

CHARACTERIZATION OF POWDERED PECTIN FROM WATERMELON (*Citrullus lanatus*) RIND

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ABSTRACT

Watermelon rind is considered as a major solid waste in the Philippines due to the high consumption of watermelon pulp. It is said to contain at least 13% of pectin. Pectin is used as a gelling agent for jam and jellies. This study aims to extract pectin from watermelon rind and to characterize the extracted pectin to determine the pectin yield, equivalent weight, methoxyl content, total anhydrouronic acid content and degree of esterification. The watermelon rind was dried using a cabinet dryer @ 50°C for 24hrs before conducting the extraction. The extraction of the pectin was conducted using microwave assisted extraction (pH 2, 700 W, 158 sec). The extracted coagulated pectin was characterized obtaining the following results: pectin yield (.17%), equivalent weight (192.31), methoxyl content (1.18%), total anhydrouronic acid content (15.84%) and degree of esterification (0.42%). Based on the results obtained, it shows that watermelon rind has a low content of pectin.

Keywords: Watermelon rind, microwave assisted extraction, pectin

INTRODUCTION

In today's time, issues about agricultural wastes have risen due to high production of perishable produces, which are not properly utilized by the consumers (Apsara and Pushpalatha, 2002). For most fruits, the pulp or the flesh is only consumed leaving the seeds, peels, or rinds behind, resulting to agricultural wastes (Gin et al., 2014). Fruit and vegetable residues became popular to be studied since the wastes are one of the important sources of polyphenols. Agricultural wastes are a great source of antioxidants and dietary fibers; thus, by using the wastes, environmental pollution is reduced (Ibrahim, Kamarrudin and Suzihaque, 2017). Watermelons are grown in different tropical and subtropical countries in the world. It is commonly grown for fresh consumption of the mature fruit (Department of Agriculture, Forestry and Fisheries, 2011).

Watermelon, with a scientific name *Citrullus lanatus*, is considered as a fruit that commonly grows during summer season and is associated to the cucurbitaceae family (Ernst and Saha, 2014) like cantaloupe, squash, cucumber and pumpkin. Watermelon is a vine-like blooming plant originally from southern Africa to Bangladesh (Hoque and Iqbal, 2015). The fruit differs in shape from round to oblong. Thus, its rind color varies from light to dark green and with or without stripes. Flesh colors vary from dark red to light red or yellow (Boyhan, Granberry and Kelley, 2017). The plant have weak stems and climb by tendrils and fruit matures on the ground and the drying of tendril at the point of attachment to the fruit is considered a sign of maturity (Alam et al., 2013).

Watermelon contains about 6% sugar and 91% water by weight (Hoque and Iqbal, 2015). Watermelons are rich in different vitamins such as vitamin C, vitamin A, vitamin B, amino acid and carotenoid lycopene (Hoque et al., 2013). Watermelons have a greater concentration of lycopene than that of lycopene in tomatoes (Perkins-Veazie and Collin, 2006). Lycopene and vitamin C are well known antioxidants present in watermelons, which provide many health benefits (Bliss, 2002). It is fat and cholesterol free and is considered as a low-calorie fruit (Bruton et al., 2009). It contains about 90% of moisture, which can help in healthy hydration (Alam et al., 2013).

Watermelon pulp and juice are used for human consumption while the rind and seeds are major solid wastes. Watermelon by-products are sources of protein, dietary fibers, and natural antioxidants (Al-Sayed and Ahmed, 2013). Watermelon rind wastes from different restaurants, cottage fruit juice producers, and different fruit industries are one of the major agricultural wastes produced (Souad et al., 2012). The rind is the white-colored flesh between the peel and the colored meat, wherein it is reported to contain at least 13% of pectin (Indah and Endah, 2015).

