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Waste to wealth: optimizing novel pectin acid extraction from honeydew (*cucumis melo l. var. inodorous*) peels as a potential halal food thickener

Abstract

Pectin is found to be widely used as additives in food, pharmaceutical and cosmetic industries. This study was carried out using a conventional acid extraction method. The influences of different acid reagents used (citric acid and nitric acid), extraction time (30, 45 and 60 min) and temperature (60, 70 and 80°C) towards the extraction yield and its degree of esterification (DE) of pectin from *Cucumis melo L. var. inodorous* peels being investigated. Citric acid gives the maximum yields of pectin (35.26%) under optimum condition at 80°C and 60 min. Extraction time and temperature were significantly ($p>0.05$) affected the amount of pectin yields, whereby the optimum condition for both acids was at 80°C and 60 min. The degree of esterification (DE) of the isolated pectin in this study was 50.38 % and 59.88 % respectively for citric acid and nitric acid where this pectin was considered as high methoxyl pectin (HMP). From the color analysis, the value for 'L', 'a' and 'b' for citric and nitric acid were significantly different ($p>0.05$) between each other. Citric acid has a lighter color of pectin extract which is preferable to be commercialized. Pectin from honeydew waste is a novel food ingredient in Malaysia and could be as an excellent alternative for replacing gelatin in food industry applications and other non-food potential interventions.

Keywords: pectin, cucumis melo l var inodorous peels, acid extraction, characterization

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Introduction

Production of honeydew was recorded to increase significantly from 2012 until 2016 due to the demand for this fruit among Malaysians. According to statistic given by Department of Agricultural Malaysia the production of melons in Malaysia was 17,398.2 metric tons.[1] This situation has led to an increase of honeydew peels as byproducts and might endanger the environment since the utilization of this byproduct has been neglected. Nowadays, there are many studies regarding the uses of wastes to extract beneficial substances such as pectin. Therefore, the fourth industrial revolution should take this opportunity to make a full utilization of this waste since it is economical and reduce the pollutants. Besides, the commercialization of the pectin has well known globally since the demands of the pectin are increasing in food, pharmaceutical and cosmetic industries.[2]

Moreover, issues that arise regarding the uses of gelatin in the industry had urged this study to be carried out. Once, gelatin had been an issue among the Muslims since it is mostly originated from porcine and bovine sources. Both animal sources are used because they provide the best quality of gelatin compared to other animal sources.[3] Although bovine is not prohibited (*haram*) in Islam, there is an issue either the bovine was properly slaughter according to shariah compliance or not which then become a doubtful (*syubhah*) for Muslim. Gelatin obtained from pig derivatives after undergoing *istihalah* (transformation) was also found to be prohibited since the characteristics of gelatin remain unchanged chemically after the transformation process has been carried out.[4] Islam forbade it followers to consume *haram* and s*yubhah* foods. It was stated in Al-Quran:

Forbidden to you (for food) are dead meat, blood, the flesh of swine, and that on which hath been invoked the name of other than Allah. That which hath been killed by strangling, or by a violent blow, or by a headlong fall, or by being gored to death; that which hath been (partly) eaten by a wild animal; unless ye are able to slaughter it (in due form); that which is sacrificed on stone (altars); (forbidden) also is the division (of meat) by raffling with arrows: that is impiety (Al-Maidah:3).

Hence, the purpose of this study was to extract and characterize the pectin from honeydew melon peels using conventional hot acid extraction method. Different acid reagents have been used to extract the pectin and two different parameters which are time and temperature were manipulated throughout this experiment. Specifically, this research is aimed to examine the influence of nitric acid and citric acid on pectin extraction yield, to optimize the parameters such as temperature and time on pectin yield and to characterize pectin based on their degree of esterification.

