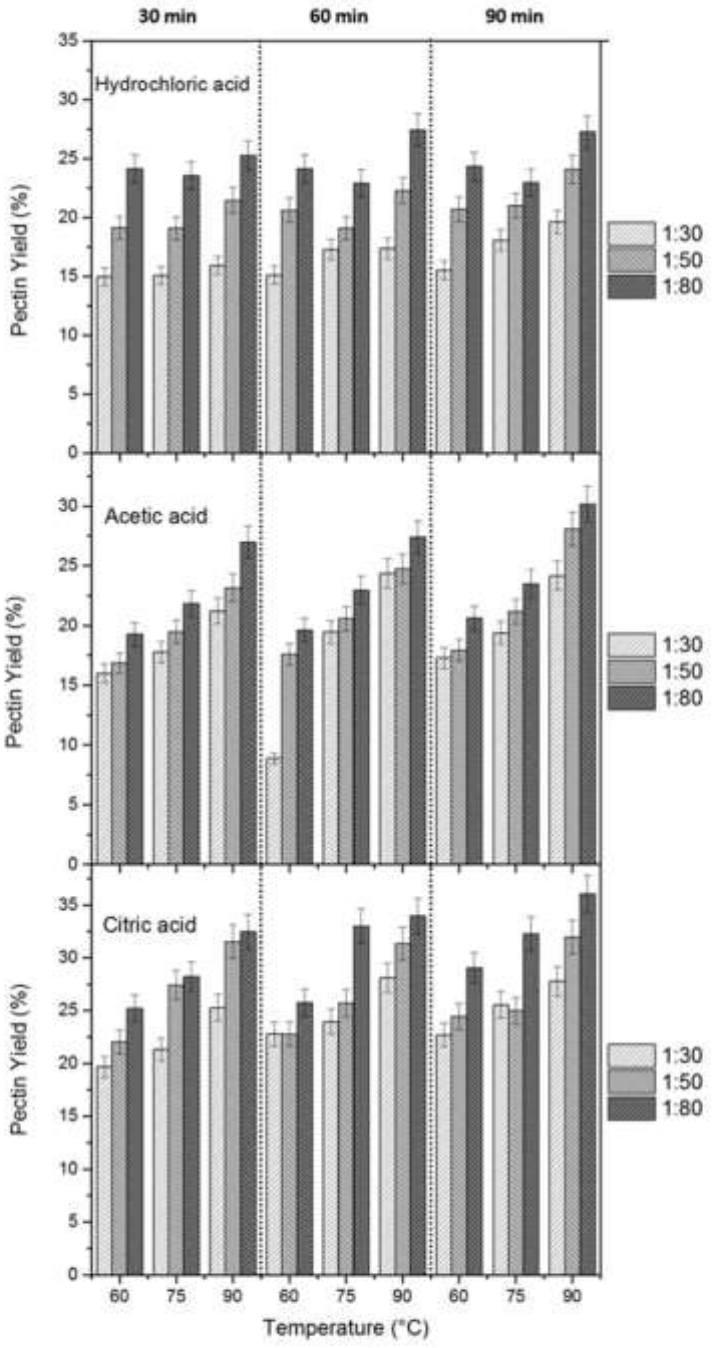

Figure 1. Pectin yield (%) under different extraction conditions: time (30, 60, and 90 min), temperature (60, 75, and 90 °C), solute:solvent ratio (1:30, 1:50, and 1:80), acid type (hydrochloric, acetic, and citric acid).

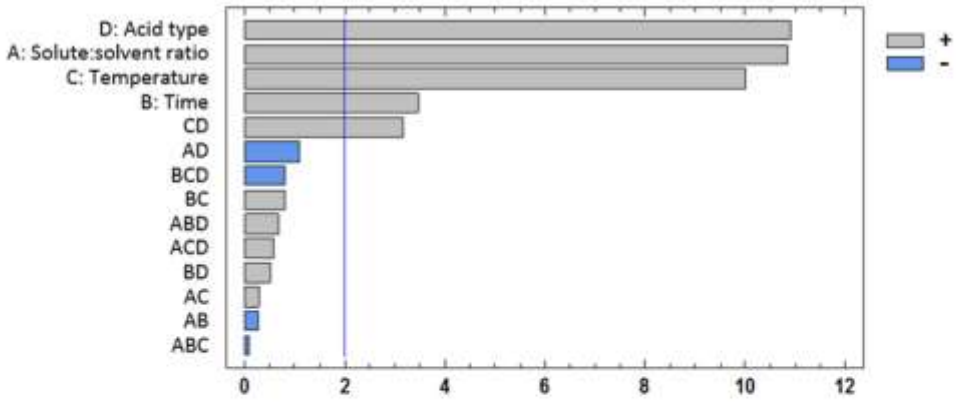

Figure 2. Standardized Pareto analysis of the pectin yield obtained from citrus waste.

3.4. Physicochemical Pectin Characterization

The higher pectin yield was obtained with pectin recuperation process A, using the two stage hydrolysis process, and C, with pH neutralization before alcohol precipitation. Moisture content values obtained with treatments A and B did not show significant differences ($p < 0.05$) between them, but were lower than the values presented in commercial citric pectin and treatment C (Table 3). The titratable acidity expressed as meq of free carboxyl/g of sample indicated that the recuperation procedure A exhibited the higher value; this could be due to the residual citric acid presented in the sample. The equivalent weight was lower using the recuperation procedure A and higher with procedure C when solution was neutralized before precipitation.

Table 3. Physicochemical characterization of pectins obtained from flour citric waste.

Physicochemical Characteristic	Pectin According to Recuperation Process			Commercial Pectin
	A *	B *	C *	
Yield (%)	36.45 ± 0.27^{a}	34.86 ± 0.21^{a}	36.21 ± 1.4^{a}	-
Moisture (%)	7.9 ± 0.01^{a}	8.17 ± 0.16^{a}	10.72 ± 0.27^{b}	10.49 ± 0.04^{b}
FA (meq free carboxyl/g)	3.01 ± 0.34^{d}	2.04 ± 0.05^{c}	1.73 ± 0.29^{b}	0.733 ± 0.0^{a}
Equivalent weight (mg)	400.37 ± 2.98^{a}	622.25 ± 0.0^{b}	706.74 ± 0.0^{c}	1364.63 ± 0.0^{d}
Methoxy (%)	10.12 ± 0.12^{b}	11.29 ± 0.29^{c}	9.00 ± 0.78^{a}	10.56 ± 0.2^{b}
ED * (%)	52.05 ± 0.59^{a}	64.09 ± 0.1^{b}	62.41 ± 1.52^{b}	82.29 ± 0.16^{c}
UA * (%)	DD *	DD *	81.59 ± 5.04^{a}	72.87 ± 0.16^{b}

Values are expressed as mean ± standard deviation. Similar letters in same line indicated no significant differences ($p < 0.05$). * A: two stages of extraction acid hydrolysis and alcohol precipitation, B: three stages of extraction acid hydrolysis alcohol precipitation and pectin washing, C: two stages of extraction with pH adjustment before alcohol precipitation, FA: Free acidity, ED: Esterification degree, UA: Uronic acid, DD: Difficult to determine.

The methoxy content and the ED are chemical parameters related to the gelification rate and pectin solidification. Procedure A showed similar methoxy % as commercial pectin; values higher than 8% are considered to be high methoxy pectin. ED (%) was significantly ($p < 0.05$) lower with the different pectin recuperation processes in comparison to commercial pectin. Nevertheless, all treatments showed high values of ED. The purity of the material can be determined by the UA content; values higher than 65% are accepted as high purity by the FAO, as is the case of the pectin obtained by procedure C.

3.5. Pectin Identification and Conformation

The qualitative test for pectin identification and conformation was performed in the sample obtained with procedure C and compared to the commercial one; all the tests indicated positive results to pectin (Table 4).

Table 4. Qualitative tests for the identification and conformation of pectin.

Test	Laboratory Pectin		Commercial Pectin	
	Description	Result	Description	Result
Pectin solution + ethanol	Yellow gelatinous pp	+	Sandy color gelatinous pp *	+
Pectin solution + NaOH 2N	Yellow gel	+	Sandy color gel	+
Precipitated gel + HCl 3N	Colorless gelatinous pp	+	Colorless gelatinous pp *	+

* pp: precipitate.

3.6. Evaluation of Pectin as Functional Ingredient

The addition of pectin in cookie elaboration was performed at 2.5%, 7%, and 10% of fat substitution. The results of water content, a_W, physical characterization, and texture of the resulting cookies are shown in Table 5. The water content in samples was between 5.3% and 8.3%; this parameter is related to the capability of the cookie to absorb water. The control and treatment with 2.5% of pectin substitution presented the lower water % content (0.45) and increased (≈0.48) for the treatments with 7% and 10% of pectin substitution. Diameter and thickness measurements of the cookies indicated that higher substitution significantly decreased these values, nevertheless, the largest spread ratio was obtained with the highest pectin substitution. Textural hardness indicated that there were not significant differences ($p < 0.05$) among the substitution concentrations and control treatments. The result values were in the interval of 18.3–20.6 N, which indicates the presence of a soft cookie that did not feature drastic fracturing of the components.

Table 5. Water content, A_W, physical characterization, and texture analysis of the resulting cookies with added citric pectin.

Treatments	Water Content (%)	A_W	Physical Characterization			Hardness (N)
			Diameter (mm)	Thickness (mm)	Spread Ratio	
Control	5.39 ± 0.3^{a}	0.44 ± 0.02^{a}	50.12 ± 0.15^{b}	10.45 ± 0.15^{c}	4.80	19.9 ± 3.3^{a}
2.5% of PS *	5.42 ± 0.5^{a}	0.45 ± 0.01^{a}	50.05 ± 0.12^{b}	10.35 ± 0.17^{c}	4.83	20.6 ± 2.1^{a}
7% of PS	8.23 ± 0.4^{b}	0.49 ± 0.02^{b}	47.50 ± 0.1^{a}	9.92 ± 0.11^{b}	4.78	18.3 ± 3.2^{a}
10% of PS	7.65 ± 0.1^{b}	0.48 ± 0.01^{b}	48.3 ± 0.11^{a}	9.68 ± 0.02^{a}	4.99	18.9 ± 3.2^{a}

Values are expressed as mean ± standard deviation. * PS: pectin substitution. Similar letters in the same line indicate no significant differences ($p < 0.05$).

4. Discussion

4.1. Flour Citrus Waste Characterization

Moisture % of the citrus waste flour is in concordance to the Mexican normativity for flour materials NOM 247-SSAI-2008 [23], which indicates a maximal value of 15%; this is an important parameter to ensure the preservation of the material, reducing the risk of microbial contamination and enzymatic degradation that could reduce polyphenols and pectin yields. pH is related to the acidity presented in citrus residues that could be reduced by the dehydration process due to the salts dissociation as reported by Badillo [22], who obtained pH values of 5.5 for fresh fruits and 3.2 after dehydration. Total titrable acidity is related to the citric acid presence that might remain in the peel waste after juice extraction [22]. The slight dark coloration of citrus waste flours could be related

to the natural pigments and sugars present in the products that could be partial degraded during waste drying, nevertheless the luminosity value higher than 60% indicates that the elaborated flour presented the possibility of preserving the quality of the by-products (polyphenols and pectin) that can be extracted from it.

4.2. TPC, Antioxidant Activity, and Polyphenol Profile

The importance of the polyphenol extraction method used in this study is related to the use of solid carbon dioxide, which favors a higher disruption of the vegetal matrix, which combines with the solvent, and promotes faster liberation of the phytochemicals compounds. A faster extraction process reduces the possibility of degradation to the polyphenols biological activity occurring due to heating and time consuming methods [24]. Furthermore, the total polyphenol content was higher than the results reported by Li et al. [25] and similar to the values obtained by Wang et al. [26] in citrus residues; in both cases, they used methanol as an extraction solvent. However, the TPC value was lower than the values mentioned by Papoutsis et al. [27]; this can be explained due to the different conditions used by these authors for drying peel preparation prior to TPC extraction with hot water, furthermore, the species of the citrus residue evaluated was also different (*C. limon*). Additionally, in our case, a lower TPC content could also be attributed to a previous essential oil extraction that could take part of the polyphenols presents in the peel and increase polyphenolic degradation before flour preparation. Although it has been reported [24] that a soft heat pre-treatment of the vegetal matrix (like the drying process) could enhance polyphenol extractions due to the previous disruption of the vegetal structures that favor polyphenol liberation, there are different conditions such as: pre-treatments methods, and different solvents and extraction conditions that can reduce or improve TPC yield [24]. Related to the antioxidant activity, the values reported herein are promising due to the preservation of more than 70% of the antioxidant activity. It has been reported that principal polyphenol compounds present in lemon residues, such as the flavonoids hesperidin and eriocitrin, may also have a major part in the antioxidant effect [28]. In this study, hesperidin is the second major compound detected, although the presence of eriocitrin was not determined due to the lack of the standard for the quantification analysis, the presence of unidentified peaks in the chromatogram was observed. If the methanolic polyphenol extraction is performed for further applications in the food industry, in all the cases a total elimination of the methanol content by evaporation is required for the restriction of methanol presence in food products, as reported by FDA. Phenolic compounds extracted from citrus waste could be excellent functional ingredients in the food industry and especially in bakery products due to the presence of antioxidant activity, nevertheless the sensitivity of the compounds suggests the use of encapsulation matrix to preserve their biological activities.

4.3. Pectin Extraction

For pectin extraction, all the factors evaluated presented a significantly effect. The solubilization of protopectin depends on a control acidic medium during the extraction process as well as the temperature and time [29]. A better pectin yield was obtained with citric acid than acetic or hydrochloric acid. Stronger acids could breakdown the polysaccharide bonds and reduce pectin yield; furthermore, the hydroxyl groups of the citric acid benefits the formation of hydrogen bonds between pectin and the citric acid favoring extraction [29]. As is indicated in results section, the Pareto analysis showed that conditions with higher values resulted in higher extraction yields; hence 90 min of time extraction and a ratio of 1:80 resulted in higher pectin yields regardless of the type of acid used. Pectin yields obtained in this work are comparable with those obtained from apple and citrus (10%–40%) at laboratory scale [29]. The use of citric acid that favors pectin yield also promotes the application of green technologies to pectin production, reducing the generation of hydrochloric acid residues.

4.4. Physicochemical Pectin Characterization

Pectin characteristics depend principally on the vegetal source as well as extraction conditions. Pectin moisture content along with all the physicochemical parameters of pectin is related to the quality of the product as it indicates the water absorption capability. Thus, pectin obtained with treatment C presented similar pectin moisture as the commercial pectin, indicating similar water absorption capability during storage at ambient temperature. Titratable acidity values were higher than those obtained in the literature [17]. This might be due to the residue evaluated and the ripening stage, as well as the residues of the citric acid used during extraction. The equivalent weight is associated with the maturity stage of the fruit and is inversely proportional to the free acidity; this parameter is related to the gelling power and viscosity of the resulted pectin. Ferreira et al. [30] determined the equivalent weight of different citric fruits, obtaining values between 528 and 1130 mg; thus, the values obtained herein between 400 and 750 mg are similar to the values reported elsewhere. Madhav and Pushpalatha [31] characterized pectins from different fruits, and found high methoxy pectins for citric sources of around 9%, as was found in this work. This value is similar to the values obtained for pomelo pectins [32]. Characterization of pectin from citrus residues (*Citrus x latifolia*) requires further analysis to study rheological parameters that can influence pectin application. However, considering its characteristic of forming gels, these pectins could also be used for medical applications [5] or to fabricate new entrapment bio-composites for probiotic delivery in the food industry [33].

