Aqueous and Microwave Assisted Extraction of Pectin from Grapefruit and Nagpur Mandarin

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ABSTRACT

Pectin is a complex polysaccharide with GRAS status which is increasingly finding its application in food and pharmaceutical industries. Two different methods (aqueous and microwave) were used for pectin extraction from the peels of grapefruit (Citrus paradisi L.) and Nagpur mandarin (Citrus reticulata L.) using four different extraction times (20, 40, 60 and 80 min), and with and without the use of cell wall degrading enzymes on yield and quality of extracted pectin. Pectin yield varied from 8.39 to 14.75% using aqueous method while it was significantly improved (8.19 to 18.58%) when microwave assisted extraction was carried out using citric acid in extraction processes, irrespective of solvent concentration, incubation time and variety. Grapefruit peel produced slightly higher yield as compared to Nagpur mandarin. The optimum condition for pectin yield was found to be 60 min and 1:15 solid to solvent ratio for both varieties. Pectin isolated of grapefruit peel behaved as high methoxyl pectin while that of Nagpur mandarin was found be low methoxyl pectin based on the degree of esterification. Equivalent weight of pectin extracted from grapefruit was higher (659.93- 737.99) as compared to Nagpur mandarin. However, anhydrouronic acid content was found to be slightly higher (64.12- 74.45%) for Nagpur mandarin as compared to grapefruit peel pectin.

Keywords: Aqueous extraction, Methoxyl percent, Microwave assisted extraction, Pectin, Yield

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Introduction

Pectins are methylated ester of polygalacturonic acid that contain 1,4-linked α-D-galacturonic acid residues. It is a part of soluble dietary fibre and widely used in the food industry as a thickener, emulsifier, texturizer and stabilizer (Levigne et al., 2002). Fresh weight of plant material contains 0.5-4.0% of pectin substances (Faravash and Ashtiani, 2008). These are the biopolymers found in the primary cell walls of most plant cells but most concentrated in citrus fruits (oranges, lemons, grapefruits) and apples. Worldwide currently commercial pectin is extracted from citrus peel and apple pomace,
which are the by-products from juice/cider manufacturing (Chakraborty and Ray, 2011; Shaha et al., 2013). Pectin polysaccharides consist of 300-1000 chains of galacturonic acid units (Yeoh et al., 2008). 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 pectin (HMP) while those less than 50% is known as low methyl ester pectin (LMP) (Joye and Luzio, 2000). An HMP forms a gel in an acidic medium (pH 2.0-3.5), if sucrose is present at a concentration 55%. In contrast, an LMP generally forms a gel in the presence of Ca\(^{2+}\) within a larger pH range (2.0-7.0) whether sugar is present or not. Commercial LMPs are manufactured from HMPs by acid, alkali, ammonia, or enzymatic de-esterification (Yapo and Koffi, 2006).

The annual production of citrus fruits is about 11.58 million metric tones with an area of cultivation of about 10.24 lac hectares. India is ranked 3rd amongst the top ten citrus fruits in terms of production after China and Brazil while in terms of productivity we fall at second last place just above Nigeria (Indian Horticulture Database, 2017). Fruit whether used for table purpose or juicing leads to a considerable amount of waste in the form of peel, rags etc. Also, percent average loss for citrus fruits is to the tune of 9.69% (Jha et al., 2015). Citrus waste comprises approximately 35-45% of the total fruit production, is highly perishable and seasonal, pose problem to the processing industries and pollution monitoring agencies (Puri et al., 2005). Meager level of processing and a sizeable post-harvest loss are responsible for a monetary loss of Rs 31,500/- crores annually in case of fruits and vegetables alone in India (Nanda et al., 2012; Jha et al., 2015; Rudra et al., 2015). By-product recovery from fruit residues can improve overall economics of processing units. Besides this, the problem of environmental pollution also can be reduced considerably (Londono-Londono et al., 2010).

Pectin extraction is the most important process in the pectin production. Mostly pectin extraction is done using hot diluted strong mineral acids like HCl, H\(_2\)SO\(_4\) etc. which are corrosive and always remain a potential threat to health, environment and overall economics due to generation of liquid waste by industrial processing (Lúcia et al., 2013). There are few reports of the extraction of pectin from remaining fruit peels using weak organic acid such as citric acid (Liew et al., 2014; Kulkarni and Vijayanand, 2010) which is health and environment friendly when compared to mineral acids. Therefore, an investigation was carried out at our laboratory to extract pectin employing citric acid, a safer alternative to inorganic acids using microwave as well as conventional aqueous extraction methods to extract pectin of grapefruit and Nagpur mandarin.