Material and methods

Materials

The starting materials, honeydew melons variety *Cucumis melo L.* were obtained from Giant Superstore at Nilai, Negeri Sembilan. The melons were washed with clean tap water to remove dirt and dust. The process was continued by peeling off the honeydew about 0.3 cm width. Only the peels and rinds parts were included in this study. The peels sample were cut into small pieces (about 1.5 x 2 cm) to increase the surface area and cleanly washed with distilled water for impurities,

immanent dirt and dust removal. The samples then were blanched in boiling water for 7 min to inactivate the enzyme and filtered through muslin cloth with the aid of hand pressure to remove excess water. The blanched peels then were dehydrated at 60°C for 24 hours and grinded into fine powder. The size of the powders was controlled by using a 44-micron (325 Mesh) sieve.

The alcohol insoluble solid (AIS) were prepared according to the methods of Koh et al.[5] The sample powder was treated with 80 % ethanol at 80°C for 45 min with the ratio of 1:4 (Sample powder: ethanol, w/v). After that, the sample was washed with 75 ml of 60 % ethanol for three times, followed by 75 ml of 95% ethanol for one time to remove the impurities, pigment and free sugar. The treated honeydew peels powder was dried in the dehydrator at 55°C for 24 hours.

Extraction of pectin

Extraction of pectin in this study was conducted by using conventional acid extraction method. The procedure for extraction of pectin was based on method by Liew,[6] with several modifications. A 10 g of honeydew peels powder was weighed and put into a 400 ml beaker, followed by the addition of 300 ml distilled water. Then, acid reagent (either citric acid or nitric acid) was added into the solution for maintaining different pH medium. To maintain the pH of the media which is 1.5, a volume of 1.6 ml of 65 % nitric acid were used. Similarly, to maintain the following pH for the media, an amount of 90 g of 99.9 % citric acid was used respectively. Hot water bath procedure was used in this experiment where the mixture of different pH media was heated at 60, 70 and 80°C at three different times 30, 45 and 60 min. After that, the hot acid extract was filtered through muslin cloth. Extraction was carried out with pH 1.5 media for each acid at three different ranges of temperatures and times. Filtrate then was cooled down at room temperature and being collected separately for further step.

Purification and centrifugation

Pectin extract was coagulated by mixing it with 95 % of ethanol in 150 ml beaker using an equal value of 1:1 ratio (v/v) at 4°C and then leave for three hours. The solution then was centrifuged using 50 ml multipurpose centrifuge comb 1514R at 6000 rpm for 10 min. The precipitate pectin (ethanol-insoluble fraction) formed after the centrifugation process was being washed with 70 % ethanol and filtered by using Buchner funnel. Then, the washed pectin was dried overnight using dehydrator (Excalibur Food Dehydrator 3000) at 40°C and grinded into powder using dry blender (Xtreme Hi-Power Blenders) at 45,000 rpm. The percentage yield of the pectin extract was calculated using the given Equation 1.

$$Y\ pectin(\%) = amount\ of\ extracted\ pectin(g) / initial\ amount\ of\ honeydew\ peels(g) x100 \quad (1)$$

Where, Y pectin (%) is the percentage of extracted pectin yield

Characterization of pectin and determination of equivalent weight

Characterization of pectin was carried out using the treatment of 80°C, 60 min of the extraction process and been carried out through titration process. These specific combination treatments had been chosen because of higher pectin yield was obtained from these parameters. The pectin was characterized to examine its suitability and functionality as an additive in food and other industries. The sample of dried pectin underwent the quantitative test to identify the following characteristic by adapting Rasidek et al[7] method.

Equivalent weight was used to calculate the anhydrouronic acid and degree of esterification which was determined by the titration with sodium hydroxide (NaOH). An amount of 0.5 g of pectin sample was weighed in 500 ml conical flask and 5 ml ethanol was added. To sharpen the endpoint, 1 g of sodium chloride was added to the solution followed by 100 ml of distilled water. Finally, six drops of phenolphthalein was added as an indicator and being titrated against 0.1 M of NaOH. Determination of equivalent weight was calculated using this formula Equation 2.

$$Equivalent\ wt = wt\ of\ sample(g) / (ml)\ alkali\ x\ normality\ of\ alkali \quad (2)$$

Determination of methoxyl content and total anhydrouronic acid content (AUA)