4.5. Evaluation of Pectin as Functional Ingredient

As reported in the results section, the addition of pectin increased water content, although up until a 3% addition of pectin the conditions evaluated did not cause a significant difference in texture analysis. Thus, these results suggest the use of pectin from *Citrus x latifolia* flour residues could be used as a fat replacement in bakery products due to the capability of this pectin to trap water and to give weight to the product without increasing calories. Further analyses to increase substitution values and sensorial analyses are needed in order to obtain a healthy and high quality product, nevertheless an approximation of a functional application is given in this work.

5. Conclusions

The interaction between the temperature and acid type, as well as the individual factors: extraction time, solute-solvent ratio, acid type, and temperature presented a significant effect ($p < 0.5$) on the yield of pectin extracted from Persian lime *Citrus x latifolia* waste flour. According to the multifactorial variance analysis, the best extraction conditions were: citric acid at 90 °C for 90 min at a ratio of 1:80 (w/v) with a yield of 36% (g of pectin recovered per g of flour used). The evaluation of pectin recuperation process on the pectin physicochemical characteristics indicated that the best treatment was obtained with neutralization before precipitation. Using these conditions, a pectin with moisture content of 10.72%, free acidity of 1.73 meq free/g carboxyl equivalent weight of 706.74 mg, methoxy content of 9.0%, esterification degree of 62.41%, uronic acid of 81.59%, and yield of 36.21% can be obtained. The pectin was also categorized as having high methoxy, slow gelation, and a high degree of purity. Polyphenols profile determination indicated the major presence of the flavonoids neohesperidin and hesperidin in the residue with a TPC of 3.9 mg of GAE/g of citrus waste flour DB and a value higher than 73% for antioxidant activity. The application of the citrus pectin as fat replacer in the cookies elaboration indicated a potential functional use of the pectin extracted using the best conditions observed. The characterization of pectin allowed for the determination of the characteristics of pectins that might be useful in other commercial applications.

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RESEARCH ARTICLE

Yield, Esterification Degree and Molecular Weight Evaluation of Pectins Isolated from Orange and Grapefruit Peels under Different Conditions

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Abstract

Orange (*Citrus sinensis*) and grapefruit (*Citrus paradise*) peels were used as a source of pectin, which was extracted under different conditions. The peels are used under two states: fresh and residual (after essential oil extraction). Organic acid (citric acid) and mineral acid (sulfuric acid) were used in the pectin extraction. The aim of this study is the evaluation the effect of extraction conditions on pectin yield, degree of esterification "DE" and on molecular weight "Mw". Results showed that the pectin yield was higher using the residual peels. Moreover, both peels allow the obtainment of a high methoxyl pectin with DE >50%. The molecular weight was calculated using Mark-Houwink-Sakurada equation which describes its relationship with intrinsic viscosity. This later was determined using four equations; Huggins equation, kramer, Schulz-Blaschke and Martin equation. The molecular weight varied from 1.538 $x10^{05}$ to 2.47$x10^{05}$ g/mol for grapefruit pectin and from 1.639 $x10^{05}$ to 2.471 $x10^{05}$ g/mol for orange pectin.

Introduction

Pectin substances are present in practically all fruits and vegetables. These substances are the major component of the middle lamella and of the primary cell walls of fruit tissues [1]. Many works reported that citrus pectin have inhibitory effects on fibroblast growth factor signal transduction [2,3], suppression of LPS-induced inflammatory responses [4] and preventive effect on cancer growth and metastasis [5–7]. Pectin has also several physiological and biological functions, such as stimulation of phagocytes and macrophages [8,9], spleen cells proliferation [10] and reduction of serum cholesterol [11]. Citrus peels are reported to be good source

of pectin [12] which is widely used in the food industry for it gel-forming properties which depends on its degrees of methyl esterification DE and molecular weight [13]. The primary structural feature of this polysaccharides is a linear chain of poly-α-(1→4)-D-galacturonic acid with varying degrees of methyl esterification (DE). Commercial pectin preparations are divided into low-methoxyl (LM) and high-methoxyl (HM) pectins according to the degree of esterification (DE). Pectins with DE less than 50% are considered to be LM pectins [14,15]. Viscous-flow properties are very important during the production and applications of pectin and the higher the molecular weight is, the higher is its viscosity, the better is its grade [16,17]. Viscosity is affected by molecular weight, degree of methylation, concentration and temperature [17–19]. Usually, the extraction of the pectin is achieved by acid treatment at high temperature, using hydrochloric acid, nitric acid or sulfuric acid. This treatment allows the extraction and the solubilization of the pectin. However, some degradation reaction such as de-esterification and depolymerization will occur. Therefore, the extraction conditions (temperature, time, and pH) should be carefully controlled to achieve the desired pectin quality. Pectin is recovered by filtration or centrifugation process. Then, pectin is separated from the purified extract by precipitation using alcohol or by insoluble salt. The pectin is washed with alcohol to remove all impurities and finally dried and milled. Various alternative or complementary extraction processes have been suggested to improve the manufacture of pectin. We cite the extrusion pretreatment of the raw material when pectin is extracted from apple pomaces [20], Ultrasonic pulsation treatment in aqueous acidic solution which allow the reduction the processing time [21] and steam injection heating under pressure [22].

The objectives of this work is the determination of pectin yield, esterification degree and the molecular weight of orange and grapefruit peels pectin extracted after juice extraction, and from the residual peel after steam distillation using two kinds of acids: a mineral one which is the sulfuric acid, and organic one which is the citric acid.

Results and Discussion

2.1 Pectin yield

According to the extraction process described in the Fig 1, the pectin yield obtained from the two citrus species (orange and grapefruit):

Based on a dry weight and all citrus peels states, grapefruit peels pectin yield was higher than that obtained from orange peels used as raw material. For both citrus species, the highest pectin yield was obtained using the residual peels. Residual Orange peels pectin yield was 29.93% and 25.92% using sulfuric and citric acid respectively, while the fresh peels give the lowest pectin yield; 23.60% using sulfuric acid and 22.69% using citric acid. The highest pectin yield obtained from grapefruit peels was 33.63% from residual one using sulfuric acid, while using citric acid gives 28.74% as pectin yield. The pectin yield obtained from fresh grapefruit peels was 25.53% and 24.54% when using sulfuric and citric acid respectively. The increase in pectin yield in both orange and grapefruit peels is ranging from 3.23% to 8,10%. The increase in pectin yield is noticed when residual peels were used can be explained by the thermal treatment during the hydro-distillation which weakened the structure of the peels thus increasing interaction between acidic solution and raw material during the extraction, therefore leading to an effective increase of pectin yield. In the process of orange essential oil and pectin extraction, it has been recommended to first extract oil using simple distillation and then isolate pectin with acid hydrolysis technique which may lead to 46.46% as pectin yield [23]. Kar has removed essential oil form orange peels using petroleum ether and used these peels as raw material for pectin extraction. The yield obtained using hydrochloric acid was 29.58% [17]. Bagherian

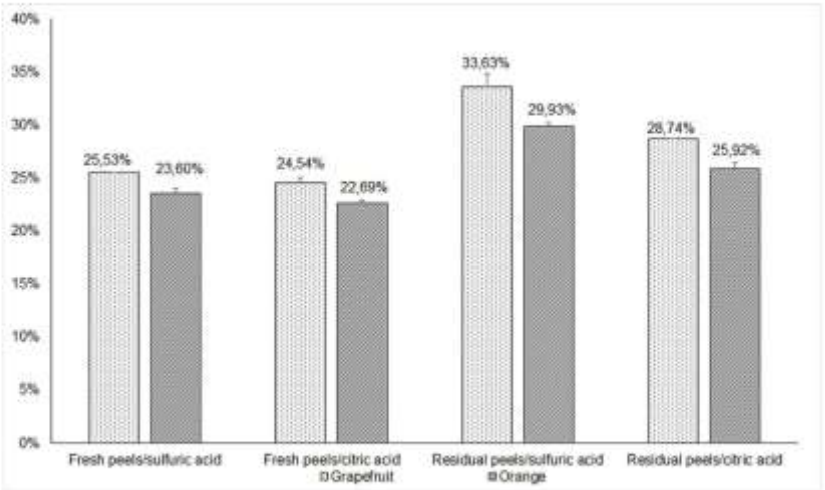

Fig 1. Effect of acids types and citrus peels stat on pectin yield.

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found that the pectin yield was 19.16% using a conventional method extraction on grapefruit peels [24].

2.2 Degree of esterification

The DE of grapefruit peels pectin ranged from 70.73±1.33% to 75.53±0.95% (Table 1) and ranged from 63.29±0.84% to 75.00±0.53% for orange peels pectin. Based on DE, pectin can be classified as high methoxyl pectin with DE >50% which is commercially available food-grade high methoxyl pectin [24,25].

From Table 1 we notice that the degree of esterification increases when we use the residual peels instead of fresh ones for pectin extraction and the DE was higher when the pectin is extracted using citric acid. The temperature and the acid concentration contribute to increase the DE of pectin [24,26]. The thermal treatment of the peels undergone during essential oils extraction affects the pectin degrees of esterification. Indeed harsh temperature conditions

Table 1. Degree of esterification of orange and grapefruit pectins.

Citrus peels	Peels stats	Acids	DE%
Grapefruit	Fresh	Sulfuric	71.72±1.06%
		Citric	70.73±1.33%
	Residual	Sulfuric	74.49±1.2%
		Citric	75.53±0.95%
Orange	Fresh	Sulfuric	63.29±0.84%
		Citric	65.49±0.57%
	Residual	Sulfuric	74.51±0.41%
		Citric	75.00±0.53%

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increases the degree of esterification [27,28]. Generally and for most of the pectins, it appears that the citric acid has a positive effect on the degree of esterification compared with that of sulfuric acid. This positive effect of citric acid has been noticed in various works [29,30].

2.3 Intrinsic viscosity determination

Intrinsic viscosity of a polymer solution is the assessment of polymer capacity to enhance viscosity [17]. Figs 2 and 3 show the method adopted for intrinsic viscosity determination. The later can be obtained using a linear regression graphic double-extrapolation procedure which involves extrapolating the course of a specific viscosity to infinite dilution [31]. As shown in Figs 2 and 3, the Huggins and Kraemer plots have a high level of linearity and extrapolate approximately to the same intercept at zero concentration for all extracted citrus pectins. These results suggest that the interference of ionic strength effects and molecular aggregation on viscosity behavior were reduced by the good choice of sodium chloride as solvent. In order to confirm and compare the results obtained from Huggins and Kraemer plots, values of intrinsic viscosity were also compared with those obtained by plotting Schulz-Blaschke Eq (8), and Martin Eq (9). The values of the intrinsic viscosity were comparable to each other and are also comparable to those obtained from Huggins and Kraemer plots for each pectin solution. For each pectin solution, the intrinsic viscosity was determined using the linear regression graphic extrapolation of the four equation mentioned Materials and Methods section (6, 7, 8 and 9). The values of intrinsic viscosity of all pectin solutions deduced from the plots are presented in Table 2 for grapefruit and Table 3 for orange peels.

The ANOVA test showed that the difference between all the peels stats/Acid is significant, F ratio was 5059.83 at ($p>0.05$). In order to compare each pair of peels stats/Acid we performed Tukey-Kramer HSD (honestly significant difference) test. Results showed that peels stats/Acid that are not connected by the same letter (a, b, c and d) are significantly different.

High degree of linearity was observed for all the plots. It shows that Huggins's equation, Kraemer's equation, the Schulz-Blaschke and Martin's equation are suitable to be applied to calculate the intrinsic viscosity [η] for all grapefruit pectin solutions (Fig 2) and also for orange pectin solutions (Fig 3). Moreover, the extrapolation plots of the four mentioned equations give approximately the same value of the intrinsic viscosity. From Table 2 and the Table 3, the intrinsic viscosity values depend on the nature of the acid used in the extraction of pectin and on the peels' states. It was seen that citric acid gives a high intrinsic viscosity value than that obtained using sulfuric acid, except for orange residual peels, where sulfuric acid extracted pectin with higher intrinsic viscosity than that obtained using citric acid. In addition, both of grapefruit and orange fresh peels give pectin with higher intrinsic viscosity than residual peels.

The ANOVA test showed that the difference between the different pectin molecular weight is significant, the F ratio was 2387.67, at $P<0.05$. The Tukey-Kramer HSD test showed that all peels stats/ acid were significantly different and that none was connected by the same latter.

To get more validity to our results, and according to Evageliou [32], we analyzed pectin chains behavior while flowing through the viscometer, by studying the variation of "zero shear" specific viscosity (η_{sp}) with degree of space-occupancy ($C[\eta]$). The concentration at which the total volume occupied by the polymer becomes equal to the total volume of the solution is known as the overlap concentration C^*. At this concentration polymer entanglement my happen [33]. Theoretical calculations showed that C^* is reached when $c[\eta] = 1$. Thus the polymer solution is defined to be dilute when $C < C^*$ and at higher concentrations ($C > C^*$) the polymer is termed semi-dilute. When $C > C^*$ an entangled network can be formed and a chain movement occur by a difficult process that changes the solution properties [32]. However for many polysaccharides significant restriction to the movement of individual chain

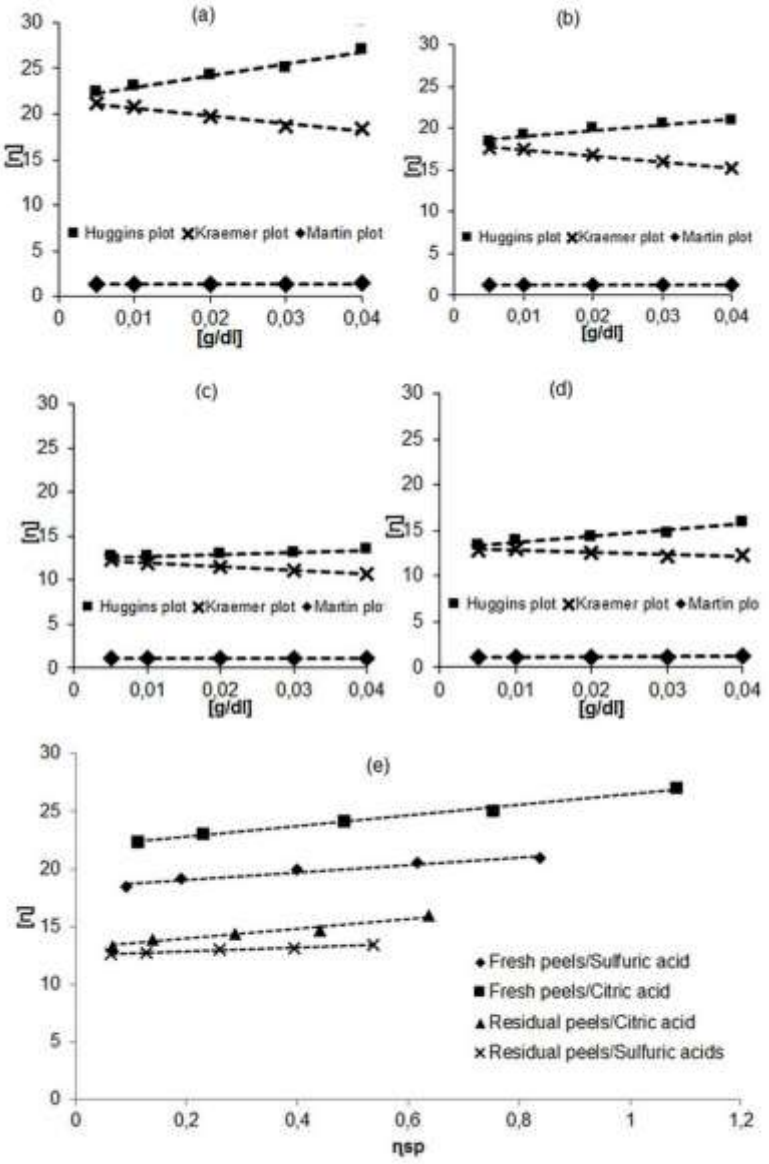

Fig 2. Huggins, Kraemer and Martin plots for grapefruit pectin extracted from fresh peels using sulfuric acid (a) and citric acid (b), and from residual peels using sulfuric acid (c) and citric acid (d)and Schulz-Blaschke plot of all grapefruit pectin solutions (e).

