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Original Article

Comparison of Extraction Techniques for Pectin from Kinnow Peel by using Convectional, Sonication, and Thermo-Sonication Techniques

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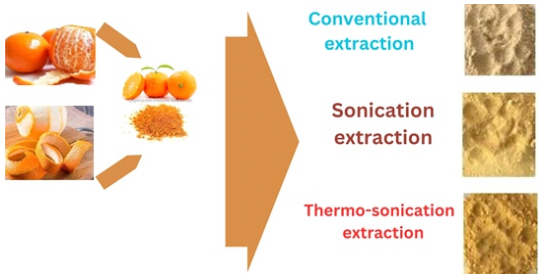
ABSTRACT

Kinnow is a species of citrus family. Pectin has been declared as a safe substance. However, conventional methods are practiced in some industries for pectin extraction from citrus peel. Innovative techniques like ultrasound-assisted extraction (UAE), sonication, and thermos-sonication may play a vital role in increasing the yield of pectin from peels of citrus. **Objective:** To conduct a comparative analysis of Kinnow peel pectin extracted through conventional, Sonication, and Thermosonication techniques. **Methods:** For this purpose raw materials (Kinnow) purchased from the local market of Faisalabad, Kinnow peels were dried and made into fine powder. This fine powder is used further to extract pectin by conventional, sonication and thermos-sonication extraction methods. **Results:** Different qualitative analyses of pectin like solubility of dry pectin in cold and hot water, and solubility in cold and hot alkali, pH, pectin color, quantitative analysis of pectins like yield and equivalent weight, and methoxyl content. During pectin testing, thermos-sonication techniques proved their excellence. Among all treatments during analysis, Moreover, thermos-sonication was found to be the best extraction technique among all treatments. The yield is maximum (15.55%) through thermo-sonicated extracted samples. The highest value of equivalent weight (345±13.08) was seen in the thermos-sonication-treated sample. The upper limit (5.33±0.12) of methoxyl content was held by thermos-sonication-treated samples. Pectin's capacity to bind sugar and spreading quality increases with increasing methoxyl content. **Conclusions:** It was concluded that the best extraction method of pectin is the thermos-sonication method for better qualitative and quantitative output.

GRAPHICAL ABSTRACT



INTRODUCTION

At present, citrus fruits are cultivated in Pakistan on 206,569 hectares of area with 2.36 million tons of production in 2015–16. In Punjab, more than 98 percent of citrus fruits are grown and 70 percent of them are under Kinnow. Indeed, Kinnow has controlled the country's citrus farming [1]. Moreover, Punjab is the core of citrus-producing areas as it covers 52,836 hectares growing over 10, 49,977 tons annually [2]. On average, the Kinnow juice

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comprises 12°Brix, 22.45% of total soluble solids (TSS), 1.38% acidity, 6.23% total sugars, 41.57 mg/100g of ascorbic acid, 0.67% ash, 5.99% reducing sugars, 13.65 mg/100g of carotenoids, 1.85% pectin, 7.43 mg/100g of β-carotene and 0.77% fat (3). It also contains about 0.42 mg / g of naringin, and about 4.69 mg / g of limonin. Various biochemical constituents like pectin help in cancer prevention, mineral balance, and glycemic control [3]. Being the most common form of pectin, homogalacturonan makes up about 50–90 percent of the plant's pectin. Pectin are complex polysaccharides comprising mostly of the esterified D-galacturonic acid in the chain of α1-4 configuration [4]. The extraction of pectin is a multi-stage process consisting of many physical and chemical steps. Selecting a suitable optimum process for pectin extraction is necessary to enhance extraction with minimal side effects on the quality of pectin [5]. Throughout the pectin extraction process, many physical and chemical processes take place which are affected strongly by temperature, solvents, pH, and number of extractions [6]. On an industrial scale, pectin is extracted from dried Kinnow peel by the use of hot water (60 to 100 °C), at 1.5 to 3 pH for 0.5 to 6 hours [7]. To attain superior quality and yield of pectin, ultrasound-assisted extraction (UAE) has been widely adopted as a novel and innovative technique [8]. UAE is a good alternative method to conventional methods. Food processing industries uses are now common in the UAE for many purposes [9]. In this, a probe system is used with deionized water that is operated at 800 W power and 20 kHz frequency. Then pectin is extracted, purified, and assessed [10].

