CHARACTERIZATION AND SAFETY ASSESSMENT OF PECTIN EXTRACTED FROM SABA (*MUSA PARADISIACA LINN.*) PEEL USING MICROWAVE ASSISTED EXTRACTION

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ABSTRACT

Banana is one of the most important fruit crop grown in the Philippines in terms of production due to its high demand, nutritive value and affordability. Its fruit can be consumed in variety of forms and processed into chips, jams, jellies, powder and flour, which results to adverse waste generation that pollutes the environment. Thus, this study aimed to utilize its peel in the aspect of food application, specifically in extracting pectin, an ingredient used as thickener, stabilizer, emulsifier and gelling agent in foods. Pectin from saba peel was extracted using microwave assisted extraction (3 pH, 700 W, 128 sec). The extracted pectin had undergone characterization and based from the results, it has 6.08% ash, 1315.79 equivalent weight and 8.06% methoxyl content indicating that it has a good ability ingel forming, jelly-forming and water dispersability, respectively. Its moisture content (5.07%) was significantly low, thus, its quality is protected due to inhibition of microbial growth and pectinase enzyme production. The degree of esterification of extracted pectin (77.38%) has shown that it is a high-ester pectin and is classified rapid-set. However, the pectin has low purity, with possible presence of protein, starch and sugars, as the value of total anhydrouronic acid (59.14%). In terms of safety assessment, the lead was not detected while the standard plate count and yeast and molds were found to be negative.

Keywords: *Saba Peel, Microwave Assisted Extraction, Pectin*

INTRODUCTION

In the Philippines, one of the most important fruit crop grown in terms of production is banana. Farmers are encouraged to continue banana production due to the high demand of Filipinos as its nutritive value and affordability is more accessible compared to mango and pineapple (Bathan & Lantican, 2010). Production rate of banana for January to March 2017 increased by 2.6% from 2.05 million metric tons in 2016 to 2.10 million metric tons this year. Distribution of production was shared by different regions such as Davao with 36.9%, Northern Mindanao with 24.5%, SOCCSKSARGEN with 12.9 % and others with 25.7% (Philippine Statistics Authority, 2017). Saba, lakatan, latundan, bangulan and cavendish are the most common cultivars in the country (Department of Agriculture, 2010). According to Philippine Statistics Authority (2017), saba ranked third in January to March production by variety with 9.0%. Moreover, its average farm price in the year 2015 was 8.47 pesos per kilogram (Philippine Statistics Authority, 2016).

Banana fruit has been widely utilized in different forms. The ripe ones could be eaten as raw, mashed and incorporated into ice cream, bread, muffins and cream pies, or could be sliced and served in fruit cups and salads, sandwiches, custards and gelatins. Furthermore, it could be processed into chips, jams, jellies, powder and flour (Abiodun-Solanke & Falade, 2010). Concurrent with the vast application of banana in food, large amount of waste is generated due to the discarded peel, which are 18-33% of the whole fruit. Banana peel is already considered a typical waste after consumption, when disposed by banana crisp industries (Toh, Leong, Chang, Khoo & Yim, 2016), or after banana fruit juice and puree processing (Swamy & Muthukumarappan, 2017). According to Ali, Ubhrani, Tagotra & Ahire (2014), for every 10 tons of bananas, approximately 1 ton of waste is produced, contributing to disposal problems requiring waste treatment to most nations.

With the interest to address environmental problems brought by banana peels, researches have been focused on investigating its compositional matrix and potent applications. According to Mohapatra, Mishra & Sutar (2010), banana peel contains 3% starch, 6-9% crude protein, 3.8-11% crude fat, 43.2-49.7% total dietary fiber, polyunsaturated fatty acids (linoleic acid and a-linolenic acid), pectin, essential amino acids (leucine, valine, phenylalanine and threonine), and micronutrients (potassium, phosphorus, calcium and magnesium). Existing applications include incorporation in livestock feeds (Wadhwa & Bakshi, 2013), bio-ethanol production (Itelima, Onwuliri, Onwuliri, Onyimba, & Oforji, 2013), biogas production (Mohapatra, *et al.,* 2010), antioxidant (Toh, *et al.,* 2016) and antifungal agents (Fugaban-Hizon, 2016), production of valuable fermented products and fertilizer (Patel, H., Patel, A., Surati & Shah, 2010). Despite of growing studies utilizing banana peel, limited local studies have been employed to test its potentiality in the aspect of food applications.