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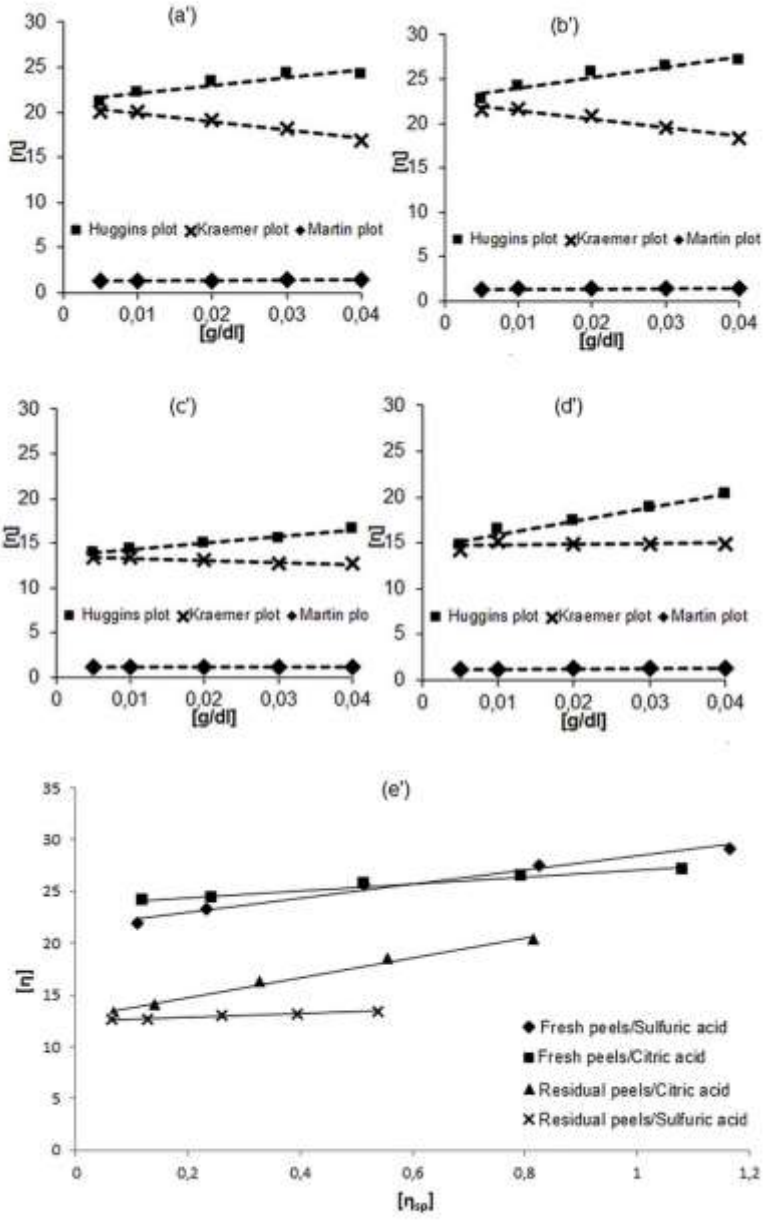
(a')
(b')
(c')
(d')
(e')
Huggins plot
Kraemer plot
Martin plot
[g/dl]
[η]
[ηsp]
Fresh peels/Sulfuric acid
Fresh peels/Citric acid
Residual peels/Citric acid
Residual peels/Sulfuric acid

Fig 3. Huggins, Kraemer, and Martin plots for orange pectin extracted from fresh peels using sulfuric acid (a'), and citric acid (b') and from residual peels using sulfuric acid (c'), and citric acid (d') and Schulz-Blacschke plot of all orange pectin solutions (e')

doi:10.1371/journal.pone.0161751.g003

occur at C = 4C* [32,34]. Fig 4 shows that all pectin solutions are termed as dilute solutions for both citrus species used in this work (a: orange; b: grapefruit) and that none of the solutions reached the four time coil-overlap concentration that causes significant changes in solution properties.

All pectin solutions are defined as dilute solutions ($C < C^*$). The specific viscosity is proportional to the parameter C [η] and it is scaled linearly. In addition, the range of concentrations used in intrinsic viscosity determination does not exceed the critical concentration C* beyond which the overlap between pectin chains are likely to occur, thereby distorting the measurement of the viscosity of pectin solutions. Various polysaccharides were studied to determine the concentration at which the behavior of their solution becomes messy because of overlaps polysaccharide chains. As a result, the transition from dilute solution behavior to the behavior of concentrated solution occurs when the specific viscosity $\eta_{sp} \approx 10$ and the parameter C [η] = 4 C* [35]. All pectin solutions are dilute solutions because they have a specific viscosity of not more than 1.5. Also the parameter "C [η]", which informs about the occupation state of volume of the solvent by the polymer, is well below the threshold 4C*. Beyond the threshold of 4 C*, the pectin solution is defined to be concentrated. Therefore, there are overlaps that cause significant restraints on individual pectin chains' movement relative to each other during the flow of the pectin solution through the capillary viscometer. Thus distorting the viscosity measurements.

2.4 Molecular weight determination

Since Huggins's equation, Kraemer's equation, the Schulz-Blaschke and Martin equation can be used to determinate the intrinsic viscosity we calculated the average of all intrinsic viscosity gotten by the Eqs (6, 7, 8 & 9) and we used this average intrinsic viscosity value in the Mark-Houwink-Sakurada Eq (10) to calculate the molecular weight Mw (Table 4). The molecular weight of pectin extracted from the residual citrus peels, after essential oil distillation, was lower than the one obtained using fresh peels for both citrus species. It can be explained by the thermal degradation of the pectin during the essential oil extraction, which has a lowering effect on pectin molecular weight. Bagherian and Fishman reported that continued heating of pectin may lead to pectin networks disaggregation, thus decreasing the molecular weight [24,36].

To support our results, we performed an exclusion chromatography using Sephadex G-150 as stationary phase. We hydrate the Sephadex (5g) with water (100ml) for one hour. After filling the column (15cm x 1.4cm), we determined the retention time of the following polymers:

- Sodium carboxymethyl cellulose (SIGMA-ALDRICH, Mw ~90000 g/mol)

Table 2. Intrinsic viscosity of grapefruit pectin solutions.

	Intrinsic viscosity [η] (dl/g)				
Grapefruit peels Stats/Acid	Huggins plot	Kraemers plot	Schulz-Blaschke plot	Martin plot	average value of [η] (dl/g)
Fresh peels/Sulfuric acid	18.385	18.173	18.454	18.412	18.356±0.091[a]
Fresh peels/Citric acid	21.231	21.332	21.691	21.449	21.426±0.144[c]
Residual peels/Sulfuric acid	12.512	12.426	12.524	12.517	12.495±0.034[c]
Residual peels/Citric acid	13.015	13.051	13.106	13.071	13.061±0.028[d]

doi:10.1371/journal.pone.0161751.t002

Table 3. Intrinsic viscosity of orange pectin solutions.

	Intrinsic viscosity [η] (dl/g)				
Orange peels Stats/Acid	**Huggins plot**	**Kraemers plot**	**Schulz-Blaschke plot**	**Martin plot**	**Average value of [η] (dl/g)**
Fresh peels/Sulfuric acid	20.687	20.961	21.361	20.980	20.997±0.18[a]
Fresh peels/Citric acid	22.874	22.479	22.906	22.699	22.739±0.15[b]
Residual peels/ Sulfuric acid	13.556	13.595	13.665	13.618	13.608±0.03[c]
Residual peels/ Citric acid	14.414	14.638	14.771	14.581	14.601±0.1[d]

doi:10.1371/journal.pone.0161751.t003

- Commercial Orange pectin (SIGMA-ALDRICH, Mw = 195000—DE = 70%)
- Locust bean gum (SIGMA-ALDRICH, MW 315000 g/mol)

A total of 17 fractions (each 15 seconds) were collected and for the revelation of the polymers presence we used the Dubois protocol [37]. This protocol allows the colorimetric determination of sugar and related substances. The aim of this experiment is obtaining a calibration curve that links the molecular weight to the retention time. The link between the molecular weight and the retention time is represented as the following equation of the calibration curve Rt = a Mw+b (Rt: retention time; Mw: Molecular weight). The equation of the calibration curve is:

$$y = -0.0009x + 292.19 \text{ and } r^2 = 0.9711.$$

The presence of the polymers was detected using Dubois protocol. While the intensity of the peak was evaluated using the absorbance reading of each fraction. After the establishment of the calibration curve, we performed the exclusion chromatography of our orange pectin which was extracted from fresh peel using citric acid (Mw = $2.405^{*}10^{05}$ g/mol) and from residual peels using sulfuric acid (Mw = $1.639^{*}10^{05}$ g/mol respectively) in order to confirm their molecular weights. Table 5 shows the results obtained according to the sampling interval:

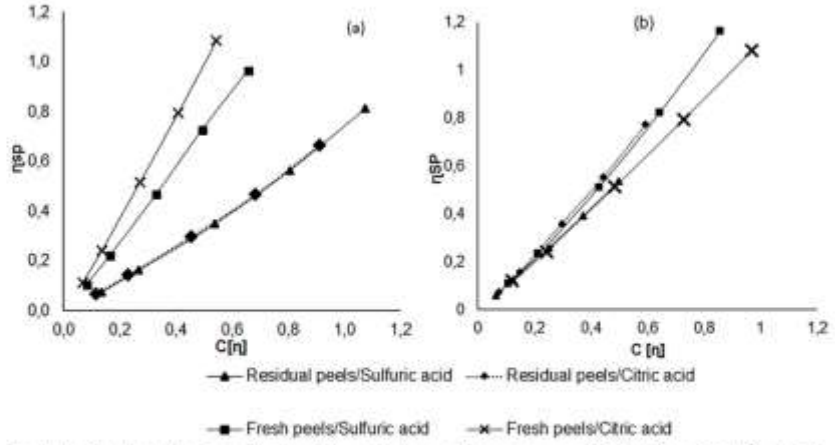

Fig 4. Variation of "zero shear" specific viscosity (η_{sp}) with degree of space-occupancy (c[η]) for Orange (a) and Grapefruit (b) pectins.

doi:10.1371/journal.pone.0161751.g004

Table 4. Orange and grapefruit pectin molecular weight (Mw).

	Mw (g/mol)	
Peels stats/acid used	**Grapfruit**	**Orange**
Fresh peels/ sulfuric	2.300 x10^{05}	2.266 x10^{05}
Fresh peels/ citric	2.472 x10^{05}	2.405 x10^{05}
Residual peels/sulfuric	1.538 x10^{05}	1.639 x10^{05}
Residual peels/citric	1.544 x10^{05}	1.728 x10^{05}

doi:10.1371/journal.pone.0161751.t004

Based on the absorbance, the peak for pectin which was extracted from fresh peels using citric acid is at 90 seconds, and at 150 seconds for the pectin which was extracted from residual peels using sulfuric acid. Using the calibration curve equation we found that the molecular weight of the orange pectin (fresh peels/citric acid) is $1.580*10^5$ g/mol and $2.247*10^5$ g/mol for the second orange pectin (residual peels / sulfuric acid). According to the viscometric measurements, the molecular weight of the orange pectin which was extracted from fresh peels using citric acid is $2.405*10^5$ g/mol while it was $1.639*10^{05}$ g/mol for the pectin which was extracted from residual peels using sulfuric acid. These results are close to the ones obtained by viscometric measurements, thus supporting them.

The use of the sulfuric acid gives pectin with a lower molecular weight than citric acid in grapefruit peels case. It was found that pectin extracted from orange had nearly the same quality as the one obtained from grapefruit based on Mw. According to Zhou, residual orange peels (after the extraction of essential oil and flavonoids) provides pectin with molecular weight of $1.65*10^5$ g/mol [38]. This result is less than what we've got using our residual orange peels $1.728*10^5$ g/mol using citric acid and nearly the same pectin molecular weight in the case of the sulfuric acid and $1.639*10^5$ g/mol. The molecular weight can vary depending on the extraction protocol conditions and the state of raw material. Haring found that the molecular weight of citrus pectin varied from 2 x10^4 to 2 x10^5 g/mol [39]. Morris found that the molecular weight of commercial pectin, with different esterification degree, was approximately constant

Table 5. Retention time Orange pectin.

	Orange pectin	
Retention time (s)	**Fresh peels / Citric acid**	**Residual peels / Sulfuric acid**
0	0	0
15	0	0
30	0,034	0
45	0,067	0
60	0,122	00
75	0,156	0,02
90	0,286	0,04
105	0,201	0,07
120	0,166	0,11
135	0,124	0,16
150	0,091	0,23
165	0,042	0,16
180	0,012	0,12
195	0	0,08
210	0	0,06
225	0	0,03

doi:10.1371/journal.pone.0161751.t005

in the range of $1.9 \times 10^5 \pm 3 \times 10^4$ g/mol [40] and $1.95 \times 10^5 \pm 5 \times 10^3$ g/mol [41]. Our results show that we were able to extract pectin that equals the commercial one based on molecular weight and even goes beyond it as in the case of pectin extracted from the fresh peels. This molecular weight decreases for pectin extracted from residual peels. High temperature and long extraction leads to a drop in the molecular weight of the pectin obtained [24,28]. In our case, this negative effect of temperature begins during the extraction of essential oils. This temperature has a positive effect on pectin yield certainly, but its impact on pectin molecular weight is negative.

Conclusion

Pectin quality varies according to the citrus species waste used as raw material. The nature of the acid used in extraction affects significantly pectin yield and the molecular weight. Residual citrus peels provide a high yield of pectin due to the thermal treatment undergone during the essential oil distillation, which weakens the primary cell walls, thus improving pectin recovery. In addition, the degree of esterification observed for all extracted pectin was higher than 50%, which classifies them as high methoxyl pectin. Pectin extracted from fresh peels had a better grade than the one extracted from residual peels in terms of molecular weight, but it does not prohibit its food industry utilization. Having pectin with a high molecular weight is important because it is possible to carry out controlled de-polymerizations reactions that allow the obtainment of low molecular weight pectin. Pectin with a low molecular weight has several benefits for the human body. Pectin consumption can potentially play an important role in detoxification of harmful chemicals, toxins and heavy metals in the body. This property makes the pectin an attractive option to treat heavy metal intoxication. Because of its large molecular weight, pectin cannot pass into the blood system. This passage is made possible by reducing the molecular weight pectin allowing it to express its chelating and detoxifying power in the human body. [42,43].

Materials and Methods

4.1 Raw Materials

In the present work, two citrus species were used: grapefruit (*Citrus paradisi*) and orange (*Citrus sinensis*). These citrus peels were collected after juice extraction process and were treated according to the method described below.

4.2 Simple preparation

In previous works [44], we have used two batches of ground citrus peels. The first batch was washed with water in order to remove impurities, dried and ground for pectin extraction, while essential oil were extracted from the second one (vapodistillation). Pectin extraction

In a previous work [45] we have optimized the pectin extraction conditions by targeting: time, temperature and acid concentration to get the highest pectin yield. We used these optimal conditions (0.1M; 80°C; 60min) in pectin extraction for its characterization. Pectin was extracted from both citrus peels states mentioned above, with aqueous sulfuric acid and citric acid (1:30, w/v) under reflux. After centrifugation (3000g for 10 min), each acid extract was filtered and the pectin was precipitated with two volumes of ethanol 96%, stirred slowly and stored in a refrigerator overnight in order to fully achieve pectin precipitation [46,47]. Afterward, the gelatinous precipitate was removed by centrifugation, washed three times with 96% ethanol to remove the monosaccharides and disaccharides [48]. The wet pectin was dried under vacuum and the weight was monitored until stabilization. All the experiments were done in triplicate and results were reproducible with an acceptable average error.