Methoxyl content is a crucial factor in controlling the setting time of pectin. It's sensitivity to polyvalent cations and its usefulness in the preparation of low solid gels, fibers & film. It was determined by using the neutralized solution obtained from the equivalent weight determination which then mixed 25 ml of 0.25M of NaOH. The mixture then was stirred using a stirring rod and kept for 30 minutes at room temperature. After 30 minutes, 25 ml of 0.25M hydrochloric acid (HCl) was added to the mix solution and titrated with 0.1M of NaOH. The percentage of methoxyl content was calculated using the following equation 3.

$$methoxyl\ content\ \% = ml\ of\ alkali\ x\ normality\ of\ alkali\ x\ 31 / wt\ of\ sample\ x\ 1000\ x\ 100 \quad (3)$$

Where, 31 is the molecular weight of methoxyl

Anhydrouronic acid content is essential for the determining purity and degree of esterification, as well as to evaluate the physical properties of the pectin substance. By using the value obtain from the titration value of NaOH in equivalent weight and methoxyl content determination, total anhydrouronic acid content was determined by using the below Equation 4.

$$AUA\ \% = 176\ x\ 0.1Z\ x\ 100 / wt\ of\ sample\ x\ 1000 + 176\ x\ 0.1y\ x\ 100 / wt\ of\ sample\ x\ 1000 \quad (4)$$

Where,

176g=molecular unit for AUA (1 unit)

Z=titration volume (ml) of NaOH from the determination of equivalent weight

y=titration volume (ml) of NaOH from the determination of methoxyl content

Determination of degree of esterification(DE)

The percentage of DE was determined by the value obtained from the percentage of methoxyl content and total anhydrouronic acid content and calculated using the following equation 5.

$$\%DE = 176\ x\%\ of\ methoxyl\ content / 31\ x\ \%\ Anhydrouronic\ Acid\ content\ x\ 100 \quad (5)$$

Color analysis

Color of pectin gel was analyzed using hunter lab colorimeter (Hunter Lab, USA). The results were reported as values of L (lightness), a (redness or greenness), and b (yellowness or blueness).

Statistical analysis

All statistical analysis in this study was performed by using Minitab 17 and was analyzed as one-way Analysis of Variance (ANOVA). A part from that means was compared by using Fisher's least significant difference (LSD). All the significances were determined at 5% level of confidence.

Results and discussion

Effect of citric acid and nitric acid on extraction time and temperature of pectin yield

The percentage yield extracted using citric acid at temperature 60°C at 30, 45 and 60 min were 23.88 %, 26.55 % and 28.08 % respectively. At the temperature of 70°C, the percentage yield at time 30, 45 and 60 minutes are 29.51 %, 31.32 % and 32.58 % respectively. Likewise, at temperature of 80°C for 30, 45 and 60 min were 30.74 %, 34.28 % and 35.26 % respectively. At the temperature of 60 to 70°C, Figure 1 had shown that they were gradually increased in pectin yield as the temperature increase for each range of extraction time. However, there was less difference on the yield at 80°C by the effect of time and temperature for range time 30 to 60 min of extraction. Maximum yield was obtained at this treatment which was at 80°C, 60 min.

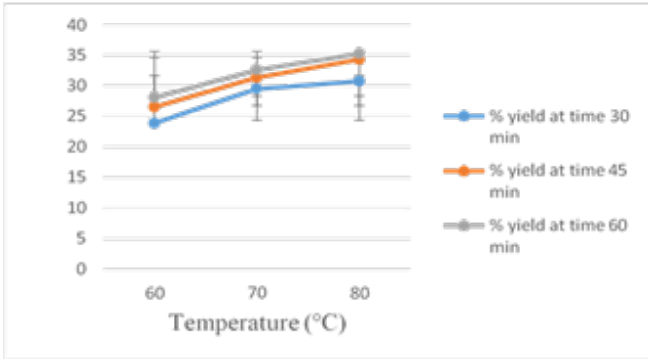

Figure 1 Effect of citric acid on time and temperature of pectin yield.