The main objective of this study was to conduct a comparative analysis of Kinnow peel pectin extracted through conventional, Sonication, and Thermosonication techniques.

METHODS

The complete detail of all the analyses of my project is as follows. Kinnow fruits (Citrus nobilis x Citrus deliciosa) of the Rutacae family) were in quite fresh form procured from local markets located in District Faisalabad. All the necessary chemicals and reagents were procured from the authenticated scientific stores. Kinnow peels were subjected to washing with water, dried, crushed, and lastly ground into a suitable size that may pass through the size of 80 mesh sieves. Kinnow peel powder was used to extract pectin. Almost 5g sample of Kinnow peel was utilized to extract pectin by using all the mentioned extraction techniques. The extraction technique mentioned in Table 1.

Table 1: Treatment plan

Treatments	Extraction techniques
T_1	Conventional extraction (Acid boiling)
T_2	Sonication (20 kHz, 30 min at 20°C)
T_3	Thermo-sonication (20 kHz, 30 min at 40°C)

We made 5 g of Kinnow peel powder. After mixing thoroughly with water its pH was set at 1.5 with the aid of 0.1 N HCl. Beakers were placed in hot water (90°C) for one and a half hours. Then, the vessels were removed from the water bath and the mixture was cooled down so that it could be centrifuged at 1500 r.p.m for 20min at 4°C in a centrifugation machine. Then the mixture was filtered and the pH of the filtrate was set at 3.5 by using 0.1 N NaOH. Ultimately the coagulation of filtrate was done with the help of 96% ethanol which was one and half of the volume of filtrate. After 6 hours (rest time) the pectin was coagulated and the whole filtrate was centrifuged to separate the coagulated pectin. Its washing was done with 96% ethanol to purify the pectin. To get the dried pectin as the end product, coagulated pectin was heated up at 50°C in the dehydrator [11]. The UAE method is used to extract the pectin from dried peel powder [10]. 5g dried peel was subjected to 150 mL solvent. To have a uniform extraction, the ultrasound pulse was set at 50% (2 sec on: 2 sec off). Extraction conditions were optimized by keeping 67 oC temperature for 28min with a power of 12.49 W/cm2 [7]. Thermosonication (TS) is a combination of heat and ultrasound application. 10 kinnow were washed and peeled off. The peel was vacuum dried by the oven (45 C for 2 hrs) after a water bath. To extract pectin from the peel, deionized water was used as an extraction solvent. TS was conducted in a 6 L ultrasonic tank at 40 k Hz and 200 W under controlled temperature. 5 g peels in 150 mL solvent were subjected to a bath at 60o C for 30min (10s on, 50s off). The temperature was periodically measured by using a digital thermometer. A nylon cloth was used to filter the extracted mixture. The residues that were insoluble in alcohol were precipitated with 96% ethanol for 3 h at 20o C and then dried, milled, and sieved. This dried sample was stored in a desiccator until analysis. TS has shown an increment in yield of pectin (26.74%) [7]. The samples of pectin (0.25 percent) were put individually in a conical flask in addition to ethanol (10ml of 95%) followed by 50ml of volume of distilled water. The mixture was continuously shaken to develop a suspension that was then heated for 15min at 85-95 ° C [12]. 5ml solution of pectin was added to 1ml and 0.1N NaOH, then heated to 85-90 °C for 15 min [12]. The pH was measured by the method of Mohamed, 2016. Samples of dried pectin were inspected visually, and sample colors were noted [12]. The yield of pectin was measured by using the subsequent equation:

$$\text{Yield (g/100g)} = \frac{\text{Weight (g) of dried pectin}}{\text{Weight of dried alcohol insoluble extraction}} \times 100$$

$$\text{The equivalent weight} = \frac{\text{Weight of the sample (mg)}}{\text{Meq of NaOH}}$$

$$\text{Methoxy content} = \frac{\text{meq. of NaOH} \times 31}{\text{Weight of sample}} \times 100$$

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All the collected data were subjected to analysis statistically through Minitab 8.1 by using ANOVA to evaluate the level of significance [13].