The rind is usually discarded as waste yet it is edible (Al-Sayed and Ahmed, 2013). A research expounded that the watermelon rind contains 93.8% moisture, 0.49% ash, 0.1% nitrogen, and 2.1% sugars. The skin of a matured watermelon contains approximately 20% cellulose, 23% hemicellulose, 10% lignin, 13% pectin, 7 mg/g silica, and 12% silica-free minerals (Campbell, 2006). The rind also contains 2-20 mg/g dry weight of the amino acid citrulline (Perkins, 2002). Watermelon rind could be a possible source of fiber in foods (Hoon and Dahri, 2016).

Pectin substances are found within the primary cell walls and intercellular regions of higher plants (Rasheed, 2008). Pectin is a polymer of α -galacturonic acid with a variable number of methyl ester groups (Liu, Shi and Langrish, 2006). It is a heteropolysaccharide, which serves as an important by-product that can be extracted from fruit and vegetable waste (Begum et al., 2014). Pectin's amount, structure and chemical composition differ between plants (Srivastava and Malviya 2011). The most common structural elements found in pectin are homogalacturonan, xylogalacturonan, rhamnogalacturonan I, rhamnogalacturonan II, arabinan, arabinogalactan I, and arabinogalactan II (Kamble, Gawande and Patil, 2017). It has effective nutritional and technological properties, whereas it is a high-value functional food ingredient used as gelling agent and emulsifier in jam and jellies, and thickener in sauces (Chakraborty and Ray, 2011). Jams that contain above 60% of sugar and soluble fruit solids use high-ester pectin (Yablokov and Alexey, 2009).

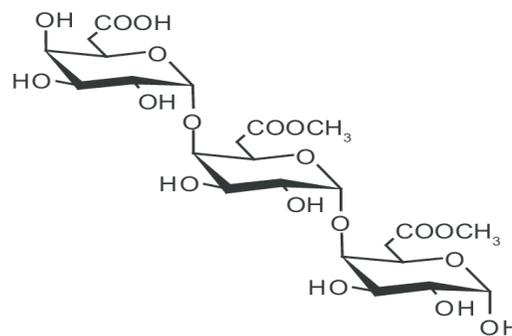


Fig. 1. Structure of Pectin (Sundar Raj AA. et al., 2012)

Aside from its function as a food enhancer, pectin is also applied in medicine wherein it helps to lower serum cholesterol level. Thus, it also helps to remove heavy metal ions from the body and stabilize blood pressure (Shaha, Punichelvana and Afandi, 2013). 6 grams of pectin per day must be consumed to have a significant effect against cholesterol level in the body; thus, less than 6 grams of pectin per day will show no significant effect. Pectin also has an effective function in removing lead and mercury from the gastrointestinal tract and respiratory organs (Raj et al., 2012). Pectin also serves as a dietary fiber wherein it helps to prevent colon cancer (Leclere et al., 2013). It also exhibits an anti-tumor activity due to its immunostimulatory activity (Majee et al., 2017).

The most important process to produce pectin is extraction. There are different techniques in extracting pectin, specifically conventional method of boiling in acidified water (Bagherian et al., 2011), microwave-assisted extraction (MAE) (Yeoh, Shi and Langrish, 2008), enzymatic extraction (Ptichkina, Markina and Rumyantseva, 2008), and high hydrostatic pressure treatment (Guo et al., 2012). The direct boiling in hot, acidified water followed by isolation of the pectin from the ensuing solution is the most commonly method for pectin extraction (Mandal, Mohan, and Hemalatha, 2007). The pectin is directly heated for a long duration subjecting to thermal degradation (Yujaroen et al., 2008; Marshal et al., 2007), so using the said method is not considered in obtaining a high quality and quantity of pectin due to the extent of time processing it (Maran et al., 2014; Adetunji et al., 2017). According to Kiss (2009), a higher yield of pectin was

achieved in using MAE in fruits like red and black currant, raspberry, and elderberry rather than conventional method of extraction. Additionally, Bako, Cserjesi, and Beszedes (2015) concluded that using MAE in pectin extraction produce a higher yield and better pectins. Therefore, microwave-assisted extraction (MAE) is a potential method that can be applied in the pectin production (Mandal, Dewanje, and Mandal, 2009).