Materials and Methods

Raw material

Grapefruits were purchased from orchard of Regional Research Station, PAU, Abohar while Nagpur mandarin from local market. The fruits were washed; removed peel manually, dried under sunlight, and stored in cool and dry place till further use for pectin extraction. The dried peel was powdered and used for experimentation. All the experiments were performed using 100 g of raw material (dried peel powder) using aqueous as well as microwave assisted extraction methods for various time periods. Until stated otherwise, each experiment was replicated thrice.

Microwave assisted extraction of pectin from citrus fruit residue

The residue/peel powder was soaked in water and processed via 3 different routes. In one experiment, soaked residue powder was
treated with 1000 units each of cellulase and xylanase for 2 h at 40 °C at native pH. Pectin extraction (of enzyme treated samples) was performed at native pH while in another experiment; the enzyme treated broth was acidified using citric acid to pH 2.0 and then extracted pectin. In the third route, soaked residue was acidified directly using citric acid to pH 2.0 (omitting enzymatic degradation step) to extract pectin.

The experiments performed using H₂SO₄ (to pH 2.0) for pectin extraction served as control. After various pre-treatments, the broth was subjected to microwave energy (power level 900 W) for 20, 40, 60 and 80 min. After microwave extraction and cooling, the pectin was precipitated from the broth using ethanol (1:1), filtered, drained and dried at 50°C overnight.

**Aqueous assisted extraction of pectin from citrus fruit residue**

For aqueous extraction, all the experiments were performed as during microwave extraction except that here after various pre-treatments, the broth was subjected to aqueous extraction (temperature 95°C) for 20, 40, 60 and 80 min. After aqueous extraction and cooling, the pectin was precipitated from the broth using ethanol (1:1), filtered, drained and dried at 50°C overnight.

**Purification of precipitated pectin**

The dried and clarified pectin samples were purified by immersing the crude pectin in ethanol: water mix (1:1) for 4 h followed by dehydrating with acetone for 4 h. Both the steps were performed with constant shaking at 150 rpm in orbital shaking incubator. Finally, the purified pectin was dried overnight at 50°C, powdered and used for physic-chemical analysis.

**Physic-chemical analysis of citrus pectin**

For physic-chemical analysis, distilled water used was boiled for 15 min to eliminate dissolved CO₂. *Yield* of pectin was estimated using precision weighing balance and expressed on per cent basis. *Equivalent weight* and *methoxyl content* were determined as per the procedure of Owens *et al.*, (1952) as given in Ismail *et al.*, (2012).

**Anhydrouronic acid (AUA) content**

The AUA content was calculated (Owens *et al.*, 1952) by using the values of equivalent weight and methoxyl content (MeO%) using the following expression:

\[
\text{AUA} \% = \frac{(\text{meq of NaOH for free acid} + \text{meq of NaOH for MeO}) \times 176 \times 100}{\text{Weight of sample (mg)}}
\]

Where 176 is the molecular weight of AUA

**Degree of esterification (DE)**

The DE was calculated (Ismail *et al.*, 2012) by using the values of MeO% and AUA% using the equation as hereunder:

\[
\text{DE} \% = \frac{\text{MeO} \% \times 176 \times 100}{31 \times \text{AUA} \%}
\]

**Results and Discussion**

Preliminary standardization of solid to liquid ratio was done with respect to time of incubation and based on this, solid to liquid ratio used was maintained at 1:10 for 20 and 40 min; 1:15 for 60 min and 1:20 for 80 min of incubation. Extraction of pectin was carried out using aqueous as well as microwave
assisted extraction under various conditions from dried and powdered grapefruit and Nagpur manadarin peels. The high concentration of hydrogen ions presents in the solvent at acidic pH stimulated the hydrolysis of protopectin. At lower pH, the highly hydrated carboxylate groups get repressed in huge hydrogen ion concentration and their charge repulsion is minimized. The addition of ethanol was able to dehydrate pectin so that the stability of its colloidal solution gets disturbed resulting in coagulated pectin (Megawati et al., 2015).

