Extraction and Characterization of Pectin from Passion Fruit Peels

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Abstract

The influence of pH and extraction time on pectin yield and composition was studied in a citric acid extraction process. The pectin yield and degree of esterification (DE) of the extracted pectin ranged from 2.25 to 14.60% and 41.67 to 67.31% respectively. It was found that extraction pH was the most important parameter influencing yield. DE was significantly affected by extraction time. Morphological analysis performed using scanning electron microscopy suggested that the dried passion fruit pectin has a smooth surface with little mound-shaped pellets on it.

Keywords: Pectin; passion fruit; peel; yield; citric acid; SEM

1. Introduction

The tropical climate creates a luxuriant plant life and produces a wide and remarkable diversity of edible and succulent fruits in Malaysia. The yellow passion fruit (Passiflora edulis f. flavicarpa) is consumed widely among the Southeast Asia population. In Malaysia, passion fruits are known as “markisa” or “buah susu”, which were first introduced by Malaysian Agricultural Research and Development Institute (MARDI) in Sungai Baging, Malaysia (Sarif et al., 2010). The passion fruit can direct consumption or made into drinks or incorporated into fruit salads, sherbets, ice cream, and a number of confectionery products. Previous studies revealed that the passion fruit contained very high amount of vitamin C and water-soluble fibers. Investigations also suggest that the passion fruit peel is a rich source of pectin, with pectin yield 10-20 % (Pinheiro et al., 2008; Seixas et al., 2014).

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Following FAO regulation, pectin must contain at least 65% galacturonic acid. It is commonly found in the cell walls and middle lamella of plants. The degree of esterification (DE) is one of the properties influencing pectin application as it determines the gelling nature of pectin. The DE percentage above 50% is classified as high methyl ester (HM) pectin while those less than 50% is known as low methyl ester (LM) pectin (Joye et al., 2000). Pectins are widely used in the food science, nutrition, cosmetics and pharmaceutical (BeMiller, 1986). The yield and DE of a pectin source need to be determined prior mass production of pectin. Pectin yield and DE vary with fruit peels, extraction parameters and extractors used.

Extraction is the most important process in the pectin production. Pectin extraction in a hot diluted strong mineral acid solution is the most commonly used method. Strong acids are corrosive and may be a potential threat to health. Moreover, the liquid waste generated from the industrial processes might lead to burden the environment and a high cost might incur for treating the strong acidic waste (Lúcia et al., 2013). The extraction of pectin from fruit peels using weak organic acid such as citric acid has been intensively conducted in recent studies (Minjares-Fuentes et al., 2014; Kulkarni et al., 2010; Pinheiro et al., 2008). This work aims to extract and characterize pectin from passion fruit peel using citric acid at various pH and time.

2. Materials and methods

2.1. Pectin extraction process

The yellow passion fruit (Passiflora edulis var. flavicarpa) randomly picked at same ripeness and with similar peel colours were selected from a same harvest from the Multi-Rich Pitaya Orchard, Selangor (Malaysia). The fruits were washed and the flesh was separated from its peel. The peels were dried in a Memmert Universal Oven (UNB 100, Memmert, Germany) at 55 °C until a constant weight was achieved. The dried peels were then milled into 500 μm powder using an electronic miller (DFT-150, Dickson, China). The fruit peel powder was packaged in a polyethylene bag and stored at -15 °C in a freezer (ACF15F, Acson, Malaysia). All the chemical reagents used were of analytical grade supplied by Systerm Sdn Bhd, Selangor (Malaysia).

Pectin was extracted at different pH and for different extraction periods. A total of 10 g fruit peel powder measured on an analytical balance (B204-S, MK II, Mettler Toledo, Switzerland) was blended with 250 ml distilled water and acidified with different volumes of 0.1 N citric acid to meet the designed pH of 2.0, 3.3 and 4.5. The mixture was then stirred using a stirrer until all the fruit peel powder was evenly wetted by acidified water in homogenous form. The pectin extraction procedure was continued treating the acidified samples at 70 °C for different duration of 30, 75 or 120 minutes in a shaking water bath (Lab Companion 37L, Jeio Tech, Korea). The mixture was kept at room temperature for 24 hours.

The precipitated pectin was recovered by refrigerated centrifuge (Mikro 22R, Hettich, Germany) at 6000 rpm for 10 minutes. Water bath heat-treated samples were then filtered and added to double volume of 95% ethanol (1:2 v/v) to allow pectin precipitation. The samples were stored in dark condition at room temperature of 25 °C for 24 hours to allow pectin flotation which was then separated by filtration and subsequently washed twice with 70% ethanol. Acetone was then added in a drop wise manner to remove unwanted pectin color (Pinheiro et al., 2008). The resulted pectin substance was dried in a conventional oven (UM500, Memmert GmbH, Schwabach, Germany) at 65 °C until a constant weight was reached. The percentage yield of the fruit peel pectin was determined as gram of product obtained per 10 g of fruit peel powder used:

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Pectin \text{ yield } (\%) = \frac{\text{Product obtained (g)}}{10 \text{ g Fruit peel powder}} \times 100
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2.2. Determination of degree of esterification (DE)