Figure 1 show that the yields of pectin were increased significantly with the increase of temperature from 60 to 80°C using citric acid. In terms of time, the yields increase gradually as the extraction time increase from 30 to 45 min. However, there was less difference in yield of the pectin as the time range increased from 45 to 60 min at each extraction temperature. From the results, it can be concluded that the pectin has reached its limit as the temperature increased to an extreme where there was less effect on pectin yield due to thermal degradation of the extracted pectin.

For the extraction using nitric acid, highest yield was obtained at temperature of 80°C and extraction for 60 minutes (Figure 2). Therefore, only this combination of treatment was used for further characterization and analysis. Highest pectin yield (35.26 %) was obtained from honeydew peel powder extracted using citric acid at temperature 80°C for 60 min of extraction time. From this study, citric acid was found to be the best for pectin extraction compared to nitric acid. This finding was supported by Devi et al.[8] who had compared the pectin yields extracted from sweet lemon (Mosambi) peels using two different types of acids (citric acid and nitric acid). Acid types strongly affect the gelling properties and macromolecular of isolated pectin. Using citric acid as an extractant gives the least amount of pectin decomposed through the alteration of physicochemical properties where it provides the best gelling properties of pectin isolate.[9]

The amount of pectin yields extracted from honeydew melon increased significantly with the increasing of extraction temperature where 80°C was observed to gives maximum yields of the pectin. As shown in the Figures 1 & 2, there are high amount of pectin yield been extracted between the temperature of 60 to 70°C for each time. Girma and Worku[10] postulated that, increasing the extraction temperature led to increase in solubility of the extracted pectin in the acid which promoted in higher rate of extraction. However, it can be observed that decrease in the tendency to isolate the pectin occurred when further increase of temperature (from 70 to 80°C) had been subjected during the extraction process. Pectin is linked by α-(1, 4) glycosidic bonds of galacturonic acid and where it is an ether bond that could hydrolyzed from high polymer to low polymer of pectin.

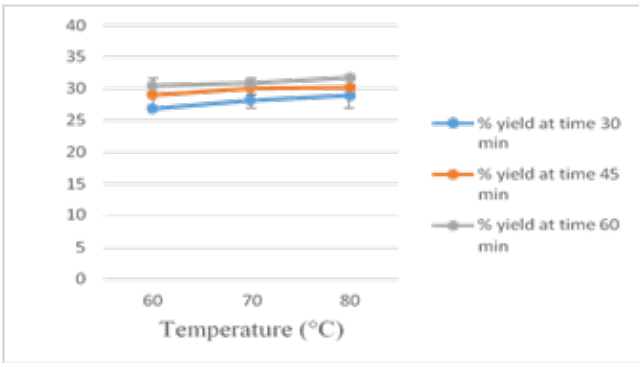

Figure 2 Effect of nitric acid on time and temperature of pectin yield.

Extreme in high temperature prompted the breakdown of the pectin configuration, which resulting in pectin with lower molecular size. Thus, this condition leads to increase solubility of the pectin in water which is imperceptible as a solid compound after the addition of ethanol. From the figures presented above, the time of extraction also significantly affected the pectin yield for both citric and nitric acid. From this study, maximum yield of the pectin was obtained at 60 min of extraction time. Pectin is a carbohydrate polymer where it needs time to soften its structure to isolate the pectin from its source.[11] At the beginning of the extraction process, the yields increase with time owing to longer time promotes more reaction time for the pectin to be isolated. Nevertheless, as the extraction time reached the peak of 60 min, the pectin was observed to be decreased in its yield. Prolonged heating of the pectin caused the thermal degradation effect on the extracted pectin. This thermal degradation predominately caused by the formation of reducing groups as well as beta-elimination[12] where the galacturonan chain of the pectin was being depolymerized into monomer. Besides, ester linkage of the pectin is removed through de-esterification process, thus the pectin became pectic acid. Therefore, less amount of pectin was perceptible by the precipitation of alcohol.