**Extraction of pectin from grapefruit peel residue**

The yield and other qualitative parameters of aqueous extracted grapefruit peel pectin are elaborated in Table 1. Among all treatment types, the highest % yield (14.75±0.19) was obtained for enzyme + citric acid combination with 60 min of incubation which was at par with only citric acid treatment (14.67±0.64) for 60 min incubation. The enzyme + citric acid combination was nearly 26% higher than control treatment whose highest pectin yield was 10.91±0.11 (Table 1). Only enzyme treatment with native pH yielded 1-3% pectin which was way below the optimum levels of pectin obtained of all other treatment types including control. The yield and other qualitative parameters of microwave assisted extracted grapefruit peel pectin are given in Table 2. Similar to aqueous extraction, the highest % yield of pectin (18.58±0.74) was obtained for enzyme + citric acid combination with 60 min of incubation which was at par with only citric acid treatment (18.54±0.56) for 60 min of incubation. This implied that enzymatic pre-treatment was not so effective for enhancing pectin yield considerably from an optimum yield. The enzyme + citric acid combination was nearly 20% higher than control treatment (14.84±0.69%) (Table 2). During microwave extraction, 20 and 40 min incubation times were insufficient to extract pectin of grapefruit peel for only enzyme treatment, however, at 60-80 min, extracted pectin yield was also negligible (0.26-0.31%) (Table 2). This might be due to absence of desired conditions for pectin breakdown at that pH or sole enzymatic treatment appeared to be too mild to yield any pectin. Pectin yield remained less at higher (alkaline or towards alkaline) pH as some pectin still remains attached to the cell wall components and un-hydrolyzed (Udonne et al., 2016). Methoxyl content (%) of extracted grapefruit peel pectin for both extraction methods (7.11-7.28%) was more than 7% and degree of esterification (60.37-63.57%) was found to be more than 50%, thus, indicating that the extracted pectin was high methoxyl in nature (Table 1 and 2). Equivalent weight of extracted grapefruit pectin was in the range of 659.93-737.99 while anhydouronic acid content ranged from 64.97-67.46% (Table 1 and 2). Among both types of extraction methods, microwave assisted extraction resulted in 21% more extraction of pectin compared to aqueous one for their optimized pectin yield at 60 min of incubation. Mohamed (2016) has reported pectin yield of 25% from grapefruit peel using a combination of HNO$_3$ and HCl at temperature 80°C and pH 2.0, for 60 min of aqueous mediated extraction. Methoxyl content of red and white type grapefruit was found to be 8.87 and 7.54 respectively, while the corresponding values for DE (%) were reported to be 55.01 and 51.24%, respectively. Thus, the grapefruit peel reported to be of high methoxyl in nature by Mohamed (2016). The AUA content was found to be 60.95% for both variants of grapefruit. Alexander and Sulebele (1980) reported pectin yield of 15-17% for Indian citrus peels, while Spanish grape fruit peels had corresponding yield of 30.7% as deduced by Iranzo et al., (1980). The differences in pectin obtained may be attributed to varietal differences and/or stage of maturity of the
fruits. Sayah et al., (2016) carried out aqueous extraction of pectin from grapefruit peel using 0.1 M each of citric acid and H₂SO₄ at 80°C for 60 min. The highest pectin yield obtained from grapefruit peel was 33.63% using sulfuric acid, while using citric acid, pectin yield was 28.74%. The corresponding values for DE (%) were 74.49 and 75.53%, respectively (Sayah et al., 2016). Khan et al., (2014) reported that maximum extraction (22.55%) of pectin was done from grapefruit peel at temperature 120°C with pH-1.5 for 30 min via aqueous (HCl) mediated extraction. Methoxyl content and equivalent weight were found to be 11.77% and 992 respectively, of the extracted pectin. However, Aina et al., (2012) reported grapefruit peel to be of low methoxyl (3.90%) in nature with equivalent weight of 293.6. Bagherian et al., (2011) inferred that highest total amount of pectin yield was found to be 27.81% (w/w) for 6 min of extraction at 900 W using microwave extraction technique. Quoc et al., (2015) extracted pectin from pumelo (Citrus maxima) peels using tartaric acid and microwave energy. The yield of pectin obtained was 23.83% at pH 1.5, rate of pumelo peel/solvent was 1/40 for irradiation time of 9 min at 660 W. Pectin extracted was rated as a high methoxyl pectin having DE of 92.75% with a low viscosity. Longer extraction time of microwave extraction (60 min) might be due to open type of microwave system. Microwave assisted extraction can be classified in to closed and open system i.e. closed system operates at high/above atmospheric pressure in a sealed-vessel with different mode of microwave radiations while open system works below atmospheric pressure. Advancements in microwave extraction such as high-pressure microwave-assisted extraction have improved the extraction rate by allowing more penetration of solvent which is accomplished through breakage of cell structure (Sundari, 2015).