4.3 Pectin yield

Pectin yield was calculated as follows:

$$Pectin\ yield\ (\%) = (m_0/m)^*100 \quad (1)$$

"m_0" (g) is the dried pectin weight and "m" (g) is the dried raw material weight.

4.4 Degree of esterification (DE)

The DE of the pectin was determined by the titrimetric method [49] with minor modifications. The dehydrated sample was moistened with 2 mL of ethanol and dissolved in 25 mL of distilled water (free of carbon dioxide). Two drops of phenolphthalein were added after complete dissolution of the sample, then we started the titration process with 0.25 M sodium hydroxide to neutralize the free carboxyl acids from anhydro-galacturonic acid and the result was recorded as (V1). Afterward, 10 mL of 0.25 M sodium hydroxide was added and stirred for 30 min for hydrolysis, followed by the addition of 10 mL of 0.25 M hydrochloric acid and stirring until the complete disappearance of the pink color of the solution. HCl Excess was titrated with 0.1 N NaOH. The number of the esterified carboxyl groups was calculated from the volume of 0.1 NaOH solution spent for titration (V2). The DE of the pectin was calculated using the following formula:

$$\%DE = \frac{V_2}{V_2 + V_1} * 100 \quad (2)$$

4.5 Viscosity measurement

For polyelectrolytes such as pectin, there is a progressive reduction in coil volume with increasing ionic strength [50]. When solutions are prepared in water, the ionic strength changes as the polymer concentration does, with a consequent variation in coil dimensions. Meaningful values of intrinsic viscosity can be obtained only if the ionic strength is maintained constant by adding extraneous salt. pectin solution (0.05, 0.1, 0.2, 0.3, and 0.4 kg/m^3) was prepared by dissolving it in 0.1 mol/L sodium chloride solution to reduce the electro-viscous effect to a minimum [38]. The mixture was then heated to 25°C and allowed to stand with mixing at ambient temperature for 12 h. After filtration, 15 ml of pectin solutions were pipetted into the capillary (Cannon-fenske) viscometer for viscosity measurements and was immersed in a thermostatic water bath at 25.0°C. The pectin solution was loaded into the viscometer and allowed to equilibrate at the bath temperature (25°C) before starting the experiment. The time for the sample to flow from one level indicator to another, known as flow time, was measured and converted to kinematic viscosity and the densities of solutions were measured using a pycnometer. All of the experiments were triplicated and the average values were taken with an acceptable average error.

4.6 Intrinsic viscosity measurements

To determinate the intrinsic viscosity, the following steps and notions are very significant:

The relative viscosity was calculated using the following equation [4,51,52] and because of the low concentration used d/d$_s$ was taken as unity:

$$\eta_r = \frac{\eta}{\eta_s} = \frac{t\,d}{t_s d_s} \quad (3)$$

Where "η_r" the relative viscosity, η the viscosity of pectin solution (Pas), "η_s" the viscosity of solvent (Pas), "t" the time taken by solvent to flow in viscometer (s). "t_s" the time taken by

solution to flow in viscometer (s). "ds" the density of solution (kg/m^3). "d_s" the density of solvent (0.1 mol/L sodium chloride).

Relative viscosity values were converted to specific viscosities (η_{sp}) using the following equation [51]:

$$\eta_{sp} = \frac{\eta - \eta_s}{\eta_s} = \eta_r - 1 \quad (4)$$

In dilute solutions, that is, in conditions of negligible interactions between pectin chains, the intrinsic viscosity [η] of the biopolymer depends only on the dimensions of the polymer chain. The intrinsic viscosity [η] is defined as the limit of η_{sp}/c or $\ln(\eta_r/c)$ as the concentration approaches zero and the principal determination method of the intrinsic viscosity magnitude is to extrapolate the reduced viscosity to its value at zero solute concentration [53–55].

$$[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c} = \lim_{c \to 0} \frac{ln\,\eta_r}{c} \quad (5)$$

Where "c" is the concentration of pectin solution and η_{sp} is the specific viscosity.

If the plot of reduced viscosity η_{red} versus concentration shows a linear trend, the Huggins Eq (6) can be used to calculate intrinsic viscosity from the intercept, and for the kramer plot using its Eq (7), where a handled value of inherent viscosity η_{inh} is plot versus solution concentration [54,56,57]:

$$\text{Huggins equation} \;:\; \eta_{red} = \frac{\eta_{sp}}{c} = [\eta]^2 K_H\, C + [\eta] \quad (6)$$

$$\text{Kramer equation} \;:\; \eta_{inh} = \frac{\ln(\eta_r)}{c} = [\eta]^2 K_k\, C + [\eta] \quad (7)$$

Moreover, the following equations were used to obtain [η] [58]:

$$\text{Schulz} - \text{Blaschke equation} \;:\; \frac{\eta_{sp}}{c} = [\eta] K_{SB}\, \eta_{sp} + [\eta] \quad (8)$$

$$\text{Martin equation} \;:\; Log\left(\frac{\eta_{sp}}{c}\right) = [\eta] c K^{'} + Log[\eta] \quad (9)$$

4.7 Determination of molecular weight

Intrinsic viscosity is one of the most important characteristic of polymers. It depends only on the molecular mass when the sample's measuring conditions (solvent and temperature) are set. The Mark-Houwink-Sakurada equation describes the relationship between intrinsic viscosity and molecular weight *Mw*.

$$[\eta] = K M_w^{\alpha} \quad (10)$$

Both K and α depend on temperature, solute and solvent characteristics. A large number of models have been used to deduce [η]-*Mw* relationships. In this work the following values were assumed $K = 1.4^{*}10^{-4}$ and $\alpha = 1.43$ [38].

4.8 Statistical analysis

For a statistical analysis, the variance analysis (ANOVA) was used to treat different averages obtained. Data was analyzed by the analysis of variance, and averages were separated by the least significant difference when significant F ($P<5\%$) values were observed.

Supporting Information

S1 Fig. Fig A of S1 Fig. Effect of acids types and citrus peels stat on pectin yield. Fig B of S1 Fig. Huggins, Kraemer and Martin plots for grapefruit pectin extracted from fresh peels using sulfuric acid (a) and citric acid (b), and from residual peels using sulfuric acid (c) and citric acid (d) and Schulz-Blacschke plot of all grapefruit pectin solutions (e). Fig C of S1 Fig. Huggins, Kraemer, and Martin plots for orange pectin extracted from fresh peels using sulfuric acid (a'), and citric acid (b') and from residual peels using sulfuric acid (c'), and citric acid (d') and Schulz-Blacschke plot of all orange pectin solutions (e'). Fig D of S1 Fig. Variation of "zero shear" specific viscosity (η_{sp}) with degree of space-occupancy ($c[\eta]$) for Orange (a) and Grapefruit (b) pectins.
(XLSX)

S1 Table. Table A of S1 Table. Degree of esterification of orange and grapefruit pectins. Table B of S1 Table. Intrinsic viscosity of grapefruit pectin solutions. Table C of S1 Table. Intrinsic viscosity of orange pectin solutions. Table D of S1 Table. Orange and grapefruit pectin molecular weight (Mw). Table E of S1 Table. Retention time Orange pectin.
(XLSX)

Author Contributions

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Methodology: MYS.

Project administration: FOC.

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Supervision: YRK FOC FE.

Writing – original draft: HT MYS.

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Lampiran 9. Jurnal 9

Efektivitas Ekstrak Pektin dari Kulit Buah Jeruk Bali (*Citrus maxima*) Sebagai Antimikroba

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Halifah Pagarra

Abstrak. Tujuan dari penelitian ini adalah untuk menyelidiki efektivitas ekstrak pektin dan aktivitas antimikroba ekstrak pektin dari kulit jeruk bali *(Citrus maxima)* dengan variasi pH, suhu, dan waktu ekstraksi. Penelitian ini adalah penelitian eksperimental dengan pengujian laboratorium menggunakan Rancangan Acak Lengkap (RAL) dengan pengulangan tiga kali faktorial 2x3x3. Fdaktor A adalah pH: 1,5 (A1), 2,5 (A2). Faktor B adalah suhu pemanasan: 60 ° C (B1), 80 ° C (B2), 100oC (B3). Faktor C adalah waktu: 60 menit (C1), 90 menit (C2), 120 menit (C3). Sedangkan pengukuran desain aktivitas antimikroba dilakukan dengan menghitung diameter zona hambat (mm). Semua data yang diperoleh dianalisis menggunakan ANOVA (α = 0,05%, kemudian dilanjutkan dengan uji lanjutan Tuckey menggunakan SPSS). Ekstraksi pektin dari kulit grapefruit menggunakan metode konvensional dengan asam klorida (0.5N HCl). Proses ekstraksi dilakukan dengan memanaskan hot plate dengan memvariasikan suhu (60 oC; 80 oC; 100oC), pH (1,5; 2,5), dan waktu ekstraksi (60; 90; 120 menit). Hasil pektin yang signifikan diperoleh diuji aktivitas antimikroba dan diukur zona penghambatan yang dihasilkan berdasarkan diameter area antimikroba. Hasil penelitian menunjukkan bahwa ekstraksi dipengaruhi oleh pH, suhu, dan waktu ekstraksi dan hasil pektin tertinggi diperoleh dengan ekstraksi dengan ph 2.5, suhu 60oC, waktu 90 menit sebanyak 27.8%. Sedangkan aktivitas antimikroba ekstrak pektin dari kulit jeruk bali (Citrus maxima) memiliki aktivitas antibakteri karena dapat menghambat pertumbuhan bakteri Escherichia coli, Staphylococcus aureus. Namun, ia tidak memiliki aktivitas antijamur terhadap Candida albicans.
Kata kunci: Kulit jeruk bali (Citrus axima), Pectin, ekstraksi, aktivitas antimikroba.

Pendahuluan

Jeruk bali (*Citrus maxima*) merupakan jenis tanaman dengan ukuran buah yang lebih besar dibandingkan jeruk yang biasa kita temui di pasar. Tanaman ini tersebar di Sumatera, Jawa, Bali, Kalimantan, dan Sulawesi. Di Sulawesi sendiri jeruk bali banyak terdapat di Desa Padang Lampe, Kecamatan Marang, Kabupaten Pangkep. Produksi jeruk bali diberbagai daerah di Indonesia mencapai 511 kg/ton pertahunnya, dari produksi tersebut dihasilkan limbah kulit buah jeruk bali sebesar 208 kg/ton. Jeruk bali mengandung banyak komponen nutrisi yang terkandung didalamnya. Sebagian besar komponen jeruk bali terletak pada kulitnya, diantaranya terdapat senyawa alkaloid, flavonoid, likopen, vitamin C, serta yang paling dominan adalah pektin dan tannin (Rahmawati dan Putri, 2013). Jeruk bali (*Citrus maxima*) dapat dikonsumsi dalam bentuk buah segar ataupun hasil olahan. Produk olahan adalah produk primer. Disamping produk primer masih tersimpan potensi yang besar yaitu produk sekunder seperti limbah

BIONATURE

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Abstract. *The aim of this study was to investigate the effectiveness of pectin extract and antimicrobial activity of pectin extract from the skin of grapefruit (Citrus maxima) with variations in pH, temperature, and extraction time. This study was an experimental study with laboratory testing using a completely randomized design (CRD) with 2x3x3 factorial three times repetition. Factor A is pH: 1.5 (A1), 2.5 (A2). Factor B is the heating temperature: 60°C (B1), 80°C (B2), 100°C (B3). Factor C is time: 60 minutes (C1), 90 minutes (C2), 120 minutes (C3). While the design measurement of antimicrobial activity is carried out by calculating the diameter of the inhibition zone (mm). All data obtained were analyzed using ANOVA (α = 0.05%, then continued by Tuckey's advanced test using SPSS). Extraction of pectin from the skin of grapefruit using a conventional method with hydrochloric acid (0.5N HCl). The extraction process was carried out by heating the hot plate by varying the temperature (60 oC; 80 oC; 100oC), pH (1.5; 2.5), and extraction time (60; 90; 120 minutes). Significant pectin yields obtained were tested for antimicrobial activity and measured inhibitory zones produced based on the diameter of the antimicrobial area. The results showed that extraction was influenced by pH, temperature, and extraction time and the highest pectin yield was obtained by extraction with ph 2.5, temperature 60oC, time 90 minutes as much as 27.8%. While the antimicrobial activity of pectin extract from the skin of grapefruit (Citrus maxima) has antibacterial activity because it can inhibit the growth of Escherichia coli bacteria, Staphylococcus aureus. However, it has no antifungal activity against Candida albicans.*
Keywords: Grapefruit skin (*Citrus axima*), Pectin, extraction, antimicrobial activity.

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kulit buah jeruk bali. Limbah dari kulit buah jeruk bali masih kurang dimanfaatkan oleh masyaraka, padahal kulit buah jeruk bali memiliki banyak manfaat. Menurut Kristiyani (2013) salah satu manfaat kulit jeruk bali adalah untuk membuat pektin.

Pektin adalah substansi alami yang terdapat pada sebagian besar tanaman pangan. Beberapa jenis buah secara alami memiliki kandungan pektin yang cukup tinggi. salah satu buah yang memiliki kandungan pektin yang tinggi adalah jeruk bali (*Citrus maxima*). Tiap tahun kebutuhan pektin mengalami kenaikan sebesar 10-15%. Di Indonesia, belum ada pabrik yang dapat mengolah pektin. Oleh karena itu Indonesia masih mengimpor pektin dari luar negeri. Sedangkan kebutuhan pektin di Indonesia semakin meningkat. Hal ini terbukti dengan semakin meningkatnya nilai impor (Sulihono, 2012).

Pektin merupakan senyawa polisakarida dengan bobot molekul tinggi, pektin digunakan sebagai pembentuk gel dan pengental dalam pembuatan jelly, marmalade, makanan rendah kalori dan dalam bidang farmasi digunakan sebagai antimikroba (Perina dkk, 2013).

Antimikroba (AM) adalah obat pembasmi mikroba, khususnya mikroba yang bersifat merugikan manusia (mikroba patogen). *Escherichia coli*, *Staphylococcus aureus*, dan *candida albicans* merupakan mikroba patogen (Ganiswara, 1995). Berbagai penelitian menyatakan bahwa tanaman jeruk memiliki aktivitas antibakteri terhadap *E. coli* dan *S. aureus*. Berdasarkan penelitiaan Sari (2015), ekstrak pektin dari kulit buah jeruk memiliki aktivitas antimikroba terhadap *Staphylococcus aureus, Saccharomyces cerevisiae, Bacillus subtilis, Escherichia coli*.

Oleh karena itu penulis tertarik untuk mengekstrak pektin dari kulit buah jeruk bali sebagai antimikroba untuk menghambat mikroba pathogen, setidaknya dengan memanfaatkan kulit buah jeruk bali sebagai bahan baku pembuatan pektin dapat mengurangi impor pektin, sekaligus memanfaatkan kulit jeruk bali yang kurang populer di kalangan masyarakat menjadi sesuatu yang bernilai ekonomis lebih tinggi.

Metode Penelitian

Penelitian ini merupakan penelitian eksperimental yang dlakukan pada bulan Agustus sam pai Desember 2018. Penelitian dilakukan di Laboratorium Jurusan Biologi FMIPA UNM. Variabel bebas dalam penelitian ini adalah variasi pH, suhu, dan waktu, sedangkan variabel terikat adalah pektin.