According to the plant authentication conducted by Bureau of Plant Industry, saba was scientifically identified as *Musa paradisiaca Linn.*, a cultivar used in cooking. The fruits are large and angular, and mature at 150 to 180 days. Its pulp is white, generally starchy but sweet when ripe. Each hand has 12 to 20 fingers with 10 to 16 hands per bunch. Saba is also a popular domestic cultivar which is most widely processed into chips or crackers. Its peel

contributes a significant amount of waste as it accounts to 40% of the total weight of fresh banana, leaving them as a solid waste at large expense and are not being used for any other purposes (Castillo-Israel, *et al.,* 2015). Despite the serious threat it implies in the environment, fruit peels, according to Castillo-Israel, *et al.* (2015), contain some valuable compounds like pectin.

Pectin is a family of complex variable polysaccharides extracted from the primary cell wall of higher plants (Canteri-Schemin, Fertonani, Waszczynskyj & Wosiacki, 2005), and its amount, structure and chemical composition differs between plants (Srivastava & Malviya, 2011). According to Nasseri, Thibault and Ralet (2008), most common structural elements in pectin include homogalacturonan, xylogalacturonan, rhamnogalacturonan I backbone, rhamnogalacturonan II, arabinan, arabinogalactan I, and arabinogalactan II. In terms of food application, it has been widely used as gelling agent, thickener, emulsifier and stabilizer (Kamble, Gawande & Patil, 2017), with specific incorporation in jams and jellies, fruit preparations, fruit drink concentrates, fruit juice, desserts and fermented dairy products (Canteri-Schemin, *et al.,* 2005).

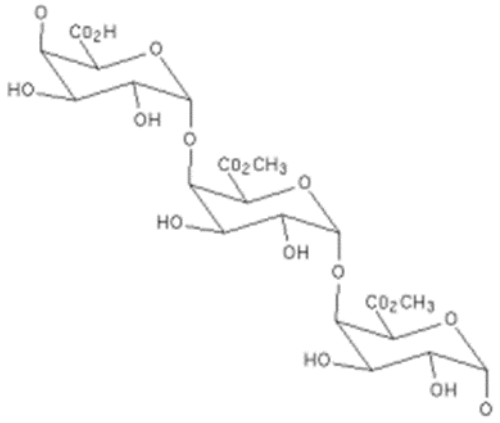


Figure 1. Structure of Pectin (Sharma, Naresh, Dhuldhoya, Merchant & Merchant, 2006)

Apart from its known function as a food ingredient, pectin has a spectrum of benefits in the aspect of health. According to Sundar Raj, Rubila, Jayabalan and Ranganathan (2012), pectin has applications in pharmaceutical industry, specifically its influence in cholesterol levels, diarrheal diseases, action against poisoning and antimicrobial activity. Based on their study, it has an effective role in the reduction of blood cholesterol, treatment of diarrheal diseases, elimination of lead and mercury from the gastrointestinal tract and respiratory organs, and light antimicrobial action towards Escherichia coli under certain in-vitro conditions. Furthermore, it has anti-cancer (Venzon, *et al.,* 2015) and antioxidant activity (Zaidel, *et al.,* 2017; Smirnov, *et al.,* 2017), and exhibits potential treatment against obesity, diabetes, and vesicle calculus (Venzon, *et al.,* 2015).

The worldwide annual pectin consumption in 2002 was approximately 45 Mt, with a global market value of at least 400 million Euros (Gama, De Farias Silva, Oliveira Da Silva & Abud, 2015). Even in the recent years, its demand has been continuously growing due to its wide range of application, especially in the field of food industry. According to Ciriminna, Fidalgo, Delisi, Ilharco and Pagliaro (2016), the food industry's demand for low-calorie and low-fat food products from consumers has resulted in increased demand for pectin from food manufacturers. Moreover, conventional pectin extraction factories are generally expensive, and require a close, large-scale source of raw material, namely dried citrus peel or apple pomace (Ciriminna, *et al.,* 2016). Thus, expansion of pectin sources and employment of alternative method for its extraction should be done to cope up with its pertinent issues.