**Extraction of pectin from Nagpur mandarin peel residue**

Among all treatment types of aqueous extraction for orange peel, the highest % yield (14.50±0.53) was obtained for enzyme + citric acid combination with 60 min of incubation which was slightly higher to only citric acid treatment (12.97±0.48) for 60 min incubation. The pectin yield of enzyme + citric acid combination was nearly 31% higher compared to the best control treatment (10.04±0.37%) (Table 3). Only enzyme treatment with native pH failed to extract any pectin for all the parameters tested. This might be due to absence of desired conditions for pectin breakdown at that pH or sole enzymatic treatment appears to be too mild to yield any pectin. Pectin yield remained less at higher (alkaline or towards alkaline) pH as some pectin still remains attached to the cell wall components and un-hydrolyzed (Udonne et al., 2016). The yield and other qualitative parameters of microwave assisted extracted orange peel pectin are given in Table 4. Similar to aqueous extraction, the highest % yield of pectin (18.56±0.55) was obtained for enzyme + citric acid combination with 60 min of incubation. The enzyme + citric acid combination was nearly 17% higher than control treatment where highest pectin yield was 15.38±0.28% (Table 4). During microwave extraction too, only enzyme treatment did not yield any pectin of Nagpur mandarin peel. Methoxyl content (%) of extracted orange peel pectin for both extraction methods (5.54-6.03%) was less than 7% and degree of esterification (45.76-49.15%) was found to be less than 50%, thus, indicating that the extracted pectin was low methoxyl in nature (Table 3 and 4). Equivalent weight of extracted orange pectin was in the range of 436.95-540.27 while anhydouronic acid content ranged from 64.12-74.45% (Table 3 and 4).
Table 1: Yield of grapefruit peel pectin during aqueous extraction and its qualitative analysis

| Combinatorial treatment type | pH for incubation | Time of incubation (min) | Pectin yield (%) | Methoxyl content (%) | Equivalent weight | Anhydrouronic acid content (%) | Degree of esterification (%) |
|-----------------------------|-------------------|--------------------------|------------------|---------------------|-------------------|-------------------------------|-------------------------------|
| *Enzyme + citric acid       | 2.0               | 20                       | 8.39±0.24        | 7.15±0.01           | 674.26±5.72       | 66.92±0.10                    | 60.69±0.14                    |
|                             |                   | 40                       | 13.43±0.31       | 7.15±0.01           | 671.29±3.97       | 66.84±0.14                    | 60.70±0.17                    |
|                             |                   | 60                       | 14.75±0.19       | 7.16±0.02           | 668.23±5.16       | 67.03±0.18                    | 60.64±0.22                    |
|                             |                   | 80                       | 13.14±0.37       | 7.16±0.01           | 666.76±5.63       | 67.11±0.30                    | 60.60±0.15                    |
| Citric acid                 | 2.0               | 20                       | 8.78±0.48        | 7.14±0.01           | 672.72±4.86       | 66.74±0.18                    | 60.73±0.19                    |
|                             |                   | 40                       | 13.43±0.75       | 7.14±0.01           | 669.75±6.04       | 66.86±0.22                    | 60.63±0.22                    |
|                             |                   | 60                       | 14.67±0.64       | 7.15±0.02           | 668.34±7.93       | 66.97±0.41                    | 60.61±0.23                    |
|                             |                   | 80                       | 13.54±0.39       | 7.16±0.03           | 663.82±5.94       | 67.21±0.38                    | 60.48±0.14                    |
| *Enzyme                     | Native            | 20                       | 1.28±0.04        | 7.12±0.02           | 676.84±7.08       | 66.47±0.34                    | 60.82±0.22                    |
|                             |                   | 40                       | 2.15±0.09        | 7.14±0.02           | 672.76±6.10       | 66.76±0.22                    | 60.75±0.24                    |
|                             |                   | 60                       | 3.23±0.12        | 7.15±0.03           | 669.24±5.60       | 66.94±0.33                    | 60.65±0.19                    |
|                             |                   | 80                       | 3.00±0.05        | 7.15±0.02           | 667.71±4.39       | 66.99±0.10                    | 60.59±0.20                    |
| Control (sulphuric acid)    | 2.0               | 20                       | 4.13±0.10        | 7.24±0.02           | 737.99±10.26      | 65.00±0.42                    | 63.21±0.28                    |
|                             |                   | 40                       | 5.28±0.22        | 7.27±0.04           | 733.00±6.02       | 65.35±0.05                    | 63.16±0.30                    |
|                             |                   | 60                       | 9.76±0.10        | 7.26±0.02           | 724.16±6.14       | 65.57±0.28                    | 62.83±0.18                    |
|                             |                   | 80                       | 10.91±0.11       | 7.28±0.03           | 720.80±9.03       | 65.82±0.37                    | 62.79±0.28                    |