Microwave-assisted extraction (MAE) has been recently investigated by many researchers. They found that it can lead to a considerable increase in the yield and quality of extracted pectin (Mohapatra and Mishra, 2011; Routray and Orsat, 2012; Kratchanova et al., 2004). It has been reported as the preferred extraction method of pectin from natural sources such as dragon fruit peels (Thirugnanasambandham et al., 2014; Rahmati et al., 2015), bagasse and pomace obtained from Mexican lime fruit (Sánchez-Aldana, Nevarez-Moorillon and Esquivel, 2013), pomelo (Quoc et al., 2015), mango (Maran et al., 2015) and papaya peels (Maran and Prakash, 2015) under different operation conditions. The principle of MAE is basically the use of microwave energy to penetrate the molecules of the solvent directly to the skin tissues of the pectin source (Rahmati, Abdullah, Momeny and Kang, 2015). It has shown potential in terms of saving time, energy and solvent consumption (Kute et al., 2015).

In this study, watermelon rind was utilized as a source of pectin. This study aims to extract pectin from the watermelon rind using microwave-assisted extraction method to characterize the pectin yield from the samples through determining its equivalent weight, methoxyl content, total anhydrouronic acid content and degree of esterification. Also, it helped to broaden the sources of pectin for food applications. This study only covered and is limited to the characterization of extracted pectin. Shelf-life and food application were not conducted.

MATERIALS AND METHODS

Collection of Samples

Fresh watermelon rinds were collected from a local vendor located in Metro Manila. To avoid deterioration before processing, the samples were stored in a container at 4°C (Mohamad, Saheed and Jamal, 2012). The materials needed for the preparation of samples were ice and large coolers for the storage of the samples while knives, plastic gloves and chopping board for the cutting of samples.

Preparation of Samples

The white part of the watermelon rinds were separated from the fresh fruits using a sterile knife (Ho and Dahri, 2016). The samples were cut ± 1 cm length, wide and thick. After cutting the rind, the samples were washed with flowing water (Sari, Ishartani and Dewanty, 2017) before drying in a ventilated dryer at 50°C for 24 hours (Ho and Dahri, 2016). The dried samples were pulverized using a 60-mesh sieve then were stored in a plastic bag for pectin extraction (Sari et al., 2017).

The extraction of pectin from the dried watermelon rind was conducted using the procedure of Sari et al. (2017) with some alterations. 10 grams of watermelon rind powder were placed in a 1000 mL beaker containing 100 mL distilled water. The pH of the mixture was adjusted to 2 by adding 1 N of acetic acid solvent. After adjusting the pH of the mixture, it was placed on the round plate inside the microwave and heated with a power level of 700 W for 2 minutes and 38 seconds. The mixture was cool down and filtered using a filter paper (Whatman Paper No. 1). Same volume of the filtrate with 96 percent concentration of ethanol was added for the precipitation of the filtrate overnight. 70 percent of ethanol was used to remove the acidity of the coagulated pectin mass by washing it. The wet coagulated pectin mass was placed in a cabinet dryer with a temperature of 45°C until constant weight was reached. The dried pectin was pulverized using a grinder to turn it into powder. The powdered pectin was placed in a sealed container (zip lock) for further testing.

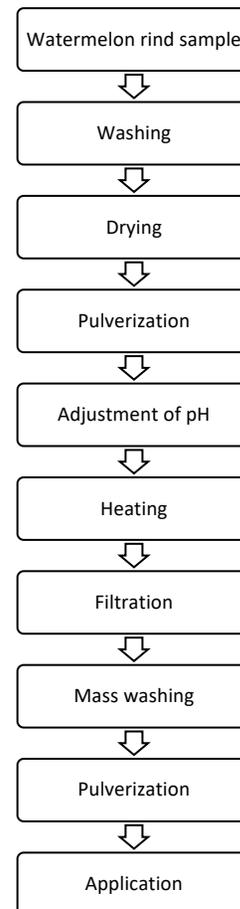


Figure 3: Schematic Diagram for Extraction of Pectin



Figure 1: Fresh Watermelon Rind



Figure 2: Dried Watermelon Rind



Figure 4: Solution after extraction



Figure 5: Coagulated Pectin



Figure 6: Dried Pectin

Characterization of Pectin Pectin Yield

The pectin yield was determined by calculating the result obtained. The formula used was based from Beegum (2016).