Alat dan Bahan

Alat yang digunakan yaitu, erlenmeyer (250ml, 500ml, dan 1000ml), gelas kimia (250ml, 500ml, dan 1000ml), gelas ukur (50ml, 500ml, dan 1000ml), pipet tetes, pipet ukur, batang pengaduk, corong, oven, autoclave, blender, cawan petri, termometer, pH meter, neraca analitik, saringan, ayakan, stopwatch, pisau, wadah, ose, tabung reaksi, rak tabung, mikropipet dan tip, spoit, botol pengencer, bunsen, laminar air flow (LAF), waterbath, hotplate, dan peralatan umum yang digunakan di laboratorium mikrobiologi.

Bahan yang digunakan yaitu kulit buah jeruk bali (*Citrus maxima*), aluminium foil, plastik, label, karet, wrap, etanol 96 %, etanol 70%, aquades, HCl 0,5 N, HCl pekat, FeCl3, R.Wagner, Magnesium, NaOH 0,5 N, nystatin, tetrasiklin, medium NA (*Nutrient Agar*), medium PDA (*Potato Dextrose Agar*), isolat bakteri *Eschericia coli, Candida albicans* dan *Staphylococcus aureus*,

Prosedur Penelitian

1. Tahap Ekstraksi Pektin

a. Ekstraksi pektin

Kulit buah jeruk bali yang sudah kering di haluskan dengan menggunakan belender dan ditimbang sebanyak 5 gram. Kemudian penambahan aquades sebanyak 100 ml, dan ditambahkan pelarut asam klorida (HCl) 0,5 N, sampai pH larutan yang sudah ditentukan (1.5 dan 2,5). Selanjutnya dipanaskan sambil diaduk pada suhu yang ditentukan (60°C ,80°C, 100°C) selama waktu yang ditetapkan (60, 90, 120 menit). Campuran yang telah diekstrak disaring dengan menggunakan saringan dan diperas untuk memisahkan filtrat dari ampasnya.

b. Pengendapan

Filtrat didinginkan sampai dengan suhu kamar kemudian dilakukan pengendapan pektin dengan menambahkan etanol 96%. Perbandingan filtrat dengan etanol yang ditambahkan adalah 1:1. Proses pengendapan dilakukan selama 24 jam.

c. Pencucian

Endapan pektin yang diperoleh dicuci dengan menggunakan etanol 96% untuk menghilangkan sisa asam. Pemisahan endapan pektin dengan etanol bekas cucian dilakukan dengan menggunakan spoid (tanda tidak lagi bereaksi dengan asam adalah ketika air bekas cucian pektin berwarna merah bila ditetesi phenolftalein).

d. Pengeringan

Pengeringan pektin basah hasil cucian dilakukan dalam oven pada suhu 50°C selama 3 hari. Tepung pektin diperoleh dengan memblender pektin kering kemudian dilakukan pengayakan. Rendemen pektin tertinggi atau signifikan yang didapat dari hasil ekstraksi pektin kulit buah jeruk bali akan dilakukan uji aktivitas antimikroba dengan konsentrasi 0,5%, 0,75%, dan 1%.

2. Tahap Analisis Fitokimia

Analisis kandungan senyawa aktif dilakukan berdasarkan metode Sari (2015). Analisis kandungan senyawa aktif dilakukan beberapa yaitu, uji alkaloid, uji tanin, uji saponin, uji flavonoid.

3. Tahap Uji Aktivitas Antimikroba

Pengujian aktivitas antimikroba dari pektin kulit buah jeruk bali (*Citrus maxima*) dilakukan dengan mengamati zona hambat menggunakan metode *paper disk*. Setiap cawan petri berisi 1 sampel dengan 3 konsentrasi berbeda (0,5%, 0,75%, 1%) serta kontrol positif dan kontrol negatif.

Gambar 1. Skema Penempatan *Paper Disk* Pada Cawan Petri.

Ket: A: Medium yang telah diinokulasi mikroba; B, C, dan D: Ekstrak 0,5%, 0,75%, dan 1%; E: Kontrol negatif; F: Kontrol positif.

Sampel yang digunakan yaitu ekstrak pektin dari kulit buah jeruk bali dengan konsentrasi (0,5%, 0,75%, 1%), langkah pertama yang harus dilakukan adalah mensterilkan kedua tangan dengan menyemprotkan alkohol 70%. Menyiapkan 3 cawan petri setiap mikroba uji dan diberi label untuk masing-masing konsentrasi ekstrak, kontrol positif, dan kontrol negatif. Kemudian tepi cawan petri dipanaskan dan medium NA maupun PDA dituang kedalam cawan petri sebanyak 15-20 ml, dan ditunggu hingga memadat. Sambil menuggu medium memadat setiap

paperdisk ditetesi larutan uji sebanyak 20µl, yaitu pektin dengan konsetrasi 0,5%, 0,75%, 1%, nystatin, dan tetrasiklin, kontrol positif (+), Aquades kontrol negatif (-) hingga *paperdisk* jenuh.

Medium NA dan PDA yang telah padat, permukaanya diinokulasikan dengan bakteri (*E. coli, S. aureus*) dan jamur (*c. albicans*) dengan metode gores sinambung secara padat. Selanjutnya masing-masing *paperdisk* yang telah ditetesi larutan uji diletakkan diatas medium, dimana masing-masing medium berisi 5 *paperdisk*. Kemudian diinkubasi selama 2 hari untuk bakter (*E. coli, S. aureus*) dan 3 hari untuk jamur (*c. albicans*), dan diukur zona hambat dengan menggunakan jangka sorong.

Analisis data

Pengolahan data dilakukan dengan Rancangan Acak Lengkap (RAL) pola faktorial 2x3x3 sebanyak tiga kali ulangan. Faktor A adalah pH: 1,5 (A1), 2,5 (A2). Faktor B adalah suhu pemanasan: 60 °C (B1), 80 °C (B2), 100 °C (B3). Faktor C adalah waktu 60 menit (C1), 90 menit (C2), dan 120 menit (C3) data yang diperoleh dianalisis menggunakan ANOVA. Sedangkan rancangan pengukuran aktivitas antimikroba dilakukan dengan menghitung diameter zona hambat (mm). Semua data yang diperoleh dianalisis menggunakan ANOVA (α= 0.05 %) kemudian dilanjutkan dengan uji lanjut *Tuckey* menggunakan program SPSS (*Statistical Package for Social Science*

Hasil dan Pembahasan

Hasil Penelitian

Hasil Ekstrak Pektin dari Kulit Buah Jeruk Bali (Citrus maxima)

Ekstraksi pektin dari kulit buah jeruk bali (*Citrus maxima*) menggunakan metode konvensional atau metode pemanasan dengan pelarut asam klorida (HCl 0,5 N). Proses ekstraksi dilakukan dengan pemanasan diatas *hot plate* dengan variasi pH (1,5 dan 2,5), suhu (60°C ,80°C, 100°C) dan waktu ekstraksi (60, 90, 120 menit). Hasil rendemen pektin dapat dilihat pada Tabel 1.

Tabel 1. Hasil Rendemen Pektin Dari Kulit Buah Jeruk Bali (*Citrus maxima*) Terhadap pH, Suhu, Dan Waktu Yang Berbeda.

Faktor 1	Faktor 2	Faktor 3	Rata-Rata Rendemen Pektin (%)
pH	Suhu (°C)	Waktu (menit)	
1,5	60	60	10
		90	18,46
		120	17,40
	80	60	13,67
		90	10,93
		120	11,13
	100	60	9,20
		90	11
		120	14,46
2,5	60	60	10,13
		90	23,06
		120	10,13
	80	60	11,13
		90	10,80
		120	10,06
	100	60	14,40
		90	15,33
		120	12,93

Ekstrak pektin yang diperoleh kemudian ditentukan rendemen pektin, rendemen pektin diperoleh dari total pektin yang dihasilkan dibagi bubuk kulit jeruk yang digunakan dikali 100%. Hasil rendemen pektin dari 54 sampel didapatkan rendemen pektin yang berbeda-beda pada setiap perlakuan, karna dipengaruhi oleh faktor pH, suhu, dan waktu. Hasil rendemen pektin tertinggi diperoleh pada ekstraksi dengan pH 2,5, suhu 60°C, waktu 90 menit sebesar 23,06%. Rendemen pektin terendah diperoleh pada ekstraksi dengan pH 1,5, suhu 100 °C, waktu 60 menit sebesar 9,20%.

Hasil Uji Fitokimia

Fitokimia adalah bahan kimia tumbuhan non-nutritif yang memiliki berbagai tingkat sifat pencegahan penyakit. Senyawa kimia metabolit sekunder merupakan sumber bahan baku yang tak ternilai untuk obat tradisional (Sirait, M. 2007). Pengujian fitokimia dilakukan untuk mengetahui kandungan senyawa kimia metabolit sekunder yang terdapat didalam ekstrak pektin dari kulit buah jeruk bali (*Citrus maxima*), sehingga dapat diketahui metabolit sekunder yang berpotensi memiliki efek terhadap aktivitas antimikroba. Hasil pengujian fitokimia dapat dilihat pada Tabel 2.

Tabel 2. Hasil Pengujian Fitokimia Ekstrak Pektin Dari Kulit Buah Jeruk Bali

Senyawa Aktif	Warna	Hasil
Alkaloid	Kuning kecoklatan, tidak terdapat endapan	-
Saponin	Kuning, terbentuk busa (berbuih)	+
Flavonoid	Jingga, terbentuk busa (berbuih)	++
Tanin	Hitam pekat	+++

Keterangan:
Tanda (-) = Tidak terkandung senyawa / tidak terbentuk endapan;
Tanda (+) = Terkandung senyawa
Tanda (++) = Terkandung banyak senyawa;
Tanda (++) = Terkandung lebih banyak senyawa;

Hasil pengujian fitokimia menunjukkan bahwa adanya golongan senyawa metabolit sekunder yang terkandung dalam ekstrak pektin dari kulit buah jeruk bali (*Citrus maxima*), seperti, flavonoid, saponin, dan tannin. Pada pengujian alkaloid, hasil dinyatakan negatif karena tidak terbentuk endapan yang berarti tidak terdapat senyawa alkaloid dalam ekstrak pektin dari kulit buah jeruk bali (*Citrus maxima*). Untuk pengujian senyawa flavonoid, saponin, dan tannin menunjukkan hasil positif.

Pada pengujian flavonoid menunjukkan terjadinya perubahan warna jingga yang menandakan bahwa pektin terkandung banyak senyawa flavonoid. Pengujian saponin, terbentuk Sedikit busa yang menandakan bahwa pektin terkandung senyawa saponin. Untuk pengujian tanin terbentuk warna hitam pekat yang menandakan bahwa dalam ekstrak pektin dari kulit buah jeruk bali (*Citrus maxima*) terkandung lebih banyak senyawa tanin.

Hasil Aktivitas Antimikroba

Pengujian aktivitas antimikroba ekstrak pektin dari kulit buah jeruk bali (*Citrus maxima*) dilakukan dengan menggunakan pelarut aquades, dan membuat larutan induk. Pembuatan larutan induk ekstrak pektin dari kulit buah jeruk bali dilakukan dengan menimbang 5 gram ekstrak pektin kedalam 100 ml aquades. Membuat konsentrasi 0,5 %, 0,75%, dan 1% dibagi berdasarkan rumus pengenceran. Pengujian ini menggunakan metode difusi *paper disc* (kertas

cakram) dengan ukuran kertas cakram 6 mm, yang dapat diamati dengan melihat diameter zona hambat yang terbentuk di sekitar kertas cakram dan diukur menggunakanJangka sorong. Mikroba uji yang digunakan yaitu bakteri gram positif (*Staphylococcus aureus*), bakteri gram negatif (*Escherichia coli*), dan jamur (*Candida albicans*).

Kontrol positif yang digunakan sebagai pembanding yaitu untuk bakteri adalah tetrasiklin sedangkan untuk jamur menggunakan nystatin dan untuk kontrol negatif menggunakan aquades steril. Pengujian aktivitas antimikroba dilakukan 3 kali ulangan. Hasil Rata-rata diameter zona hambat ekstrak pektin dari kulit buah jeruk bali (*Citrus maxima*) pada akivitas antmikroba dapat dilihat pada Tabel 3. (Hasil olah data dapat dilihat di Lampiran 4).

Tabel 3. Rata - rata Diameter Zona Hambat Ekstrak Pektin dari Kulit Buah Jeruk Bali (*Citrus maxima*) pada Akivitas Antmikroba.

Perlakuan	Rata-Rata Diameter Zona Hambat (mm)		
	Escherichia coli	*Staphylococcus aureus*	*Candida albicans*
EP (0,5%)	9,0000^{a}	7,6666^{ab}	6,0000^{a}
EP (0,75%)	9,3333^{ab}	9,6666^{b}	6,0000^{a}
EP (1%)	10,3333^{b}	16,0000^{c}	6,0000^{a}
KP	6666^{c}	21,0000^{d}	15,0000^{b}
KN	6,0000^{a}	6,0000^{a}	15,0000^{b}

***Keterangan**: EP: Ekstrak Pektin; KP: Kontrol Positif; KN: Kontrol Negatif. Huruf yang sama dalam satu kolom menunjukkan "tidak berbeda nyata". Huruf yang berbeda dalam satu kolom menunjukkan "berbeda nyata". Huruf yang berbeda antar kolom menunjukkan" sangat berbeda nyata". Berdasarkan uji Tuckey dengan taraf kepercayaan α: 0,05.*

Hasil pengujian aktivitas antimikroba menunjukkan, ekstrak pektin dari kulit buah jeruk bali (*Citrus maxima*) memiliki aktivitas antibakteri dengan adanya peningkatan konsentrasi larutan ekstrak uji, namun tidak menunjukkan adanya aktivitas antijamur dengan tidak terbentuknya zona hambat disekitar paper diks.

Bakteri *Escherichia coli*, dapat dilihat bahwa kontrol negatif tidak berbeda nyata terhadap ekstrak pektin 0,5%, berbeda nyata terhadap ekstrak pektin 0,75%, dan sangat berbeda nyata terhadap ekstrak pektin 1% dan kontrol positif. Sedangkan terhadap bakteri *Staphylococcus aureus* menunjukkan bahwa kontrol negatif tidak berbeda nyata terhadap ekstrak pektin 0,5%, dan sangat berbeda nyata terhadap ekstrak pektin 0,75%, 1 %, dan kontrolpositif.

Hasil pengujian aktivitas antijamur dari ekstak pektin kulit buah jeruk bali terhadap *Candida albicans* dapat dilihat bahwa kontrol negatif, ekstrak pektin 0,5%, 0,75%, dan 1% tidak menunjukkan adanya aktivitas antijamur dengan tidak bertambahnya ukuran diamater dari kertas cakram sehingga hasil yang diperoleh tidak berbeda nyata. Hasil yang sangat berbeda nyata hanya diperoleh dari kontrol positif.

Pembahasan

Ekstrak Pektin dari Kulit Buah Jeruk Bali (Citrus maxima)

Sampel yang digunakan dalam penelitian ini adalah kulit buah jeruk bali (*Citrus maxima*). Kulit buah jeruk bali yang digunakan adalah kulit yang belum terlalu matang atau berwarna hijau kekuning kuningan dan belum ada tanda kebusukan, karna kulit yang belum terlalu matang mengandung pektin cukup tinggi dibandingan kulit buah jeruk bali yang sudah matang (Cahyanto, 2017).