*Enzyme = 1000 units each of cellulase and xylanase added to soaked peel powder and incubated at 40°C for 2 h prior to adding citric acid while in only enzyme treatment type, no citric acid was added after enzymatic pre-treatment; - means no yield; n= 3 (value±SEm)
Table 2 Yield of grapefruit peel pectin during microwave extraction (900W) and its qualitative analysis

| Combinatorial treatment type | pH for incubation | Time of incubation (min) | Pectin yield (%) | Methoxyl content (%) | Equivalent weight | Anhydrouronic acid content (%) | Degree of esterification (%) |
|-----------------------------|-------------------|--------------------------|------------------|----------------------|-------------------|-------------------------------|-------------------------------|
| *Enzyme + citric acid       | 2.0               | 20                       | 14.30±0.61       | 7.11±0.03            | 675.32±7.05       | 66.47±0.46                    | 60.70±0.13                    |
|                             |                   | 40                       | 17.61±0.67       | 7.12±0.06            | 671.39±9.11       | 66.72±0.39                    | 60.61±0.42                    |
|                             |                   | 60                       | 18.58±0.74       | 7.16±0.07            | 663.38±7.11       | 67.25±0.23                    | 60.44±0.45                    |
|                             |                   | 80                       | 18.02±0.37       | 7.18±0.07            | 659.93±5.97       | 67.49±0.19                    | 60.40±0.44                    |
| Citric acid                 | 2.0               | 20                       | 14.07±0.71       | 7.12±0.05            | 671.20±4.61       | 66.68±0.39                    | 60.59±0.15                    |
|                             |                   | 40                       | 17.93±0.61       | 7.11±0.04            | 663.40±7.65       | 66.84±0.20                    | 60.42±0.53                    |
|                             |                   | 60                       | 18.54±0.56       | 7.14±0.04            | 663.74±3.05       | 67.08±0.13                    | 60.41±0.24                    |
|                             |                   | 80                       | 17.85±0.47       | 7.18±0.05            | 660.49±7.87       | 67.46±0.60                    | 60.42±0.17                    |
| *Enzyme                     | Native            | 20                       | -                | -                    | -                 | -                            | -                            |
|                             |                   | 40                       | -                | -                    | -                 | -                            | -                            |
|                             |                   | 60                       | 0.26±0.02        | 7.12±0.05            | 665.79±6.12       | 66.94±0.17                    | 60.42±0.38                    |
|                             |                   | 80                       | 0.31±0.02        | 7.15±0.05            | 662.39±6.87       | 67.21±0.12                    | 60.37±0.43                    |
| Control (sulphuric acid)    | 2.0               | 20                       | 9.52±0.78        | 7.22±0.04            | 734.90±8.73       | 64.97±0.15                    | 63.06±0.40                    |
|                             |                   | 40                       | 14.70±0.56       | 7.25±0.06            | 730.66±7.33       | 65.31±0.08                    | 63.57±1.07                    |
|                             |                   | 60                       | 14.84±0.69       | 7.26±0.03            | 716.77±8.71       | 65.71±0.17                    | 62.76±0.39                    |
|                             |                   | 80                       | 14.71±0.24       | 7.27±0.04            | 718.45±7.94       | 65.86±0.15                    | 62.70±0.36                    |