The most widely used method for pectin extraction is boiling in acidified water, complemented by coagulation with ethanol. However, this conventional method consumes a lot of time and energy (Kute, Mohapatra, Babu & Sawant, 2015). Therefore, microwave assisted extraction (MAE) method, a reliable alternative to conventional extraction techniques, is employed. The principle of MAE is basically the use of microwave energy to penetrate the molecules of the material directly, or the energy transfer system. Specifically, it utilizes the microwave radiation to partition target components into the solvent (Rahmati, Abdullah, Momeny & Kang, 2015). It has shown potential in terms of saving time, energy and solvent consumption (Kute, *et al.,* 2015). This method has been used in extracting pectin from dragon fruit peel (Rahmati, *et al.,* 2015), mango peel (Rojas, *et al.,* 2015), jackfruit rind (Koh, Leong & Noranizan, 2014), and Balinese orange peel (Megawati, Widiastuti, Jannah & Rahayuningtiyas, 2015). According to studiesby Liu, Shi, & Langrish (2006), and Zhongdong, Guohua, Yunchang, & Kennedy (2006), a destructive change in the plant tissue can be observed in fresh orange peels when subjected to MAE. This leads to a significant increase in the yield of extractable pectin, proving that MAE can absorb microwave energy on different areas or components of a sample containing a non-homogenous structural characteristic. Due to this, MAE is currently used on several plant extractions for its effectiveness and promising results, however, very few are yet to be used in industrial-scale utilizations.

In the present study, saba peel was utilized as a source of pectin. This study aims to extract pectin from the saba peel using microwave assisted extraction, to characterize the pectin yield from the samples through determining its ash content, moisture content, equivalent weight, methoxyl content, total anhydrouronic acid content and degree of esterification, and to conduct safety assessment by determining its lead content, standard plate count and yeast and molds. Subsequently, this study will help in waste utilization to significantly decrease ecological problems, expand local sources of pectin for food industrial applications and will also contribute knowledge about potent functional uses of fruit wastes, specifically banana peel.