RESULTS

The solubility of pectin was analyzed in both cold and hot water. The results are in Table 2. The table shows that the pectin is completely insoluble in cold water. It swells a bit and on vigorous shaking forms clots. Pectin was then put to test for its solubility in hot and cold alkali. More or less, a similar trend was observed. Its results are shown in Table 2.

Table 2: Solubility of Pectin in water and alkali

Parameter	Pectin extraction methods		
	Acid boiled extraction	Ultrasound-Assisted Extraction	Thermo-sonicated extraction
Color	Brownish	Slightly reddish	Slightly reddish
Solubility in cold water	Insoluble, form suspension, imbibes, swells with strong shaking,	Insoluble, form suspension, imbibes, swells with strong shaking,	slightly dissolved and formed suspension after vigorous shaking
Solubility in hot water	Dissolved	Dissolved	Dissolved
Solubility in cold alkali	yellow precipitate formed by pectin suspension	yellow precipitate formed by suspension of pectin	Pectin suspension formed a yellow precipitate
Solubility in hot alkali	Pectin suspension dissolved and turned milky	Pectin suspension dissolved and turned milky	pectin suspension dissolved and turned milky

pH values were evaluated and the data were subjected to statistical analysis. Table (3) shows that the values of pH were highly significant. The mean values of pH taken from all three pectin types are presented in Table 3. The pH of the pectin is seen in the range of 3.17-3.55. The highest value is seen in the case of the acid-boiled sample while the lowest value was seen in the sample extracted with thermo-sonicated technique.

Table 3: Means for pH of Pectin Extracted from Kinnow Peel (p=0.01)

Treatments	Means ± SD
T_1	3.55 ± 0.04^{A}
T_2	3.35 ± 0.05^{B}
T_3	3.17 ± 0.06^{C}
*=Significant (p ≤ 0.05-0.01) **= High Significant (p ≤ 0.01) NS= Non-Significant (p > 0.05)	

This means carrying different letters are significantly different from each other

T1: Acid Boiling Extraction of Pectin from Kinnow Peel

T2: Ultrasound-Assisted Extraction of Pectin from Kinnow Peel

T3: Thermo-Sonication Extraction of Pectin from Kinnow Peel

The color of the pectin was analyzed visually and found that acid-boiled pectin was slightly different from the other types. The acid-boiled pectin exhibited a brownish color while the color of thermo-sonicated and ultrasound-assisted extraction was slightly reddish. This distinction is possibly due to the heat treatment directly applied in case of acid boiling. Other reasons may include human errors, surface contamination, fruit types, or environmental factors. Table 4 describes that the results are highly significant; The results produced differ significantly from each other. Mean values of eq. weights are displayed in Table 4. This table describes that the data set contains values in the range of 290-345. The highest value was seen in the thermo-sonicated -treated sample while the lowest equivalent weight was seen in the UAE treated sample. These values show the degradation of pectin. The sample with a higher value means that there is less degradation of the sample while the low value of pectin shows more degradation of pectin.

Table 4: Means for Equivalent Weight of Pectin Extracted from Kinnow Peel (p=0.01)

Treatments	Means ± SD
T_1	297 ± 07.55^{B}
T_2	280 ± 07.77^{C}
T_3	345 ± 13.08^{A}
*=Significant (p ≤ 0.05-0.01) **= High Significant (p ≤ 0.01) NS= Non-Significant (p > 0.05)	

The table data describes that the results are highly significant. The mean values of yield are displayed in Table 5. The data exhibits that the maximum value (15.55%) is owned by T3 while the minimum value (12.49) is obtained by T1. These values show that the yield is maximum through the thermo-sonicated extracted sample. So, this may be concluded that the thermo-sonicated method will give maximum yield.