Ekstarksi pektin dilakukan menggunakan metode konvensional atau metode pemanasan dengan pelarut asam klorida (HCl 0,5 N). Proses ekstraksi dilakukan dengan pemanasan diatas

hot plate dengan variasi pH (1,5 dan 2,5), suhu (60°C ,80°C, 100°C) dan waktu ekstraksi (60, 90, 120 menit) dengan tujuan untuk mendapatkan pektin yang signifikan. Menurut Sulihono (2012), prinsip dasar ekstraksi pektin adalah pektin dalam jaringan tanaman sebagai protopektin yang tidak larut dalam air (insoluble), dilakukan hidrolisis protopektin dalam air yang diasamkan dan dipanaskan untuk mengubah protopektin menjadi pektin yang bersifat larut dalam air. Dari hasil penelitian yang dilakukan dengan jumlah perlakuan sebanyak 54 sampel, didapatkan rendemen pektin yang berbeda–beda pada setiap perlakuan yang diberikan. Pektin yang dihasilkan dengan perlakuan yang berbeda akan menghasilkan berat yang berbeda pula, ini membuktikan bahwa Faktor pH, suhu, dan waktu berpengaruh terhadap rendemen pektin yang dihasilkan. Hasil rendemen pektin tertinggi diperoleh pada ekstraksi dengan pH 2,5, suhu 60°C, waktu 90 menit sebesar 27,8 %.

Berdasarkan hasil diatas pengontrolan pH dalam ekstraksi pektin memiliki peranan penting karena dapat mempengaruhi rendemen pektin. Rentang pH untuk ekstraksi pektin bervariasi tergantung kepada bahan yang akan diekstraksi. Umumnya ekstraksi pektin dari kulit jeruk dilakukan pada pH 1,5 – 3 (Towle dan Christensen, 1973). Hal ini sesuai dengan penelitian yang dilakukan Prasetyowati (2009), menunjukkan bahwa pada pH 2,5 jumlah pektin yang diperoleh lebih banyak dibandingkan pH 1,5, dimana pH yang terlalu rendah atau terlalu asam dapat merusak pektin sehingga rendemen yang dihasilkan sedikit.

Jadi semakin rendah pH yang digunakan maka semakin sedikit pektin yang dihasilkan. Sedangkan pH diatas 3 yang kurang asam lebih sedikit ion hidrogen sehingga kalsium dan magnesium yang disubtitusi lebih sedikit. pH 2,5 memiliki banyak kalsium dan magnesium yang tersubtitusi sehingga jumlah pektin yang didapat makin banyak.

Suhu yang tinggi selama ekstraksi dapat meningkatkan rendemen pektin. Batas suhu ditentukan untuk mencegah kerusakan pada bahan. Secara umum, suhu ekstraksi untuk pektin adalah 60 – 90°C. Sedangkan Waktu ekstraksi, Semakin lama waktu yang dibutuhkan untuk ekstraksi dalam pelarut, perolehan (yield) yang diperoleh semakin tinggi. Tetapi, penambahan waktu ekstraksi tidak sebanding dengan yield yang diperoleh (Herdigenarosa, 2013).

Hal ini sesuai dengan penelitian yang dilakukan Wang (2014), menunjukkan bahwa pada suhu 60°C dan waktu 90 menit memberikan rendemen pektin tinggi. Penggunaan suhu yang terlalu tinggi untuk kulit jeruk juga dapat mengakibatkan degradasi pektin dan waktu ekstraksi dilakuan pada waktu optimum, ekstraksi dilakukan selama pelarut yang digunakan belum jenuh. Pelarut yang telah jenuh tidak dapat mengekstraksi lagi atau kurang baik kemampuan untuk mengekstraksinya karena gaya pendorong (driving force) semakin lama semakin kecil. Akibatnya waktu ekstraksi semakin lama dan yield yang dihasilkan tidak bertambah lagi secara signifikan.

Fitokimia

Uji fitokimia bertujuan untuk mengetahui golongan senyawa metabolit sekunder yang terkandung dalam ekstrak pektin dari kulit buah jeruk bali (*Citrus maxima*). Menurut Septiani (2017) menunjukkan bahwa pektin dari kulit buah jeruk (*Citrus sp*) memiliki kandungan senyawa metabolit sekunder seperti saponin, flavonoid, dan tannin Adapun pengujian yang dilakukan yaitu kandungan senyawa alkaloid, flavonoid, saponin, tannin.

Hasil uji fitokimia ekstrak pektin dengan perekasi wagner dan meyer menunjukkan tidak adanya endapan coklat dan endapan putih kekuningan. Menurut Tiwari *et al* (2011), hal ini disebabkan karna alkaloid bersifat gugus basa yang mengandung nitrogen, sedangkan pektin bersifat asam sehinnga pada pektin tidak terdapat senyawa alkaloid.

Flavonoid adalah golongan pigmen organik yang tidak mengandung molekul nitrogen. Pigmen ini merupakan antraktan bagi serangga dan merupakan agen polinasi. Pigmen juga bermanfaat bagi manusia dan salah satu manfaat yang penting adalah sebagai antioksidan (Markham, 2011).

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Flavonoid mempunyai aktivitas antimikroba dengan mengganggu fungsi metabolisme melalui perusakan dinding sel dan mendenaturasi protein mikroba. Menurut Cowan (1999) senyawa flavon, flavonoid dan flavonol merupakan senyawa fenolik yang diketahui disintesis oleh tanaman sebagai respon terhadap infeksi mikroba. Mekanisme kerja sebagai antibakteri karena kemampuan untuk membentuk kompleks dengan protein ekstraseluler dan terlarut dengan dinding sel mikroba.

Berdasarkan hasil yang didapatkan ekstrak pektin menghasilkan busa, yang menandakan bahwa pektin mengandung saponin. Menurut Agoes (2012), Saponin adalah senyawa aktif yang kuat dan menimbulkan busa jika dikocok dalam air sehingga bersifat seperti sabun dan mempunyai kemampuan antibakterial. Saponin dapat meningkatkan permeabilitas membran sel bakteri sehingga dapat mengubah struktur dan fungsi membran. Saponin memiliki aktivitas antimikroba dengan mengganggu tegangan permukaan dinding sel. Saat tegangan permukaan terganggu zat antimikroba akan dengan mudah masuk kedalam sel dan akan mengganggu metabolisme hingga akhirnya terjadilah kematian sel bakteri (Karlina *et al*., 2013).

Berdasarkan hasil yang didapatkan, untuk pengujian tannin terbentuk warna hitam pekat yang menandakan bahwa dalam ekstrak pektin dari kulit buah jeruk bali (*Citrus maxima*) terkandung lebih banyak senyawa tanin. Tanin diketahui mempunyai beberapa khasiat yaitu sebagai astringen, antidiare, anti bakteri dan antioksidan (Desmiaty *et al*., 2008). Tanin mempunyai aktivitas antimikroba dengan targetnya adalah merusak dinding sel mikroba (Tiwari *et al*., 2011).

Aktivitas Antimikroba

Tetrasiklin merupakan antibiotik berspektrum luas yang dapat melawan pertumbuhan bakteri gram positif dan gram negatif. Mekanisme kerja tetrasiklin adalah menghalangi terikatnya RNA pada bagian spesifik dari ribosom, akibatnya sintesis protein mengalami hambatan (Widjajanti, 1988). Mekanisme kerja nistatin yaitu dengan mengadakan ikatan yang kompleks dengan ergosterol di membran sitoplasma jamur yang sensitif. Hal tersebut akan menyebabkan perubahan permeabilitas membran dengan membentuk pori-pori intra membran dan dengan demikian kehilangan intrasel penting senyawa, seperti ion dan molekul kecil, dan kemudian sel akan mengalami kematian (Brescansin, *et al*., 2013).

Hasil pengujian antimikroba pada tabel 4.3. menunjukkan bahwa ekstrak pektin dari kulit buah jeruk bali (*Citrus maxima*) memiliki aktivitas antibakteri dengan adanya peningkatan konsentrasi larutan ekstrak uji, namun tidak memiliki aktivitas antijamur dengan tidak terbentuknya zona hambat disekitar kertas cakram.

Berdasarkan tabel 4.3. Bakteri *Escherichia coli*, dapat dilihat bahwa kontrol negatif tidak berbeda nyata terhadap ekstrak pektin 0,5%, berbeda nyata terhadap ekstrak pektin 0,75%, dan sangat berbeda nyata terhadap ekstrak pektin 1% dan kontrol positif. Sedangkan terhadap bakteri *Staphylococcus aureus* menunjukkan bahwa kontrol negatif tidak berbeda nyata terhadap ekstrak pektin 0,5%, dan sangat berbeda nyata terhadap ekstrak pektin 0,75%, 1 %, dan kontrol positif. Hal ini sesuai dengan penelitian yang dilakukan Sari (2015), menunjukkan bahwa ekstrak pektin dari buah kulit jeruk memiki aktivitas antibakteri terhadap bakteri uji *Staphylococcus aureus* dan *Escherichia coli*.

Hasil pengujian aktivitas antijamur dari ekstak pektin kulit buah jeruk bali terhadap *Candida albicans* dapat dilihat bahwa kontrol negatif, ekstrak pektin 0,5%, 0,75%, dan 1% tidak menunjukkan adanya aktivitas antijamur dengan tidak bertambahnya ukuran diamater dari kertas cakram sehingga hasil yang diperoleh tidak berbeda nyata. Hasil yang sangat berbeda nyata hanya diperoleh dari kontrol positif. Berdasarkan hasil penelitian Silvia (2018) menunjukkan pada konsentarsi 5%, 10%, tidak menunjukkan adanya aktivitas antijamur, namun pada konsentarsi 20% terdapat aktivitas antijamur, karna konsetrasi yang rendah.

Hasil negatif yang ditunjukan ekstrak pektin antijamur dapat disebabkan oleh konsentrasi ekstrak uji. Menurut Silvia (2018) konsentrasi ekstrak uji dapat mempengaruhi aktivitasnya

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sebagai antimikroba, konsentrasi yang tinggi dapat meningkatkan bahan aktif yang berfungsi sebagai antimikroba sehingga kemampuannya dalam menghambat pertumbuhan mikroba juga semakin besar.

Kesimpulan

Berdasarkan hasil penelitian, kesimpulan yang dapat diperoleh yaitu Faktor pH, suhu, dan waktu berpengaruh nyata terhadap rendemen pektin yang dihasilkan. Hasil rendemen pektin tertinggi diperoleh pada ekstraksi dengan pH 2,5, suhu 60°C, waktu 90 menit sebesar 27,8 %. Bakteri *Escherichia coli* dan *Staphylococcus aureus,* menunjukkan bahwa ekstrak pektin dari kulit buah jeruk bali (*Citrus maxima*) memiki aktivitas antibakteri, sedangkan *Candida albicans* tidak menunjukkan adanya aktivitas antijamur.

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PENGARUH JENIS ASAM DAN pH PELARUT TERHADAP KARAKTERISTIK PEKTIN DARI KULIT LEMON (*Citrus limon*)

The Effect of Acid Type and pH Solvent to The Pectin Characteristic of Lemon Peel (Citrus limon)

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ABSTRACT

This research was conducted with the aim to know the influence of acid and solvent pH on the extraction of pectin from lemon peel and find the right kind of acid and solvent pH to produce pektin of lemon peel with the best characteristics. The experimental design used in this research was a factorial Completely Randomized Design (CRD), which consisted of two factors. The first factor was a type of acid consisting of chloride acid and citric acid. The second factor was a solvent pH consisting of a pH 1,5; 2; 2,5 and 3. All treatments were repeated twice to obtain 16 units of experiments. The data were analyzed with analysis of variance and followed by Duncan test. The best results showed that extraction using chloride acid and pH of solvent 1,5 resulted in 22,35% of pectin yield, 11, 55% of water content, 22,11% of ash content, 1052,47% of equivalent weight, 10,81% of methoxyl content, 78,07% of galacturonic content, and 78,58% of esterification degree.

Keywords : lemon peel, pectin, characteristic, acid, solvent pH

PENDAHULUAN

Pektin merupakan asam poligalakturonat yang berbentuk rantai panjang dan tidak bercabang serta memiliki gugus metil ester. Penggunaan pektin paling banyak pada industri pengolahan pangan karena kemampuannya membentuk gel dan sumber serat dalam makanan. Berbagai produk makanan yang menambahkan pektin yaitu jeli, selai, makaroni, coklat, dan kembang gula. Variasi makanan yang semakin meningkat menyebabkan peningkatan kebutuhan pektin dalam industri pangan. (Anon., 2004)

Pektin memiliki nilai ekonomis yang cukup tinggi tetapi di Indonesia industri penghasil pektin belum ada sehingga saat ini masih mengandalkan impor dari mancanegara seperti Jerman dan Denmark. Jumlah impor pektin di Indonesia dari tahun 2008 hingga 2011 secara berurutan yaitu 147,6 ton; 147,3 ton; 291,9 ton; dan 240,8 ton. Jumlah impor pektin paling banyak terjadi pada tahun 2010 yaitu 291.870 kg dengan harga 2.977.479 US Dollar (Anon., 2011).

Usaha mengurangi impor pektin yaitu dengan mencari sumber bahan baku pektin yang diduga memiliki potensi untuk dikembangkan yakni pektin yang berasal dari kulit jeruk lemon. Lemon *(Citrus limon)* adalah salah satu produk hortikultura yang paling banyak diolah menjadi sari buah lemon. Sekitar 70% dari berat buah dalam industri pengolahan sari buah lemon akan terbuang (meliputi kulit, biji, pulp dan air lemon yang tersisa).

Limbah sari lemon terutama kulit lemon memiliki prospek yang tinggi untuk diolah

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menjadi sumber bahan baku pektin. Fitriani (2003) mengungkapkan rendemen pektin pada kulit jeruk lemon berkisar antara 16,32-32,61%, dengan perlakuan variasi suhu dan waktu ekstraksi.

Pada umumnya ekstraksi pektin dilakukan dengan ekstraksi asam. Peranan asam dalam ekstraksi pektin adalah untuk memisahkan ion bivalen, memutus ikatan antara asam pektinat dengan selulosa, menghidrolisis protopektin menjadi pektin yang larut dalam air. Asam yang digunakan dalam ekstraksi pektin adalah asam organik seperti asam tartrat, asam malat, asam sitrat, asam laktat, asam asetat, dan asam fosfat. Asam organik digunakan karena memiliki sifat toksik yang lebih rendah dibandingkan asam mineral. Asam organik memiliki tingkat keasaman yang rendah sehingga tidak mendegradasi pektin menjadi asam pektat.

Menurut Kertesz (1951) dalam Hariyati (2006), ada kecenderungan untuk menggunakan asam mineral seperti asam sulfat, asam khlorida, dan asam nitrat. Penggunaan asam mineral akan mempercepat terlepasnya ion H^+ sehingga dapat menghidrolisis protopektin menjadi pektin yang mudah larut dan menyatukan molekul pektin dengan molekul pektin lain sehingga terbentuk sebuah jaringan pektin. Namun penggunaann asam mineral kuat cenderung menyebabkan pektin terdegradasi menjadi asam pektat. Tuhuloula, *et al.* (2003) mengungkapkan tingkat keasaman yang lebih tinggi tidak baik dalam ekstraksi pektin karena akan menyebabkan kecenderungan terjadinya degradasi pektin menjadi asam pektat sehingga perolehan pektin menjadi lebih sedikit.

Asam klorida dan asam sitrat merupakan asam yang paling sering digunakan pada ekstraksi pektin. Asam klorida merupakan asam mineral yang murah dan digunakan secara luas dalam bidang industri termasuk industri penghasil pektin. Asam sitrat merupakan asam organik yang berasal dari daun dan buah genus Citrus (jeruk-jerukan). Asam sitrat sangat mudah ditemukan serta sering digunakan pada ekstraksi pektin. Penelitian Kalapathy dan Proctor (2001) pada ekstraksi pektin kedelai menggunakan asam klorida 0,1 N dengan pH larutan 3,5 menghasilkan hasil pektin tertinggi sebesar 28%. Begitu pula pada penelitian Madjaga, *et al.* (2017) pada ekstraksi pektin dari kulit buah sukun dengan pelarut asam sitrat pada konsentrasi 7% menghasilkan rendemen pektin sebesar 39,585%.