Characterization and determination of equivalent weight

The equivalent weight of pectin extracted from honeydew peels was found to be significantly difference ($p>0.05$) between both citric and nitric acid by referring to Table 1. It could be claimed that nitric acid was analyzed to have higher equivalent weight compared to citric acid. Similar results were obtained by Devi et al.[8] in the study on pectin extracted from sweet lemon. According to Devi et al.[8] the equivalent weight of citric and nitric acid was found to be 312.5 and 833.33 respectively. In similar veins, Ramli and Asmawati[13] claimed that, the equivalent weight of the pectin varies in a broad range of number since it is depending on the method and nature of the fruit used

during the extraction. Pectin with high equivalent weight could have a higher gel forming effect when being added to food or pharmaceutical product. Meanwhile, lower equivalent weight of pectin could cause the higher partial degradation of pectin. Differences in the value of equivalent weight were being affected by the amount of free acid presence in the pectin extract.

Table 1 Results for the characterization of pectin extracted with citric acid and nitric acid under optimum conditions (80°C and 60 minutes)

Characteristics	Citric acid	Nitric acid
Equivalent weight (g/mol)	562.43±20.33[b]	1317.5±39.80[a]
Methoxyl content (%)	5.61±0.40[a]	3.52±0.15[b]
Total anhydrouronic acid (%)	63.20±3.1[a]	33.33±0.85[b]
Degree of esterification (%)	50.38±1.36[b]	59.88±1.39[a]

[ab]Means that do not share a letter are significantly different.

Determination of methoxyl content and total anhydrouronic acid content (AUA)

Methoxyl content is a crucial factor that influence the setting time of pectin and indicator in determining the gel forming ability of the pectin.[14] Sugar binding capacity as well as spreading quality also determined by this characteristic whereby they were increase significantly with the increasing of methoxyl content.[12] Table 1 had shown that the percentage of methoxyl content for citric acid (5.82 %) and 3.79 % for nitric acid. The results were approximately like the methoxyl content in dragon fruit pectin (2.98 % to 4.34 %) but slightly lower than those as found in pumpkin peels (6.2 % to 7.23%) and grapefruit peels (8.875 % to 7.542 %). From the results, it was determined that the percentage of methoxyl content were significantly ($p>0.05$) affected by the type of pectin.

The highest amount of total anhydrouronic acid content (AUA) was found to be pectin extracted using citric acid (63.2 %). There were significantly different ($p>0.05$) in the percentage of AUA between both acid that been used during the extraction stage. Percentages of AUA obtained in this study do not accept for the minimum limits of pectin purity since the value must be greater than 65 %.[14] Lower value of AUA indicates that the isolated pectin might have high amount of protein content, starch and sugars that existed in the pectin precipitate.[15]

Determination of DE

The mechanism of gelation in pectin was dominated by their degree of esterification. From the data shown in Table 1, there is significant difference ($p>0.05$) between pectin extracted from both citric and nitric acid. This means that the value of DE was affected by the type of acid used for extraction of the pectin. In this work, nitric acid has a higher DE compared to citric acid and this result is supported in the literature by Devi et al.[8] where nitric acid has DE of 58.62 % when extracted from sweet lemon peel. Pectin can be classified into two categories depending on percentage of DE. Pectin within the percentage of DE 50 % and below are recognized as low methoxyl pectin (LMP), while pectin that has DE higher than 50 % are indicated as high methoxyl pectin (HMP).

Therefore, pectin that been isolated in this study was recorded to be categorized as HMP for both acids since the value of DE is more than 50 %. HMP can form gel with the presence of sugar and acid which it is called as sugar acid pectin gels. Immobilization of water (solvent) with sugar and acid in pectin molecules resulting in system resisting deformation where it causes stress-strain relationship for small deformation. Formation of junction zones in pectin structure helps to build up the 3-dimensional network where associated chain was stabilized by hydrogen bonding between dissociated carboxyl and secondary alcohol groups as well as hydrophobic interaction between methyl esters.[15]