The degree of esterification, DE is defined as the ratio of esterified galacturonic acid groups to the galacturonic acid groups present (Van et al., 1991). The DE of pectin was determined by the titrimetric method of Food Chemical Codex with slight modification. 0.2 g of dried pectin sample was moistened with ethanol and dissolved in 20 ml distilled water. The sample was placed in an automatic shaking water bath at 45 °C until the pectin dissolved completely. Three drops of phenolphthalein were added into the sample. The sample was titrated with 0.1 N sodium...
hydroxide. The result was recorded as the initial titration volume once some pink color appeared. The number of free carboxy group was calculated from the volume of 0.1 N sodium hydroxide solution spent for initial titration. Then, 10 ml of 0.1 N sodium hydroxide was added to neutralize polygalacturonic acid. The sample was plugged with a stopper and shaken vigorously, then allowed to stand at room temperature for 2 hours to de-esterify pectin. 10 ml of 0.1 N hydrochloric acid was added to neutralized sodium hydroxide and the sample was shaken until its pink color disappeared. Three drops of phenolphthalein were added into the sample and the sample was further titrated with 0.1 N sodium hydroxide. The volume of titration was recorded as final titration volume once some pink color appeared. The number of the esterified carboxy group was calculated from the volume of 0.1 N sodium hydroxide solution spent for final titration. The DE was calculated from the following formula:

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DE (\%) = \left( \frac{\text{Final titration volume (ml)}}{\text{Initial titration volume (ml)} + \text{Final titration volume (ml)}} \right) \times 100 \%
\]  

(2)

2.3. Preparation of the samples for examination using SEM

Passion fruit powder and the pectin samples were observed using scanning electron microscopy (S-3400N, Hitachi, Japan). The SEM was used to elucidate morphological changes of samples which were extracted by acidic extraction method. Dry pectin sample was dried using microwave and wet pectin sample was the crude pectin. The morphological of wet pectin sample and dry pectin sample were analyzed to obtain the microwave heating effect on pectin structure. The preparation of pectin samples for SEM include mounting the pectin samples on the aluminum studs, pectin samples coating with gold in vacuum and observing the pectin samples by SEM.

2.4. Design of experiment

The experiment started with selected pH of 3.3 based on literatures of Tang et al. (2011), Lucia et al. (2012) and a series of preliminary tests. At each pH level, including a lower and higher pH of 2.0 and 4.5, the optimum extraction time based on experiment result which gave the highest yield was determined. Each treatment was conducted in duplicates and completely randomized design (CRD) was used in this study.

3. Results and discussion

3.1. Image study on fruit peel powder and extracted pectin

Fig. 1 shows the photograph and Fig. 2 the micrograph of passion fruit powder and extracted pectin. The micrograph of passion fruit peel powder (Fig. 2(a)) shows that the peel was compact and flaky in shape. The extracted wet pectin (Fig. 2(b)) has a nano-structure which was smooth and compact with a little wrinkle on the surface. The extracted pectin dried using oven (Fig. 2(c)) indicated that drying has some destructive and swelling effects on pectin structure with mound-shaped pellets formed on smooth pectin surface. A similar image of wet pectin was obtained by Xu et al. (2014) who extracted pectin from grapefruit peel pectin using ultrasound heating method and similar image of dry pectin was obtained by Luo et al. (2014) and Minjares-Fuentes et al. (2014) who extracted pectin from Catharanthus roseus leaves and grape pomace.
3.2. The influences of extraction time and pH on pectin yield and degree of esterification

Fig. 3(a) shows that extraction time of 75 minutes gave a maximum yield of pectin. Pectin, a carbohydrate polymer which is composed of α-(1, 4) linked units of galacturonic acid needs time to soften its structure for pectin extraction. The pectin yield increase with time at the beginning of extraction was because longer times provide more reaction time opportunity. However, pectin yield decreased after the peak point 75 minutes, possibly explained by the effect of acid which may have destroyed the glycoside bond and ester bond (Xue et al., 2011) of pectin which lead to lower yield. This trend of optimum pectin yield point is in agreement with Xue et al. (2011); Kulkarni et al. (2010); Agarwal et al. (1968) who have used citrus peel, passion fruit peel and mandarin orange waste respectively. Fig. 3(b) shows DE decreased with extraction time. The shortest extraction time of 30 minutes at pH 3.3 demonstrated the highest DE of 58.92%. Pectin above 50% DE which is also known as HM pectin, is advantageous for making high sugar products (Canteri-Schemin et al., 2005). Thus, higher DE was obtained by sample treated for shorter extraction time.

pH is considered as one of the more crucial parameters affecting the amount and properties of extracted pectin. Fig. 4(a) shows that pectin yield decreased with increasing pH which the highest pectin yield of 14.60% was obtained at pH 2. The presence of high concentration of hydrogen ions in the solvent has stimulated the hydrolysis of protopectin (Kertesz, 1951). Protopectin is a compound formed by the combination of cellulose with pectin molecules. At low pH, as the hydrogen ion concentration of the solution is increased, ionization of the carboxylate groups is repressed, i.e., the highly hydrated carboxylate group is converted into hydrated carboxylic acid groups. The lost of carboxylate groups is able to reduce the repulsion of the polysaccharide molecules which promotes the gelation properties of pectin giving more precipitated pectin at lower pH (BeMiller et al., 1986). This observation agreed with Canteri-Schemin et al. (2005) and Yapo et al. (2007)’s work who extracted pectins from apple pomace and sugar beet pulp where the yield increased with increasing acid strength. pH has less influence on DE value of the extracted pectin (Fig. 4(b)). At an average DE of 59.8 ± 9.8%, the passion fruit peel pectin can be considered as HM pectin, similar to other pectins from fruit waste such as apple pomace (68.8%) by Canteri-Schemin et al. (2005), passion fruit peels (69.7%) by D’Addosio et al. (2005) and (71.6%) by Corona et al. (1996).
4. Conclusions

In terms of pectin yields from passion fruit peels, pH was more than the extraction time as an extraction factor. An optimum pectin extraction using citric acid was found at 75 minutes at the lowest pH 2, which gave 14.60% yield. The DE of extracted pectin across the varied time and pH was quite consistent at average about 54.78% and can be considered as HM pectin. The image of passion fruit pectin provided structural information of dried passion fruit pectin which is smooth surface with little mound-shaped pellets on it.

References