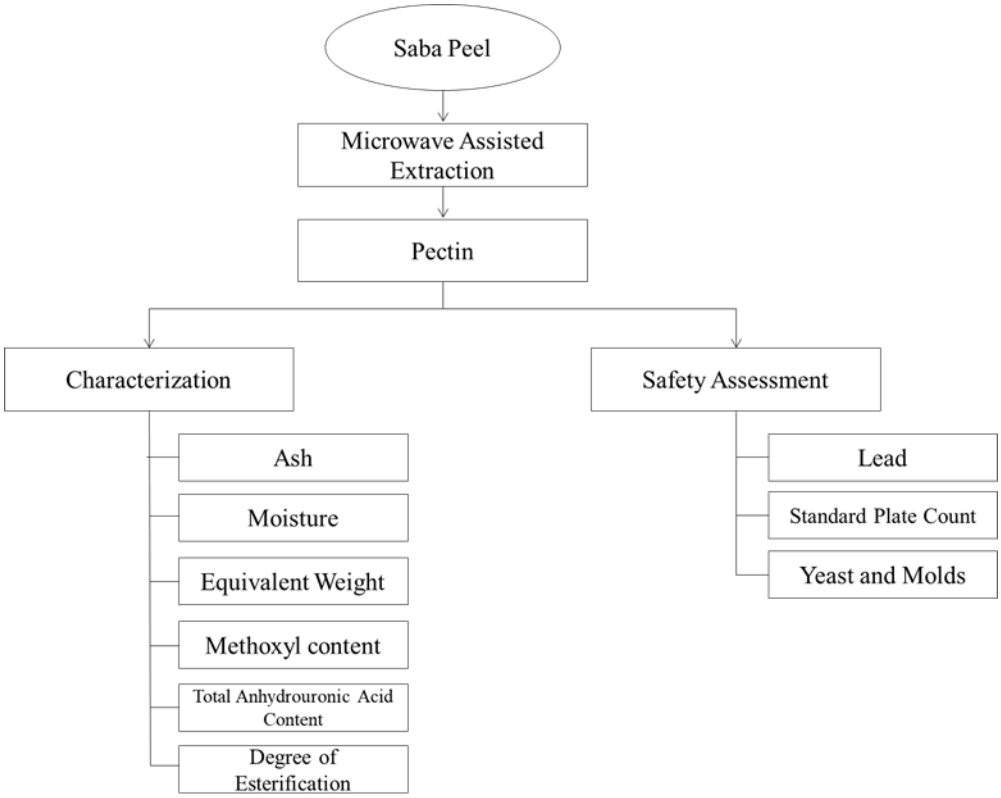


Figure 2. Research Design

In overview, the research design of the study involves the extraction of pectin from the Saba (*Musa paradisiaca Linn.)* peel using Microwave Assisted Extraction. The resulting powder was characterized in terms of Ash, Moisture, Equivalent Weight, Methoxyl Content, Total Anhydrouronic Acid content, and Degree of Esterification. In addition, a safety assessment was conducted for the following: Lead, Standard Plate Count, and Yeast and Molds. The methods were mainly adapted from Swamy, *et. al.* (2017) and Kamble, *et. al.* (2017).

METHOD

Sample Preparation

Ripe Saba peel was collected from Quezon City, Philippines. Preparation of ripe banana peel powder was done following the procedures of Swamy, *et. al.* (2017) with some modifications. The ripe banana skins were cut into fine slices (1 mm). They were washed in flowing water and blanched for 2 min. in 90°C water. The blanched peels were dried in a TD-009 cabinet dryer at a temperature of 60°C until constant weight was obtained. Then, the dried ripe banana peel was powdered using a high speed VFS-DAS06 Fitzmill with 0.5 mm screen mesh and was stored in an airtight container before conducting the experiments.

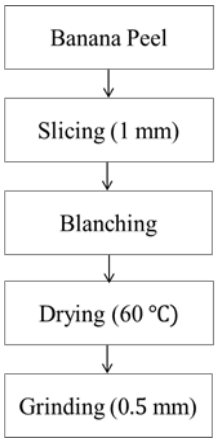


Figure 3. Schematic diagram for sample preparation

Figure 4. *Musa paradisiaca Linn***.** Peel

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

Figure 5. Sample preparation. (a) cutting into fine slices (b) blanching (c) drying (d) milling (e) banana peel powder

Microwave Assisted Extraction of Pectin

Extraction of pectin was conducted following the optimized procedures of Swamy, *et. al.* (2017) with some modifications. 80g of powdered ripe banana peels were added to a 1000 ml beaker containing 800 cc distilled water. The distilled water was adjusted to pH 3 using 0.90g of citric acid for the dispersion and absorption of pectin powder and effective penetration of energy transfer system of the microwave to its molecules (Nehru & Sanjeeviraja, 2013). Then, the beaker was placed at the middle of the rotating table with microwave power level at 700 W for 128 sec. After the extraction process, the beaker was cooled to room temperature and was filtered, discarding the residue, following the procedure of Kamble, *et. al.* (2017). Afterwards, the solution was filtered through an ordinary two layer-cheesecloth. The filtrate was washed thrice with 95% (v/v) ethanol, leaving the coagulated mass of pectin. Afterwards, the sample was dried in the Memmert hot air oven at 65°C until a constant weight was obtained. The pectin yield was calculated as:

$$\text{Yield (\%)} = \frac{\text{Weight of dry sample (g)}}{\text{Weight of initial sample (g)}} \text{ x } 100$$