*Enzyme = 1000 units each of cellulase and xylanase added to soaked peel powder and incubated at 40°C for 2 h prior to adding citric acid while in only enzyme treatment type, no citric acid was added after enzymatic pre-treatment; - means no yield; n= 3 (value±SEm)
Table 3: Yield of orange peel pectin during aqueous extraction and its qualitative analysis

| Combinatorial treatment type | pH for incubation | Time of incubation (min) | Pectin yield (%) | Methoxyl content (%) | Equivalent weight | Anhydrous uronic acid content (%) | Degree of esterification (%) |
|-----------------------------|-------------------|---------------------------|------------------|----------------------|------------------|---------------------------------|-------------------------------|
| *Enzyme + citric acid       | 2.0               | 20                        | 8.66±0.34        | 5.85±0.10            | 450.93±2.62      | 72.28±0.34                      | 45.92±0.55                    |
|                             |                   | 40                        | 12.10±0.47       | 5.95±0.06            | 446.12±5.51      | 73.30±0.72                      | 46.09±0.25                    |
|                             |                   | 60                        | 14.50±0.53       | 5.98±0.05            | 441.88±3.46      | 73.71±0.48                      | 46.06±0.11                    |
|                             |                   | 80                        | 14.49±0.71       | 6.01±0.06            | 436.95±3.40      | 74.45±0.10                      | 45.83±0.44                    |
| Citric acid                 | 2.0               | 20                        | 9.12±0.12        | 5.84±0.03            | 450.52±3.98      | 72.30±0.44                      | 45.89±0.24                    |
|                             |                   | 40                        | 12.83±0.76       | 5.91±0.04            | 445.16±3.61      | 73.14±0.56                      | 45.87±0.09                    |
|                             |                   | 60                        | 12.97±0.48       | 5.93±0.04            | 441.91±4.46      | 73.53±0.21                      | 45.76±0.42                    |
|                             |                   | 80                        | 12.45±0.59       | 6.03±0.03            | 439.28±3.05      | 74.37±0.45                      | 46.03±0.06                    |
| *Enzyme                     | Native            | 20                        | -                | -                    | -                | -                              | -                            |
|                             |                   | 40                        | -                | -                    | -                | -                              | -                            |
|                             |                   | 60                        | -                | -                    | -                | -                              | -                            |
|                             |                   | 80                        | -                | -                    | -                | -                              | -                            |
| Control (sulphuric acid)    | 2.0               | 20                        | 3.07±0.31        | 5.55±0.06            | 540.27±3.75      | 64.12±0.57                      | 49.11±0.11                    |
|                             |                   | 40                        | 6.83±0.37        | 5.63±0.04            | 532.97±5.42      | 65.04±0.17                      | 49.15±0.42                    |
|                             |                   | 60                        | 7.75±0.34        | 5.66±0.06            | 524.54±4.18      | 65.74±0.43                      | 48.88±0.32                    |
|                             |                   | 80                        | 10.04±0.37       | 5.68±0.05            | 515.52±4.03      | 66.29±0.40                      | 48.64±0.11                    |

*Enzyme = 1000 units each of cellulase and xylanase added to soaked peel powder and incubated at 40°C for 2 h prior to adding citric acid while in only enzyme treatment type, no citric acid was added after enzymatic pre-treatment; - means no yield; n= 3 (value±SEM)*
Table 4: Yield of orange peel pectin during microwave extraction (900W) and its qualitative analysis