Detection of color

The color of the pectin extracted from honeydew peels was measured using Hunter Lab Colorimeter. All the data were presented in Figure 3. Color of the pectin is a crucial factor in determining the appearance of its gel produced. It is varied depending on the source that been used to extract the pectin. The 'L' values for pectin extracted with citric acid and nitric acid observed in this study to be in the range of 25.03 to 29.34 and 28.50 to 30.63 respectively. 'L' value measures the level of brightness of the color where 0 to 50 indicates dark, while 50 to 100 indicates light. In this work, the color of pectin extracted by both acids are dark where the color was brownish in visual observation as shown in Figure 4. Lighter colors are much preferable for the pectin since it gives minimal effect on the appearance of final product. A part form that, the 'a' value varies from 3.65 to 4.27 for citric acid and 4.19 to 5.32 for nitric acid. When measuring color using hunter lab colorimeter, 'a' value gives result on red versus green color. Positive number of 'a' value indicates red and negative value indicates green. Pectin that being extracted from honeydew peels was identified to be positive value where the color intensity lies in red color. The 'b' values also vary for citric acid and nitric acid where it ranges from 24.45 to 25.27 and 23.60 to 24.73. The 'b' value measure the color intensity between yellow to blue color. Positive number of 'b' value indicates yellow while negative number indicates blue. The intensity color of pectin in this work was found to be positive value which means it lies in range of yellow color.

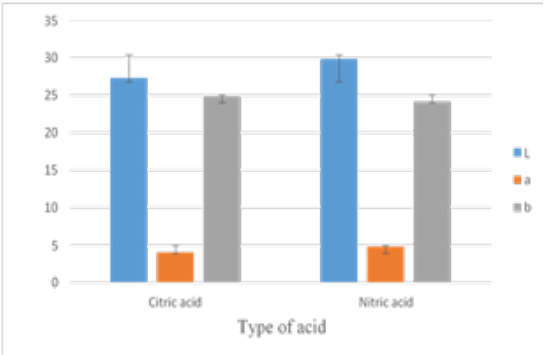

Figure 3 'L', 'a' and 'b' values of pectin.

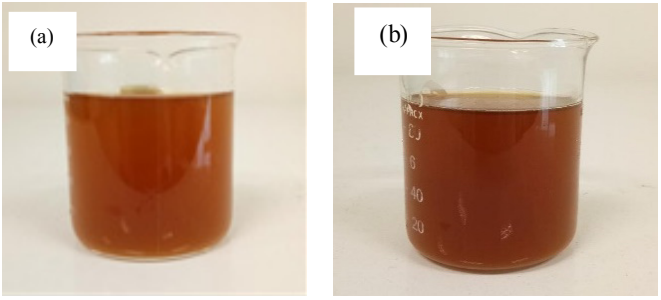

Figure 4 Gel of pectin extracted from honeydew melon peels using citric acid (a) and nitric acid (b).

Statistical analysis of pectin extracted from honeydew peels was significantly ($p > 0.05$) affected by the type of acid used in this study since the p-value was lower than 0.05. During the precipitation process, there was entrapment of polyphenols or water soluble pigments in the pectin which had caused the color to be appeared. The color of the pectin could be improved by conducting the filtration using filter aid, activated carbon and others.[5,15–19]

Conclusion

This study emphasized on the extraction and characterization of pectin from honeydew peels. From the results that were obtained, it is shown that citric acid produced higher yield of pectin (35.26 %) compared to nitric acid. The best condition for pectin extraction was found to be the combination treatment of 80°C and 60 minutes of extraction. All the characteristics of the pectin are significantly affected by the types of acid used during the treatment. Pectin obtains in this study were characterized as high methoxyl pectin (HMP) since the DE was higher than 50 % which can form gel with the presence of acid and sugar. From the results, honeydew melon peels produced a good source of quality of pectin and can be contemplated as commercial pectin in the industry like other citrus fruit sources. There were a few studies on pectin extraction using acids other than citric, nitric and hydrochloric acid. In future study, it is highly recommended for the academicians to compare the extraction yields of the pectin using other acids such as oxalic, acetate, sulphuric acid etc. Pectin and gelatin share the same function where both are mostly used in food product as thickener and stabilizer agents. Since gelatin has become an issue for Muslims owing to its doubtful source and Halal status, government should monitor regularly the uses of gelatin in the industries. Besides, the uses of pectin could replace the gelatin in the industries to solve the Halal issue since pectin is derived from plant sources.[20–22]

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Conflicts of interest

The authors declare that there was no conflict of interest.

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