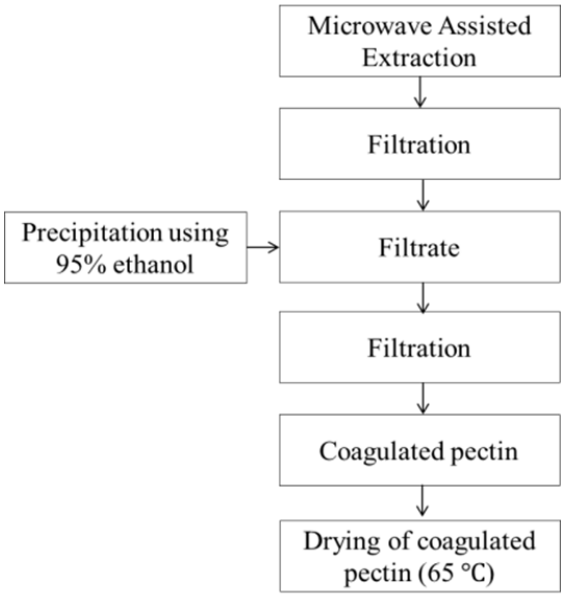
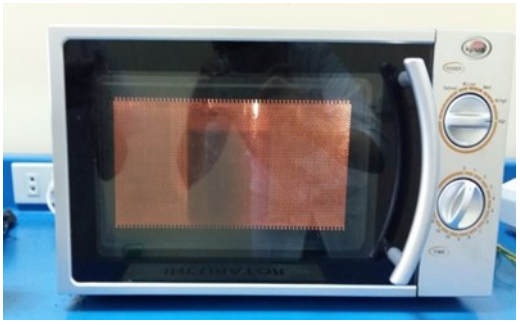


Figure 6. Schematic diagram for pectin extraction



(a)

(c) (d)

(e) (f)

Figure 7. Pectin extraction. (a) microwave extraction (b) solution after extraction (c) filtration of solution (d) coagulated pectin (e) drying of coagulated pectin

Figure 8. Dried pectin

Pectin Characterization

The extracted pectin from saba peel was characterized in terms of ash content, moisture content, equivalent weight, methoxyl content, total anhydrouronic acid content and degree of esterification.

Ash Content

Ash content was determined using gravimetric method according to the procedure of AOAC (2005). Ash content was calculated as (Kamble, *et al.,* 2017):

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \text{ x } 100$$

Moisture Content

A 3 g of sample was placed in KERN DBS moisture analyzer. Moisture content testing was done thrice and the average was taken based from the data obtained.

Equivalent Weight

A 0.5 g of sample was placed in a 250 ml conical flask and 5 ml of ethanol was added. Then, 1 g of sodium chloride and 100 ml of distilled water were further added. Finally, 6 drops of phenol red were added and was titrated against 0.1 N NaOH. Titration point was indicated by purple color. This neutralized solution was stored for determination of methoxyl content & anhydrouronic acid content (Kamble, *et al.,* 2017).

$$\text{Equivalent weight} = \frac{\text{Weight of sample x 1000}}{\text{ml of alkali x Normality of alkali}}$$

Methoxyl Content

The neutral solution was collected from determination of equivalent weight, and 25 ml of 0.25 N NaOH was added. The mixed solution was stirred thoroughly and was kept at room temperature for 30 min. Afterwards, 25 ml of 0.25N HCl was further added and was titrated against 0.1N NaOH (Kamble, *et al.,* 2017). Methoxyl content was calculated as:

$$\text{Methoxyl content (\%)} = \frac{\text{ml of alkali x Normality of alkali x 3.1}}{\text{Weight of sample}}$$

Total Anhydrouronic Acid Content

The total AUA of pectin was determined by the formula (Kamble, *et al.,* 2017):

$$\text{AUA (\%)} = \frac{176 \text{ x } 0.1z \text{ x } 100}{w \text{ x } 1000} + \frac{176 \text{ x } 0.1y \text{ x } 100}{w \text{ x } 1000}$$

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 (DE)

The DE of pectin was measured on the basis methoxyl and AUA content, and was calculated as (Kamble, *et al.,* 2017):

$$\text{Degree of esterification (\%)} = \frac{176 \text{ x \%MeO}}{31 \text{ x \% AUA}} \text{ x } 100$$

Safety Assessment

Lead Content

Determination of lead content was done by Inductively Coupled Plasma – OES following the procedures of A.O.A.C. (2005).

Standard Plate Count

Detection of standard plate count was done by petrifilm based from the procedures of A.O.A.C. (2005), Bacteriological Analytical Manual On-Line (2001) and Compendium of Methods for the Microbiological Examination of Foods (2001).

Yeast and Mold Count

Detection of yeast and molds was done by petrifilm based from the procedures of A.O.A.C. (2005), Bacteriological Analytical Manual On-Line (2001) and Compendium of Methods for the Microbiological Examination of Foods (2001).