Table 5: Means for Yield (%) of Pectin Extracted from Kinnow Peel (p=0.01)

Treatments	Means ± SD
T_1	12.49 ± 0.16^{C}
T_2	14.51 ± 0.10^{B}
T_3	15.55 ± 0.20^{A}
*=Significant (p ≤ 0.05-0.01) **= High Significant (p ≤ 0.01) NS= Non-Significant (p > 0.05)	

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The Mean Table (6) shows that the values are significant as analyzed by applying statistics on the triplicates of data. The maximum values of the methoxyl content are found in T3(5.33±0.12) while the minimum found in T1(3.46±0.06) are shown in Table 6. Methoxyl content lies in the range of 3.46-5.33%. The lower limit of this analysis is achieved by acid-boiled pectin and the upper limit is held by a thermo-sonicated treated sample.

Table 6: Means for Methoxyl Content (%) of Pectin Extracted from Kinnow Peel (p=0.04)

Treatments	Means ± SD
T_1	3.46 ± 0.06^{C}
T_2	4.28 ± 0.10^{B}
T_3	5.33 ± 0.12^{A}
*=Significant (p ≤ 0.05-0.01) **= High Significant (p ≤ 0.01) NS= Non-Significant (p > 0.05)	

DISCUSSION

Our result of solubility in hot and cold water resembles the result of López-Ordaz et al., 2019 [14]. According to him pectin is completely and easily dissolved in hot water rather than in cold water. The result of Lin et al., 2005 [15] indicates that the pectin originally belong to cell wall of tomato fruit extend to increase the water solubility. In contrast to this, a hot alkali solution leads to the formation of milky precipitates in the suspension. The results were compared with those produced in the study by Ma et al., 2008 [16]. The results of the study of pH were resembling with Chan et al., 2017 [17]. He performed his study on the evaluation of apple peel and developed alkali-soluble pectin. This work reveals some closeness with Gao et al., 2014 [18]. He also describes the acidic nature of pectin and extends his work on the cross-linking of pectin in the presence of Ca+ as a linking agent. This study was well explained with the results of Ma et al., 2008 [16]. He got the color of pectin brownish light. Observed by de Oliveira et al., 2016 [19] that the yield of extraction can go up by 1.6 fold when ultrasound used for extraction. The results obtained demonstrated that ultrasound was a competent and time-saving technique for the extraction of pectin from passion fruit peel. Table 4 (equivalent weight) describes that the data set contains values in the range of 290-345. The sample with a higher value means that there is less degradation of the sample while the low value of pectin shows more degradation of pectin. These results show a resemblance to Chan et al., 2017 [17]. His work was on the lemon pomace-extracted pectin. Toma et al., 2001 [20] Stated that ultrasonic treatment increases the swelling and softening method of cell walls via the hydration of pectin material from middle lamella, which leads to break down of vegetal tissue during sonication. Therefore, it appears clear that sonication plays an important role in splitting the vegetal tissue, enhancing the extraction yields. The yield data exhibits that the maximum value (15.55%) is owned by T3 while the minimum value (12.49) is obtained by T1. These values show that the yield is maximum through thermo-sonicated extracted samples. This study showed similar results as produced by Arrutia et al., 2020 [21]. They worked on the characterization and extraction of pectin from grapefruit and used it as a gelling agent in different foods. In first 10 minutes, the amount of pectin extracted increased while a small amount decreased after extraction of 6 minutes but after long period of time the extraction amount become slow by de Oliveira et al., 2016 [19]. Methoxyl content lies in the range of 3.46-5.33% (Table 6). Pectin's capacity to bind sugar increases with increasing methoxyl content and hence the spreading quality as well Alkhatib et al., 2017 [22].

CONCLUSIONS

The conventional methods are practiced in some industries for pectin extraction from citrus peel. Innovative techniques like ultrasound-assisted extraction (UAE) may play a vital role in increasing the yield of pectin from peels of citrus. The maximum amount of pectin was obtained from thermos-sonication. During pectin testing thermos-sonication techniques proved their excellence.

Authors Contribution

Conceptualization: TS
Methodology: TS, FH, MUK
Formal analysis: MSM, HAM
Writing-review and editing: MYQ, SM, NF, RB, AS
All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The author declares no conflict of interest.

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