$$\text{Pectin Yield} = \frac{\text{weight of pectin (g)}}{\text{weight of the dried rind}} \times 100$$

Equivalent Weight

The determination of the Equivalent Weight was obtained from the method of Ranganna (1995). In a 250 mL conical flask, 0.5 g of pectin, 5 mL of ethanol, 1 g of sodium chloride and 100 mL of distilled water were placed. 6 drops of phenol red were also placed into the conical flask. The mixture was titrated into the 0.1 N NaOH. The titration point was indicated by purple color. The neutralized solution was stored for the determination of the methoxyl content. The formula used was based from Wonago (2016).

$$\text{Equivalent Weight} = \frac{\text{weight of sample (g)} \times 100}{\text{ml of Alkali} \times \text{Normality of Alkali}}$$

Methoxy Content

The determination of the methoxyl content was obtained from the method of Ranganna (1995). The neutralized solution and the 25 mL of Sodium Hydroxide (0.25 N) were collected from the determination of the equivalent weight. Before storing the mixed solution for 30 minutes in room temperature, the solution was properly stirred. After 30 minutes, 30 mL of 0.25 N hydrochloric acid was added into the mixture. The mixture was titrated into the 0.1 N NaOH. The formula used was based from Azad et al. (2014).

$$\text{Methoxyl Content} = \frac{\text{ml of alkali} \times \text{Normality of alkali} \times 3.1}{\text{weight of sample}}$$

Total Anhydrouronic Acid Content

The determination of the Total AUA was determined using the formula from Mohammad and Hasan (1995) with some modification of Azad et al. (2014).

$$\% \text{ of AUA} = \frac{176 \times 0.1 z \times 100}{w \times 1000} + \frac{176 \times 0.1 y \times 100}{w \times 1000}$$

When molecular unit of AUA (1 unit) = 176 g

Where,

z = ml (titre) of NaOH from equivalent weight determination

y = ml (titre) of NaOH from methoxyl content determination

w = weight of sample

Degree of Esterification

The degree of esterification was determined by computing the data obtained from the result of the Methoxyl and the Total Anhydrouronic Acid Content. The formula used was based from Girma and Worku (2016).

$$\text{Degree of Esterification} = \frac{176 \times \text{MeO} \times 100}{31 \times \text{AUA}}$$

RESULT AND DISCUSSION

As shown on Table 1, the initial weight of fresh watermelon rind was 5 kg and obtained 320 g after the drying procedure. The weight of dried coagulated pectin extracted from dried watermelon rind was 8.32 g after the washing and drying procedures and obtained 0.17% pectin yield. Therefore, obtaining a very low result in yield was due to the use of dried peel in pectin extraction wherein the drying condition can greatly affect the yield (Campbell, 2006).

Table 1
Pectin yield from Watermelon Rind

Weight of Watermelon Rind (Grams)	Weight of Pectin After Drying (Grams)	Pectin Yield (%)
5000 (Fresh)		0.17(Fresh Rind)
320 (Dried)	8.32	2.6 (Dried Rind)

Table 2
Characterization of Pectin from Dried Watermelon Rind

Characteristics	Result	Standard
Equivalent Weight	192.31	N/A
Methoxyl Content (%)	1.18	<8 (Castillo-Israel et al., 2015)
Total Anhydrouronic	15.84	> 65%
Acid Content (%)		(Food Chemical Codex)
Degree of	0.42	High-ester pectin > 50%
Esterification (%)		Low-ester pectin < 50% (Mohammed H., 2016)

Equivalent weight is another indicator of jelly-forming ability of extracted pectin (Vaclavik and Christian, 2008). Equivalent weight of pectin is the total content of free galacturonic acid (not esterified) in the molecular chains of pectin (Altat et al., 2015). The computed equivalent weight of pectin extracted from watermelon rind is 192.31. The researchers obtained low equivalent weight due to the increased microwave power energy, which resulted to decreased equivalent weight. Compared to the study of Sari et al. (2017), they obtained a high amount of equivalent weight wherein the microwave power they used was 279.3 watts for 12 minutes.