RESULTS AND DISCUSSION

Pectin Yield

Microwave assisted extraction was the method employed in extracting pectin from saba peel. The optimized procedure of Swamy, *et. al.* (2017) led to higher pectin yield compared to the previous studies that used conventional method. Moreover, microwave assisted extraction overcomes laborious time since conventional method requires an hour or more, compared to a few minutes of pectin extraction using MAE.

Table 1. Pectin yield of Ripe Saba Banana Peels

Weight of Sample Before Drying (g)	Weight of Sample After Drying (g)	Pectin Yield (%)
1792.2	459.4	25.63%

Characterization

The extracted pectin from *Musa paradisiaca Linn.* peels were characterized and summarized in the table below:

Table 2. Pectin Characteristics of Ripe Saba Banana Peels

Characteristics	Results	Standard
Ash (%)	6.08	<10 (Azad, Ali, Akter, Rahman & Ahmed, 2014)
Moisture content (%)	5.07	N/A
Equivalent Weight	1315.79	N/A
Methoxyl Content (%)	8.06	>8 (Castillo-Israel, *et al.,* 2015)
Total Anhydrouronic Acid Content (%)	59.14	>65 (Castillo-Israel, *et. al.*, 2015; Kamble, *et al.,* 2017).
Degree of Esterification (%)	77.38	High-ester: >50 Low-ester: <50 (Mohamed, 2016)

According to Azad, *et al.* (2014), ash content indicates a good criterion for gel formation of pectin, wherein the maximum limit is set at 10%. Based on the result, the ash content of the extracted pectin was 6.08g/100g, and following the formula by Kamble, *et. al.* (2017), it has 6.08% ash. Thus, the said pectin has a good gel forming characteristic as it is less than the maximum limit. It is universally accepted that the gel formation of pectin is highly dependent on the degree of methoxylation; the higher the methoxyl content, the lower ash content it needs to form a gel with good characteristics (Urias-Orona, *et al.,*2010).

Based from the result, the saba peel pectin has 5.07% moisture content, which is found to be comparable to the study of Kamble, *et. al.* (2017) and significantly lower compared to the study of Castillo-Israel, *et. al.* (2015). The moisture content of pectin extracted from unripe banana peel is in the range of 4% to 6% depending on the extraction time (Kamble, *et al.,* 2017), while those from ripe and unripe banana peel is found to be 10.00% and 14.13%, respectively (Castillo-Israel, *et al.,* 2015). High moisture content could enhance the growth of microorganisms and production of pectinase enzymes that can further affect the pectin quality (Azad, *et al.,* 2014), thus, this should be low for safe storage and prevention of quality degradation.

Equivalent weight of pectin is another indicator of its jelly-forming ability, with high molecular pectin having better ability (Castillo-Israel, *et al.,* 2015). The computed equivalent weight of pectin is 1315.79, higher than the ripe banana peel pectin which is at 953.89 but lower than the unripe banana peel 1503.16 (Castillo-Israel, *et al.,* 2015). The extracted pectin has shown comparability with the study of Kamble, *et al.,* (2017) at 1 hour and 2 hour extraction time of unripe banana peel pectin which is 1315.78 and 1388.88, respectively.

The methoxyl content is an important factor in controlling the setting time and ability of pectin to form gel (Mohamed, 2016; Azad, *et al.,* 2014). Moreover, it influences the dispersability of pectin in water; higher methoxyl content makes the pectin readily dispersible in water than those with less than 7.0% methoxyl content (Castillo-Israel, *et al.,* 2015). According to Aina, *et al.* (2012), extracted pectin could have 0.2% to 12% methoxyl content depending on the source and mode of extraction. Based from the result, the methoxyl content from the extracted pectin is 8.06%, indicating that it has good water dispersability. This result is higher compared to the study of Castillo-Israel, *et al.,* (2015), wherein the ripe and unripe banana peels pectin was at 6.40% and 5.25%, respectively (Castillo-Israel, *et al.,* 2015). Moreover, the result is the same with the study of Kamble, *et al.,* (2017) at 3 hour extraction time of unripe banana peel.

The purity of the extracted pectin is indicated by AUA and its value should not be less than 65% (Castillo-Israel, *et. al.*, 2015; Kamble, *et al.,*2017). Based from the result, the extracted pectin only has 59.14% AUA.The low value of AUA means that the extracted pectin might not be sufficiently pure, with the possible presence of protein, starch and sugars in the precipitated pectin (Mohamed, 2016).