Pektin berbentuk protopektin yang tidak larut dalam air pada jaringan tanaman. Penambahan asam dengan pH rendah pada ekstraksi akan menghidrolisis peotopektin menjadi pektin yang larut dalam air. Ekstraksi pektin sayur-sayuran dan buah-buahan dilakukan pada kisaran pH 1,5 sampai 3,0 dengan suhu pemanasan 60 – 100°C selama setengah jam sampai satu setengah jam (Towle dan Christensen, 1973). Oleh karena itu diperlukan jenis asam dan pH yang tepat pada ekstraksi kulit lemon sehingga dapat menghasilkan pektin dengan karakteristik terbaik.

METODE PENELITIAN

Tempat dan Waktu

Penelitian ini dilakukan di Laboratorium Pengolahan Pangan, Laboratorium Analisis Pangan, dan Laboratorium Bioindustri, Universitas Udayana. Waktu pelaksanaan April sampai Juni 2018.

Bahan dan Alat

Bahan baku yang digunakan dalam penelitian ini adalah limbah kulit lemon yang didapatkan dari pengolahan sari buah lemon di UD Fenny Denpasar. Bahan kimia yang digunakan untuk ekstraksi pektin adalah etanol 96%, asam khlorida (HCl), asam sitrat, akuades, indikator phenolphtalein (PP), 0,1 N NaOH, 0,25 NaOH, akuades, NaCl, 0,25 HCl, dan asam oksalat.

Alat yang digunakan dalam penelitian ini

adalah termometer, timbangan analitik (*Shimadzu*), oven, pH-meter (PHS -3D pH Meter), tanur, kain saring tebal (*Hero*), desikator, blender (*Philips*), *stopwatch*, *hot plate magnetic strirrer* (IKA C-MAG HS 7), toples, *aluminium foil* serta alat-alat gelas (pyrex).

Analisis Data

Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) faktorial. Faktor pertama adalah jenis asam (A), yaitu asam klorida dan asam sitrat sedangkan faktor kedua adalah pH pelarut (P) yaitu pH 1,5; 2; 2,5; dan 3. Seluruh perlakuan diulang sebanyak dua kali sehingga diperoleh 16 unit percobaan. Data yang diperoleh dianalisis dengan sidik ragam, dan apabila berpengaruh terhadap parameter yang diamati, dilanjutkan dengan uji Duncan.

Pelaksanaan Penelitian

a. Tahap Persiapan Sampel

Limbah kulit lemon dibersihkan dan dikeringkan dengan menggunakan oven pada suhu 55°C selama 15 jam. Kulit lemon yang telah kering kemudian dihancurkan hingga mendapatkan bubuk kulit lemon.

Akuades diasamkan dengan jenis asam sesuai perlakuan yaitu asam khlorida (A1) dan asam sitrat (A2) dengan konsentrasi masing-masing 1 N sehingga terjadi perubahan pH menjadi 1,5 (P1), pH 2,0 (P2), pH 2,5 (P3), dan pH 3,0 (P4) sesuai perlakuan.

b. Ekstraksi

Ekstraksi dilakukan dengan menambahkan 30 gram bubuk kulit lemon pada 1.050 ml air yang telah diasamkan (Perbandingan bubuk kulit lemon dengan air yang diasamkan 1:35). Ekstraksi kemudian dilakukan selama 40 menit pada suhu ±80°C di *hot plate magnetic strirrer*. Hasil ekstraksi kemudian disaring dengan menggunakan kain saring tebal untuk memisahkan filtrat dengan ampas kulit lemon. Kemudian filtrat dikentalkan sampai volume menjadi setengahnya dengan pemanasan pada *hot plate magnetic strirrer* pada suhu 90°C.

c. Pengendapan

Filtrat kemudian didinginkan sampai suhu ruang dan dilakukan pengendapan dengan menambahkan etanol 96% yang telah diasamkan dengan menambahkan 2 ml asam klorida 37% per satu liter etanol. Perbandingan etanol yang telah diasamkan dengan filtrat adalah 1,5 : 1. Pengendapan ini dilakukan selama 24 jam. Pemisahan endapan pektin dari larutan etanol dilakukan dengan disaring menggunakan kain saring tebal.

d. Pencucian

Endapan pektin yang diperoleh dicuci dengan menggunakan etanol 96%. Pencucian dilakukan dengan menambahkan etanol sampai endapan pektin terendam kemudian diaduk. Selanjutnya endapan pektin disaring. Hal ini diulang kembali sampai pektin bersifat netral. Pektin yang netral ialah pektin yang tidak berwarna merah bila ditambahkan indikator phenolphtalein (PP).

e. Pengeringan

Pektin basah dikeringkan dalam oven pada suhu 40°C selama 8 jam. Pektin yang sudah kering dihaluskan dan ditimbang untuk kemudian dilakukan pengujian lanjut.

Parameter yang Diamati

Parameter yang diamati pada penelitian ini meliputi rendemen (Ranganna, 1977), kadar air (SNI 01-1891-1992), kadar abu (SNI 01-1891-1992), berat ekivalen (Ranganna, 1977), kadar metoksil (Ranganna, 1977), kadar asam galakturonat (Owens, *et al*., 1952), dan derajat esterifikasi (Schultz, 1965 dalam Fitriani, 2003).

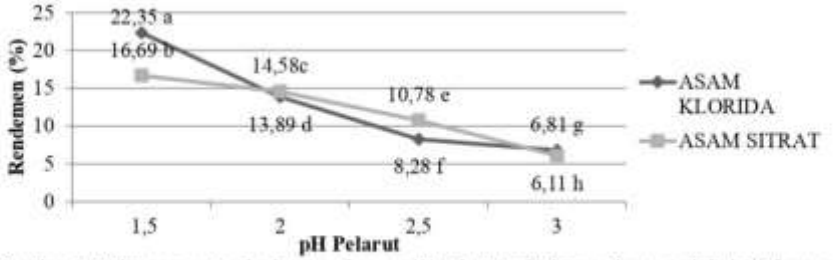
HASIL DAN PEMBAHASAN

Rendemen

Hasil sidik ragam menunjukkan bahwa interaksi antara jenis asam dan pH pelarut berpengaruh sangat nyata ($P<0,01$) terhadap rendemen pektin kulit lemon. Grafik interaksi antara jenis asam dengan pH pelarut terhadap

rendemen pektin kulit lemon dapat dilihat pada Gambar 1.

Gambar 1 menunjukkan bahwa rendemen pektin dari kulit lemon dari berbagai perlakuan berkisar antara 6,11 - 22,35%. Rendemen pektin tertinggi terdapat pada perlakuan ekstraksi menggunakan asam klorida dengan pH 1,5 yaitu 22,35% sedangkan rendemen terendah diperoleh dari perlakuan ekstraksi pektin kulit lemon menggunakan asam sitrat dengan pH 3 yaitu 6,11%. Hal ini menunjukkan bahwa rendemen pektin yang dihasilkan mengalami kenaikan seiring dengan penurunan pH pelarut, baik dengan menggunakan asam klorida maupun asam sitrat.



Gambar 1. Hubungan antara jenis asam dengan pH pelarut terhadap rendemen pektin kulit lemon
Keterangan : Notasi yang sama dibelakang nilai rata-rata menunjukkan pengaruh perlakuan tidak berbeda nyata (P>0,05)

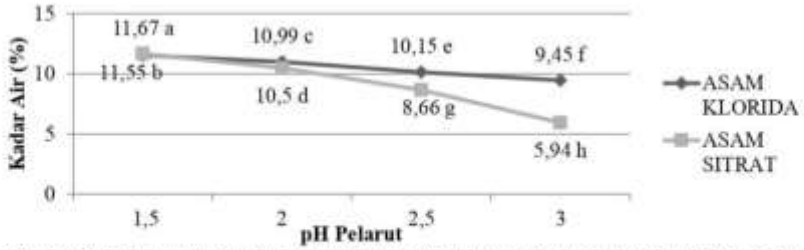
Semakin rendah pH pelarut, maka rendemen pektin kulit lemon yang dihasilkan semakin tinggi. Hal ini disebabkan karena semakin rendahnya pH pelarut menyebabkan semakin banyak ion hidrogen sehingga proses hidrolisis protopektin menjadi pektin menjadi lebih cepat dan meningkatkan rendemen pektin yang dihasilkan. Menurut Hanum *et al.* (2012), pH pelarut yang rendah menyebabkan semakin banyak ion hidrogen yang mensubstitusi kalsium dan magnesium dari protopektin, proses hidrolisis protopektin menjadi pektin lebih cepat, sehingga dapat menghasilkan pektin yang lebih banyak.

Rendemen pektin yang dihasilkan pada ekstraksi menggunakan asam klorida lebih tinggi dibandingkan asam sitrat. Asam klorida merupakan asam mineral yang memiliki tetapan keseimbangan (K) lebih tinggi dibandingkan asam sitrat sebagai asam organik. Nilai K untuk asam klorida sebesar 10^7 sedangkan asam sitrat sebesar $7{,}21 \times 10^4$ (Hesti, 2003). Semakin besar nilai K maka akan meningkatkan jumlah suatu asam berdisosiasi dan semakin kuat pula asam untuk menarik ion divalen dan menggantinya dengan ion hidrogen. Ion hidrogen berfungsi untuk menghidrolisa protopektin menjadi pektin yang larut sehingga rendemen pektin yang dihasilkan akan semakin tinggi. Menurut Hesti (2003) pada kondisi asam, protopektin cenderung terhidrolisa menjadi asam pektinat atau pektin yang larut. Proses pelarutan pektin menjadi asam pektinat ini dapat terjadi karena adanya subsitusi ion divalen protopektin menjadi ion hidrogen ataupun karena putusnya ikatan antara asam pektinat dengan selulosa.

Kadar Air

Hasil sidik ragam menunjukkan bahwa interaksi antara jenis asam dan pH pelarut berpengaruh sangat nyata (P<0,01) terhadap kadar air pektin kulit lemon. Grafik interaksi antara jenis asam dengan pH pelarut terhadap kadar air pektin kulit lemon dapat dilihat pada Gambar 2.

Berdasarkan Gambar 2 dapat dilihat bahwa kadar air pektin dari kulit lemon dari berbagai perlakuan berkisar antara 5,94 - 11,67 %. Kadar air pektin tertinggi terdapat pada perlakuan ekstraksi menggunakan asam klorida dengan pH 1,5 yaitu 11,67% sedangkan kadar air terendah diperoleh dari perlakuan ekstraksi pektin kulit lemon menggunakan asam sitrat dengan pH 3 yaitu 5,94 %. Kadar air maksimum pektin kering menurut IPPA (2003) adalah 12%, dengan demikian kadar air pektin hasil penelitian ini masih di bawah syarat maksimum yang ditetapkan.



Gambar 2. Hubungan antara jenis asam dengan pH pelarut terhadap kadar air pektin kulit lemon
Keterangan : Notasi yang sama dibelakang nilai rata-rata menunjukkan pengaruh perlakuan tidak berbeda nyata (P>0,05)

Semakin rendah pH pelarut maka akan meningkatkan kadar air pektin yang dihasilkan. Rendahnya pH pelarut menyebabkan banyaknya ion hidrogen yang menghidrolisa protopektin menjadi pektin. Semakin banyaknya pektin yang terbentuk maka ikatan hidrogen yang terbentuk juga semakin meningkat. Menurut Prasetyowati, *et al.* (2009), molekul air tunggal atau kelompok air dapat terikat pada permukaan pektin melalui ikatan hidrogen atau gugus –OH pada molekul pektin dengan atom H dari molekul air.

Kadar air pektin yang dihasilkan dengan menggunakan asam klorida lebih tinggi dibandingkan dengan dengan asam sitrat. Ini dikarenakan asam klorida memiliki tetapan keseimbangan (K) yang lebih tinggi sehingga jumlah ion hidrogen semakin tinggi. Ion hidrogen tersebut akan meningkatkan kinetika reaksi hidrolisis protopektin menjadi pektin yang larut. Semakin banyak pektin yang terbentuk akan meningkatkan ikatan hidrogen yang terbentuk. Menurut Prasetyowati, *et al.* (2009), molekul air tunggal atau kelompok air dapat terikat pada permukaan pektin melalui ikatan hidrogen atau gugus –OH pada molekul pektin dengan atom H dari molekul air.

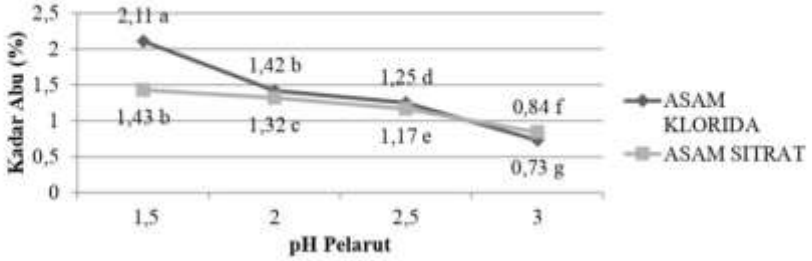
Kadar Abu

Hasil sidik ragam menunjukkan bahwa interaksi antara jenis asam dan pH pelarut berpengaruh sangat nyata (P<0,01) terhadap kadar abu pektin kulit lemon. Grafik interaksi antara jenis asam dengan pH pelarut terhadap kadar abu pektin kulit lemon dapat dilihat pada Gambar 3.

Hasil penelitian menunjukkan kadar abu pektin yang diperoleh dari ekstraksi menggunakan asam klorida berkisar antara 0,73 – 2,11 %. Kadar abu pektin dalam IPPA (2003) yaitu tidsk lebih dari 10%, dengan demikian kadar abu pektin kulit lemon hasil penelitian ini memenuhi standar yang telah ditetapkan. Kadar abu pektin tertinggi terdapat pada perlakuan ekstraksi menggunakan asam klorida dengan pH 1,5 yaitu sebesar 2,11%. Kadar abu terendah terdapat pada perlakuan

ekstraksi menggunakan asam klorida dengan pH 3 yaitu sebesar 0,73%.

Semakin rendah pH pelarut maka semakin tinggi kadar abu pektin yang dihasilkan. Perlakuan dengan asam yang memiliki pH rendah akan mengakibatkan hidrolisis protopektin menjadi pektin. Reaksi hidrolisis protopektin yang semakin meningkat mengakibatkan komponen ion Ca dan Mg juga bertambah. Dengan begitu, akan meningkatkan kadar mineral sehingga kadar abu pektin yang dihasilkan semakin meningkat. Menurut Kalapathy dan Proctor (2001), asam memiliki kemampuan untuk melarutkan mineral alami bahan yang diekstrak dan semakin meningkat dengan meningkatnya konsentrasi asam, suhu dan waktu ekstraksi. Mineral tersebut akan larut dan mengendap kemudian bercampur dengan pektin pada saat pengendapan alkohol.



Gambar 3. Hubungan antara jenis asam dengan pH pelarut terhadap kadar abu pektin kulit lemon
Keterangan : Notasi yang sama dibelakang nilai rata-rata menunjukkan pengaruh perlakuan tidak berbeda nyata (P>0,05)

Jenis asam mempengaruhi kadar abu pektin. Kadar abu pektin yang dihasilkan dengan menggunakan asam klorida lebih tinggi dibandingkan dengan menggunakan asam sitrat. Ini menunjukkan semakin kuat asam akan meningkatkan reaksi hidrolisis protopektin yang mengakibatkan bertambahnya mineral berupa Ca dan Mg sehingga kadar abu pektin akan semakin meningkat. Menurut Hanum (2012), semakin kuat asam yang digunakan dalam ektraksi pektin akan meningkatkan reaksi hidrolisis protopektin oleh asam yang akan meningkatkan komponen Ca dan Mg dalam larutan ekstrak.