| Combinatorial treatment type | pH for incubation | Time of incubation (min) | Pectin yield (%) | Methoxyl content (%) | Equivalent weight | Anhydouronic acid content (%) | Degree of esterification (%) |
|-----------------------------|-------------------|--------------------------|------------------|---------------------|------------------|-----------------------------|-----------------------------|
| *Enzyme + citric acid       | 2.0               | 20                       | 13.75±0.44       | 5.91±0.04           | 455.71±2.02      | 72.20±0.43                  | 46.44±0.08                  |
|                             |                   | 40                       | 17.20±0.48       | 5.87±0.03           | 453.48±4.04      | 72.20±0.53                  | 46.18±0.10                  |
|                             |                   | 60                       | 18.56±0.55       | 5.94±0.07           | 449.60±3.37      | 72.78±0.55                  | 46.34±0.31                  |
|                             |                   | 80                       | 16.76±0.32       | 5.97±0.04           | 442.53±3.33      | 73.74±0.16                  | 45.99±0.35                  |
| Citric acid                 | 2.0               | 20                       | 8.19±0.51        | 5.87±0.04           | 453.66±2.53      | 72.18±0.44                  | 46.17±0.06                  |
|                             |                   | 40                       | 10.69±0.50       | 5.92±0.04           | 449.59±2.83      | 72.79±0.07                  | 46.12±0.31                  |
|                             |                   | 60                       | 16.56±0.66       | 5.98±0.05           | 445.84±4.16      | 73.47±0.59                  | 46.18±0.22                  |
|                             |                   | 80                       | 15.20±0.67       | 6.01±0.04           | 442.58±4.78      | 73.96±0.69                  | 46.18±0.12                  |
| *Enzyme                     | Native            | 20                       | -                | -                   | -                | -                           | -                           |
|                             |                   | 40                       | -                | -                   | -                | -                           | -                           |
|                             |                   | 60                       | -                | -                   | -                | -                           | -                           |
|                             |                   | 80                       | -                | -                   | -                | -                           | -                           |
| Control (sulphuric acid)    | 2.0               | 20                       | 12.13±0.26       | 5.57±0.06           | 532.95±5.03      | 64.71±0.63                  | 48.87±0.03                  |
|                             |                   | 40                       | 13.14±0.38       | 5.54±0.05           | 529.81±4.68      | 64.71±0.08                  | 48.65±0.44                  |
|                             |                   | 60                       | 14.48±0.10       | 5.61±0.04           | 522.28±7.91      | 65.55±0.45                  | 48.60±0.50                  |
|                             |                   | 80                       | 15.38±0.28       | 5.65±0.06           | 516.11±3.93      | 66.23±0.22                  | 48.43±0.42                  |

*Enzyme = 1000 units each of cellulase and xylanase added to soaked peel powder and incubated at 40°C for 2 h prior to adding citric acid while in only enzyme treatment type, no citric acid was added after enzymatic pre-treatment; - means no yield; n= 3 (value±SEm).
Among both types of extraction methods, microwave assisted extraction resulted in 22% more extraction of pectin compared to aqueous one for their optimized pectin yield at 60 min of incubation. Yadav et al., (2015) found that pectin extraction (aqueous) from orange peel was optimum with extraction conditions: 85°C (temperature), 2.0 (pH) and 60 min (time of incubation). Equivalent weights for control (HCl) and citric acid treatments were found to be 625 and 416, respectively. Devi et al., (2014) carried out pectin extraction from orange peel using citric and nitric acid for different time, temperature and pH combinations and found 80°C temperature and 1.5 pH for an incubation time of 60 min to be optimum conditions for pectin extraction. Methoxyl content of extracted pectin was 5.89 (citric acid) and 5.58 (nitric acid). Khan et al., (2015) extracted pectin from sweet orange using aqueous extraction method and reported a yield of 21% using extraction conditions of 70°C temperature and 2.5 pH with 30 min of incubation. The extracted pectin had methoxyl content of nearly 70%. Similarly, Aina et al., (2012) reported orange peel to be of low methoxyl (5.79%) in nature with equivalent weight of 534. Luzio (2008) extracted pectin form orange peel (albedo) using closed vessel reactor heated with microwave irradiation. The highest yield was 17% at 110°C for 2 min at pH 1.7. Degree of methylation was 50.3% for the same. Mohamed and Hasan (1995) extracted pectin from green and yellow orange peels and found total pectin to be 16.06 and 14.48%, respectively with corresponding degree of esterification 72.5 and 73.8%, respectively. The anhydrogalacturonic acid content was found 69.49 and 68.99% respectively, for green and yellow type peel while the corresponding equivalent weight was estimated to be 920.73 and 974.60, respectively. Similarly, Yeoh et al., (2008) used microwave extraction of pectin for orange peel for 15 min extraction period at various pH values (1.5, 2.0, 5.5 and 10.0). Maximum pectin (5.27%) was extracted at pH 1.5. Megawati et al., (2015) extracted pectin from Balinese orange peel via microwave extraction and found an optimum yield of 40.5% with a power level of 300 W and extraction time of 20 min.

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