Degree of esterification determines methylated carboxyl group for gel setting (Sharma, *et al.*, 2006). Pectin is divided into two major groups: high-ester pectin (DE>50%) and low-ester pectin (DE<50%). A more rapid setting of gel is caused by a higher degree of esterification (Mohamed, 2016). Furthermore, according to Kamble, *et al.* (2017) and Castillo-Israel, *et. al.* (2015), pectin is classified as rapid-set (DE>72%) and slow-set (DE 58-65%). The extracted pectin has 77.38% DE, which means it is a high-ester pectin and is classified rapid-set.

Safety Assessment

Table 4. Safety Assessment of Pectin from Ripe Saba Banana Peels

Parameters	Results	Standard
Lead, ppm (By Inductively Coupled Plasma – OES)	ND	5 mg/kg (Food Chemicals Codex, 2004)
Standard Plate Count, CFU/g (By Petrifilm)	<10	N/A
Yeast and Mold Count, CFU/g (By Petrifilm)	<10	N/A

*count of <10 CFU/g is synonymous to negative detection at 10^1 dilution factors

Lead is a soft metal that can be contained particularly on foods specifically fruits which primary causes brain damages, heart damages and mental retardation commonly for children (Tiwari, Seema & Tripathi, Indra & Tiwari, Hl., 2013). Based from the result, the lead content of pectin from saba peel was not detected, thus, it follows the standards according to Food Chemicals Codex (2004), wherein lead content of pectin should not be more than 5 mg/kg.

Factors affecting microbial growth consist of bacteria, yeast, molds, intrinsic (moisture content, nutrient content, biological structure and redox potential) and extrinsic factors (Moral, U., Nagar, P., Maan, S., and Kaur, K., 2017). Pectin from saba peel was found to be negative from standard plate count at 10^1 dilution factors. Additionally, various studies have been performed on the different parts of banana, including the peels, demonstrating the inhibitory effect against pathogenic bacteria. Hence, aside from the hygienic process done to produce pectin from sample collection, preparation, drying and extraction, the banana peels have been considered to exhibit antimicrobial and antioxidant agent (Fagbemi, Ugoji, Adenipekun, & Adelowaotan, 2009). This outcome suggests that aerobic bacterial growth was hindered as a result of having low moisture, as they thrive in high moisture environments.

Yeast and molds were also found to be negative from the sample at 10^1 dilution factors. According to Sumathy, N. H. and Sumathy, J. H. (2011) banana peels contain fatty acids that add up its antimicrobial activity against yeast and molds. In addition, yeast was prevented due to having low moisture content in the extracted pectin. Some molds, however, are prevalent in fruits and dried products, especially those having high sugar/low water activity/low pH products. But due to the results on the yeast and mold count test, it can be shown that these were also prevented.

CONCLUSION

Microwave assisted extraction shows potential in extracting pectin from saba (*Musa paradisiaca Linn*.) peel, having 25.63% yield. However, due to low purity, the actual amount of pectin may be significantly lower which may need further tests. Despite that, it has shown to be compliant with the standard safety requirements. Additionally, based on the results from the other tests, with the current parameters, the saba peel could be a potent raw material to extract good quality pectin for use in food applications. It could also be a solution to the ever-growing ecological problems brought upon by several food wastes, thus opening up several other possibilities for saba in many other different applications.

RECOMMENDATIONS

The study was focused on the use of MAE and its effectivity in extracting pectin. However, in order to clearly prove that MAE poses the optimum yield, a study involving the use of the conventional method must be done side-by-side with MAE while following similar parameters and conditions as much as possible. Conclusively, the extracted pectin was of low purity (based on the Total Anhydrouronic Acid content) hinting at the presence of several other compounds (which may be protein, starch, and fibre). As such, it is also recommended to identify them, as well as quantify how much of them contribute to the extracted powder. With the identification of the remaining compounds, the claim on the gel-forming capability of the pectin will be further clarified, thus, opening up more opportunities for utilization and improvement in the future studies.

In addition, it can be suggested to conduct a test for water activity, which will further strengthen the claim on the safety assessment tests, and will prove that there is very little chance of microbial growth in the product.

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