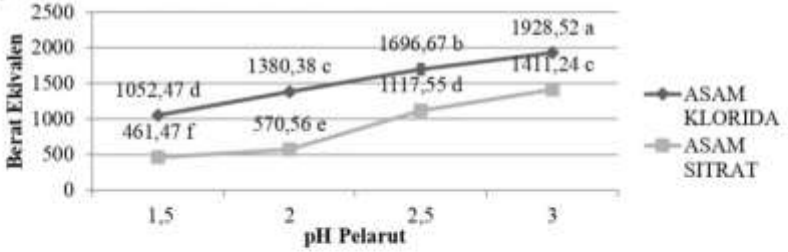
Berat Ekivalen

Hasil sidik ragam menunjukkan bahwa interaksi antara jenis asam dan pH pelarut berpengaruh sangat nyata (P<0,01) terhadap berat ekivalen pektin kulit lemon. Grafik interaksi antara jenis asam dengan pH pelarut terhadap berat ekivalen pektin kulit lemon dapat dilihat pada Gambar 4.

Gambar 4 menunjukkan bahwa berat ekivalen pektin dari kulit lemon dari berbagai perlakuan berkisar antara 461,47 – 1928,52. Berat ekivalen pektin tertinggi terdapat pada perlakuan ekstraksi menggunakan asam klorida dengan pH 3 yaitu 1928,52 sedangkan berat ekivalen terendah diperoleh dari perlakuan ekstraksi pektin kulit lemon menggunakan asam sitrat dengan pH 1,5 yaitu 461,47.

Semakin rendah pH pelarut maka berat ekivalen pektin kulit lemon yang dihasilkan juga semakin rendah. pH yang rendah dapat menyebabkan terjadinya deesterifikasi pektin menjadi asam pektat, di mana jumlah gugus asam bebas semakin banyak sehingga berat

ekivalen semakin rendah. Menurut Fitriani (2003), perubahan berat ekivalen dipengaruhi oleh deesterifikasi. Peningkatan proses deesterifikasi berarti peningkatan jumlah gugus asam bebas dan hal ini berarti penurunan berat ekivalen karena asam pektat yang memiliki berat ekivalen yang lebih rendah semakin meningkat.



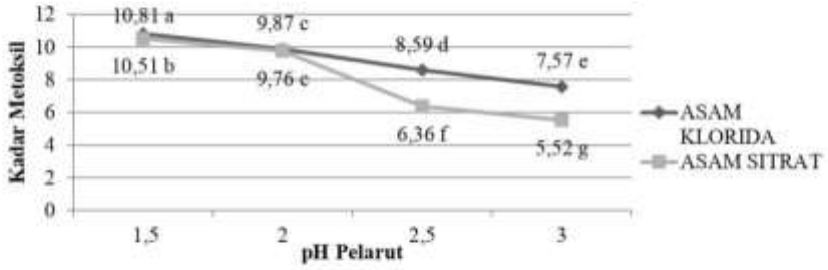
Gambar 4. Hubungan antara jenis asam dengan pH pelarut terhadap berat ekivalen pektin kulit lemon

Keterangan : Notasi yang sama dibelakang nilai rata-rata menunjukkan pengaruh perlakuan tidak berbeda nyata (P>0,05)

Sifat asam mempengaruhi berat ekivalen pektin. Berat ekivalen pektin menggunakan asam klorida lebih tinggi dibandingkan asam sitrat. Semakin kuat asam maka akan meningkatkan hidrolisa protopektin menjadi asam pektinat atau pektin yang larut. Pektin yang larut memiliki berat ekivalen yang tinggi. Asam pektat murni memiliki berat ekivalen 176, sedangkan pektin murni memiliki berat ekivalen yang tinggi yaitu sebesar 1886 (Fitriani, 2003).

Kadar Metoksil

Hasil sidik ragam menunjukkan bahwa interaksi antara jenis asam dan pH pelarut berpengaruh sangat nyata (P<0,01) terhadap kadar metoksil pektin kulit lemon. Grafik interaksi antara jenis asam dengan pH pelarut terhadap kadar metoksil pektin kulit lemon dapat dilihat pada Gambar 5.



Gambar 5. Hubungan antara jenis asam dengan pH pelarut terhadap kadar metoksil pektin kulit lemon

Keterangan : Notasi yang sama dibelakang nilai rata-rata menunjukkan pengaruh perlakuan tidak berbeda nyata (P>0,05)

Hasil penelitian menunjukkan kadar metoksil pektin yang diperoleh dari ekstraksi menggunakan asam klorida berkisar antara 5,52 – 10,81%. Kadar metoksil pektin tertinggi terdapat pada perlakuan ekstraksi menggunakan asam klorida dengan pH 1,5 sebesar 10,8 %. Kadar metoksil terendah terdapat pada perlakuan ekstraksi menggunakan asam sitrat dengan pH 3 sebesar 5,52 %. Berdasarkan standar IPPA, pektin bermetoksil tinggi memiliki kadar metoksil >7,12% sedangkan pektin bermetoksil rendah yaitu 2,5 – 7,12%. Pektin dengan ekstraksi menggunakan asam sitrat pada pH 2,5 dan pH 3 termasuk ke dalam pektin bermetoksil rendah sedangkan pektin lainnya termasuk dalam kategori pektin bermetoksil tinggi.

Kadar metoksil pada pektin memiliki peranan penting yaitu menentukan sifat fungsional pektin seperti struktur dan tekstur dari gel pektin. Pektin bermetoksil tinggi dapat membentuk gel dengan penambahan gula dan asam. Kondisi yang diperlukan untuk pembentukan gel adalah kadar gula 58-75% dengan pH 2,8-3,5. Pektin bermetoksil rendah tidak memiliki kemampuan membentuk gel dengan adanya gula dan asam, tetapi dapat membentuk gel dengan adanya kation polivalen seperti kalsium (Fitria, 2013).

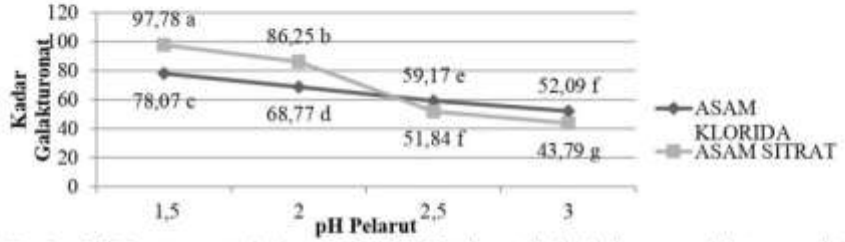
Semakin rendah pH pelarut maka semakin tinggi kadar metoksil pektin yang dihasilkan. Hal ini dapat disebabkan oleh gugus karboksil yang teresterifikasi semakin meningkat dengan semakin rendahnya pH pelarut sehingga kadar metoksil pektin semakin tinggi. Menurut Sufy (2015), kadar metoksil merupakan jumlah metanol yang terdapat dalam pektin dan jumlah gugus karboksil teresterifikasi. Semakin meningkatnya konsentrasi asam akan meningkatkan kadar metoksil pektin karena gugus karboksil teresterifikasi semakin meningkat.

Kadar metoksil pada perlakuan ekstraksi menggunakan asam klorida lebih tinggi dibandingkan dengan kadar metoksil pada menggunakan asam sitrat. Hal ini disebabkan karena asam klorida memiliki nilai K yang lebih tinggi sehingga meningkatkan ion hidrogen yang menghidrolisa protopektin menjadi pektin. Semakin banyak pektin maka jumlah metanol pada gugus karboksil tersesterifikasi juga semakin banyak sehingga meningkatkan kadar metoksil. Menurut Hanum (2012), semakin banyak jumlah ion hidrogen pada asam yang digunakan untuk menghidrolisa protopektin menjadi pektin yang larut, maka akan semakin meningkatkan jumlah gugus karboksil teresterifikasi pada pektin sehingga kadar metoksil semakin meningkat.

Kadar Asam Galakturonat

Hasil sidik ragam menunjukkan bahwa interaksi antara jenis asam dan pH pelarut berpengaruh sangat nyata (P<0,01) terhadap kadar asam galakturonat pektin kulit lemon. Grafik interaksi antara jenis asam dengan pH pelarut terhadap kadar asam galakturonat pektin kulit lemon dapat dilihat pada Gambar 6.

Hasil penelitian menunjukkan kadar asam galakturonat pektin yang diperoleh berkisar antara 43,79 – 97,78 %. Kadar asam galakturonat pektin tertinggi terdapat pada perlakuan ekstraksi menggunakan asam sitrat dengan pH 3 yaitu sebesar 97,78%. Kadar asam galakturonat terendah terdapat pada perlakuan ekstraksi menggunakan asam sitrat dengan pH 1,5 yaitu sebesar 43,79%.



Gambar 6. Hubungan antara jenis asam dengan pH pelarut terhadap kadar asam galakturonat pektin kulit lemon

Keterangan : Notasi yang sama dibelakang nilai rata-rata menunjukkan pengaruh perlakuan tidak berbeda nyata (P>0,05)

Semakin rendah pH pelarut pada ekstraksi pektin maka semakin tinggi juga kadar asam galakturonat pektin yang dihasilkan. Hal ini disebabkan semakin rendah pH pelarut yang digunakan, maka kinetika reaksi hidrolisis protopektin semakin meningkat sehingga dapat meningkatkan kadar asam galakturonat pektin. Menurut Ariesti, *et al.* (2015), kadar galakturonat pektin semakin meningkat seiring dengan bertambahnya konsentrasi asam dikarenakan meningkatnya reaksi hidrolisis protopektin menjadi pektin yang komponen dasarnya D-galakturonat.

Kadar asam galakturonat pada ekstraksi dengan menggunakan asam klorida lebih tinggi dibandingkan dengan asam sitrat pada pH 2,5 dan 3. Hal ini dikarenakan asam klorida pada pH tersebut masih menghidrolisis protopektin menjadi pektin lebih baik dibandingkan dengan asam sitrat. Ini menyebabkan kandungan asam galakturonat juga lebih tinggi. Menurut Hesti (2003), asam klorida memiliki nilai K yang lebih tinggi dibandingkan dengan asam sitrat. Nilai K ini menunjukkan semakin kuat asam dalam disosiasi ion divalen menjadi ion hidrogen yang membantu hidrolisis protopektin menjadi pektin. Kandungan asam galakturonat yang terdapat di dalam pektin juga semakin meningkat dengan meningkatnya pektin yang dihasilkan.

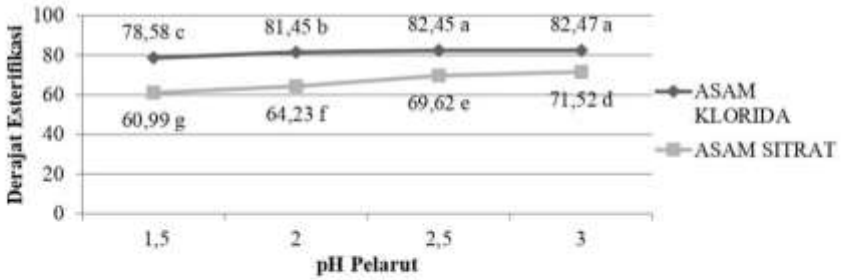
Kadar asam galakturonat pada ekstraksi menggunakan asam klorida lebih rendah dibandingkan dengan asam sitrat pada pH 1,5 dan 2. Hal ini diduga karena hidrolisis pektin pada asam kuat dengan pH rendah cenderung terus-menerus sehingga dapat mendegradasi pektin menjadi asam pektat. Degradasi pektin menjadi asam pektat menyebabkan penurunan asam galakturonat pektin. Menurut Nurhikmat (2003) degradasi pektin menjadi asam pektat dapat terjadi karena hidrolisis pektin oleh asam yang cenderung terus-menerus sehingga menurunkan kadar asam galakturonat.

Derajat Esterifikasi

Hasil sidik ragam menunjukkan bahwa interaksi antara jenis asam dan pH pelarut berpengaruh sangat nyata (P<0,01) terhadap derajat esterifikasi pektin kulit lemon. Grafik interaksi antara jenis asam dengan pH pelarut terhadap derajat esterifikasi pektin kulit lemon dapat dilihat pada Gambar 7.

Gambar 7 menunjukkan derajat esterifikasi pektin kulit lemon yang diperoleh berkisar antara 60,99 – 82,47 %. Derajat esterifikasi pektin tertinggi terdapat pada perlakuan ekstraksi menggunakan asam klorida dengan pH 3 yaitu sebesar 82,47 %. Derajat esterifikasi terendah terdapat pada perlakuan ekstraksi menggunakan asam sitrat dengan pH 1,5 yaitu sebesar 60,99 %. Standar mutu pektin

dalam IPPA (2003), pektin disebut memiliki ester tinggi apabila derajat esterifikasi di atas 50% sedangkan pektin ester rendah memiliki derajat esterifikasi di bawah 50%. Pektin yang dihasilkan dalam penelitian ini termasuk pektin ester tinggi karena memiliki derajat esterifikasi di atas 50%.



Gambar 7. Hubungan antara jenis asam dengan pH pelarut terhadap derajat esterifikasi pektin kulit lemon

Keterangan : Notasi yang sama dibelakang nilai rata-rata menunjukkan pengaruh perlakuan tidak berbeda nyata (P>0,05)

Semakin rendah pH pelarut maka semakin rendah derajat esterifikasi pektin. Ini disebabkan karena pH yang rendah akan menyebabkan degradasi pektin menjadi asam pektat sehingga gugus metil ester berkurang. Menurut Budiyanto dan Yulianingsih (2008) ikatan glikosidik gugus metil ester dari pektin cenderung terhidrolisis menghasilkan asam galakturonat. Jika ekstraksi dilakukan pada pH yang rendah, pektin akan terdegradasi menjadi asam pektat dimana asam galakturonat bebas dari gugus metil ester. Jumlah gugus metil ester menunjukkan jumlah gugus karboksil yang tidak teresterifikasi atau derajat esterifikasi.

Derajat esterifikasi pektin dengan esktraksi menggunakan asam klorida lebih tinggi dibandingkan dengan asam sitrat. Hal ini dikarenakan asam klorida memiliki nilai K yang tinggi sehingga meningkatkan proses hidrolisis protopektin menjadi pektin yang larut. Tingginya jumlah pektin yang dihasilkan akan meningkatkan gugus metil ester atau derajat esterifikasi pektin. Menurut Hesti (2003) asam klorida memiliki tetapan keseimbangan disosiasi (K) sebesar 10^7. Nilai K yang tinggi akan meningkatkan jumlah suatu asam berdisosiasi dan meningkatkan jumlah ion hidrogen sehingga hidrolisis protopektin menjadi pektin menjadi lebih cepat. Pektin terdiri dari asam poligalakturonat yang mengandung metil ester. Kandungan gugus metil ester yang meningkat dengan ekstraksi asam klorida menunjukkan derajat esterifikasi yang semakin meningkat.

SIMPULAN DAN SARAN

Simpulan

Berdasarkan penelitian yang telah dilakukan maka dapat disimpulkan beberapa hal sebagai berikut:

1. Interaksi antara jenis asam dan pH pelarut berpengaruh sangat nyata terhadap rendemen, kadar air, kadar abu, berat ekivalen, kadar metoksil, kadar asam galakturonat, dan derajat esterifikasi pektin kulit lemon.

2. Hasil penelitian terbaik pektin kulit lemon adalah perlakuan kombinasi ekstraksi dengan menggunakan asam klorida pada pH pelarut 1,5. Kombinasi ini memberikan rendemen pektin sebesar 22,35%, kadar air 11,55%, kadar abu 2,11%, berat ekivalen 1052,47 , kadar metoksil 10,81%, kadar asam galakturonat 78,07%, dan derajat esterifikasi 78,58%.

Saran

Berdasarkan hasil penelitian ini disarankan dilakukan penelitian yang mengkaji pengaruh jenis asam lain untuk menghasilkan pektin dengan rendemen yang lebih tinggi serta berat ekivalen yang sesuai standar IPPA (2003) yaitu sebesar 600-800.

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