Methoxyl content serves a vital factor in controlling the setting time of pectin and the ability of the pectin to form gels (Constenla and Lozano, 2003). Increase in methoxyl content also increases the spreading quality and sugar-binding capacity of pectin (Madhav and Pushpalatha, 2002). Extracted pectin could only range from 0.2% to 12% methoxyl content depending on the source and how it was obtained (Aina et al., 2012). As shown on Table 2, the result of methoxyl content extracted from watermelon rind is 1.18%. In comparison with commercial pectin, which commonly has a methoxyl content ranging from 8-11% and has the ability to form high sugar gels, low methoxyl pectin (>7%) can form gels with low concentration of sugars (Castillo-Israel et al., 2015). This result means that the sample can only form gels with low concentration of sugar due to its low methoxyl content.

The Total Anhydrouronic Acid Content was used as an indicator of purity of pectin used for jams and jellies (Ahmed et al., 2017). According to the Food Chemical Codex, the result of the Total Anhydrouronic Acid Content should be greater than 65%. As shown on Table 2, the computed result of the Anhydrouronic Acid Content was 15.84%, which was below the standard value. This result means that the extracted pectin might have the presence of protein, starch and sugar because according to Ismail et al. (2012), having a

low purity of extracted pectin can be caused by possible presence of protein, starch and sugar in the precipitated pectin.

The degree of esterification helps to determine the gelling property of pectin (Altaf et al., 2015). According to Azad et al. (2014), the result of the degree of esterification depends on the maturity of the raw material used. There are two degrees of esterification: high-ester pectin (> 50%) and low-ester pectin (< 50%) (Mohamed, 2016). Based on the table shown, the computed result of the degree of esterification was 0.42%, which is considered to be on the low-ester pectin. The higher the value of degree of esterification, the faster it forms gel (Thakur, Singh and Handa, 1997). The result means that the sample used has high level of maturity, which leads to low degree of esterification content, and due to low degree of esterification, the extracted pectin has low setting time property.

CONCLUSION

Pectin extraction from the watermelon (*Citrullus Lanatus*) rind using microwave-assisted method was influenced by microwave power and extraction time. Based on the results of the study, it shows the potential of microwave-assisted extraction in extracting pectin from the watermelon rind. The extracted pectin from the watermelon rind obtained a yield of .17%. Equivalent weight, methoxyl content, total anhydrouronic acid content and degree of esterification was determined obtaining 192.31, 1.18%, 15.84%, and 0.42%, respectively. Moreover, the present work revealed that watermelon rind has pectin that can be used for food applications and could help ecological problems due to food wastes. However, since the obtained yield is low, it is not a practical source of pectin.

RECOMMENDATION

The study was only focused on the characterization of pectin due to limited time and raw materials. MAE was the only extraction method used. It is recommended to use conventional method for pectin extraction from watermelon rind. Also, a comparison between MAE and conventional method can be done to clearly prove that MAE can produce a higher yield of pectin.

It is also suggested to produce more samples to obtain a larger amount of pectin for the conduction of additional suggested tests for characterization of pectin such as ash content, moisture content, and galacturonic acid to serve as an additional proof of the gelling ability of pectin extracted from watermelon rind. Pectin analysis is recommended to determine if the sample acquired is pure pectin. Also, safety assessment tests are suggested to be conducted to prove that there is low chance of microbial growth. Conducting a product development to ensure that the sample can be truly used as a gelling agent for food products is also recommended.

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