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Pectin extraction from orange peels with microwave assisted extraction technique & its characterization

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Abstract
An investigation was undertaken with orange peel powder to extract pectin and study the yield of pectin and its characters. The peel powder was mixed with 0.5M HCL maintaining 1.5 pH to create solution which was centrifuge at 4000 rpm for 15 minutes. To determine the MAE conditions practical was performed. Solution was placed in microwave oven. Parameters maintained were microwave power 540 watt for time of 2, 3, 4 minutes. The solution was irradiated in microwave and thereafter let it cool to room temperature. After pectin formation, pectin was washed by ethanol solution in 95%, 90% and 85% to get more purified form of pectin. Then it was dried in hot air oven in 70degree till dried. Among the three determination it was concluded that with 540watts for time 4 minutes gave the best yield of pectin. According to our other test highest yield was also provided with 480watts with 9 minutes.

Keywords: Pectin, microwave assisted extraction, esterification

Introduction
Alpha1, 4-D-galacturonic acid chains make up the structural heteropolysaccharide known as pectin, which is present in higher plants' main cell walls and intercellular spaces (Karbuz and Tugrul, 2021). It primarily consists of neutral sugars like L-rhamnose, L-arabinose, and D-galactose and D-galacturonic acid (Hosseini *et al*., 2015) [15]. Pectin is a colloidal, white, amorphous carbohydrate that is found in fruit, specially apples and citrus fruits. Fruit peels, also referred to as agricultural waste, include an indoors shape that can be utilised to produce a powerful, non-hazardous, easy-to-use, kinetically brief, and low-volume adsorbent. Due to its thickening and emulsifying characteristics, it's far often used in the food, cosmetic, and pharmaceutical sectors. Because of its characteristics and potential makes use of, it also significantly impacts the biopolymer enterprise. It makes up over 35% of the primary mobile walls in dicotyledonous and non-granular plant life, 2-10% within the grass, and 5% in woody tissues, making it the maximum complex polysaccharide in nature (Karbuz and Tugrul, 2021). Commercially, pectin is produced as a white to light brown powder that is mostly derived from orange fruits. They can be made from orange peels in a variety of ways, including the standard procedure and a mixture of chelators such citric acid, microwave, ultrasound, high pressure, subcritical water, enzyme use, and electromagnetic induction heating (Putnik *et al*., 2017) [2]. A polysaccharide, pectin is used in a variety of ways in the culinary, cosmetic, and pharmaceutical industries. It comes from plant cells, such as corn heads and hulls as well as fruit peels and pomace. Pectin can be utilised as a stabiliser in acidic protein drinks and enhance the mouthfeel and pulp balance in glasses made from juice. Additionally, pectin boosts the gel energy of low-calorie jams and decreases syneresis in jams and marmalades. In confectionery jellies, pectin is employed to offer a strong gel shape and a smooth bite. Pectin is normally utilized in food applications at a dosage of 0.5% to 1%. (Tiwari *et al*., 2017) [21].
In terms of each economic worth and normal manufacturing, citrus fruits lead the %. Certainly, one of them, specifically the oranges (*Citrus sinensis* L.), is a tree fruit this is extensively grown round the world. In tropical and subtropical regions, oranges are commonly grown for consumption either whole or processed to extract the juice and scent. These essential oils (fragrances) are a blend of terpenes, volatile chemicals, and oxygenated derivatives such alcohols, esters, and aldehydes (citral) (Tiwari *et al*., 2017) [21].

The citrus family (Rutaceae), which also includes other fruits including mandarins, lemons, grapefruits, and limes, includes the sweet orange. The most valuable fruit is an orange, followed by grapefruit, lemons, mandarins, and limes. Sweet orange was planted in many places throughout the prehistoric era, including the regions that are now occupied by modern China, India, Bhutan, Burma, and Malaysia. In 2015, Awanda *et al*. By 2500 BC, oranges had likely arrived from South East Asia and were being grown in China, where they were known as "Chinese apples" (Etebu and Nwauzomae, 2014) [6]. The sweet orange is a tree fruit that grows widely over the world. The possibility of extracting pectin and essential oils from orange peels is investigated in the current work. According to experimental findings, the peel source for pectin extraction yields more when used after orange oil has been extracted using a straightforward distillation method rather than leaching residue (Tiwari *et al*., 2017) [21]. Vitamins, particularly vitamin C, as well as phytochemicals are responsible for these health advantages. A single orange is said to contain more than 60 flavonoids and about 170 phytonutrients. (Yerou *et al*., 2017) [23].

Microwave-assisted extraction (MAE) has numerous benefits, such as improved extract yield, smaller equipment, lessened temperature gradients, and faster heating to extract bioactive compounds from matrices. In comparison to conventional methods of extraction, MAE takes less time to remove bioactive chemical substances. Moreover, extracting organic and organometallic compounds is selective (Cravotto *et al*., 2008). This technique likewise seems like a green technology because it uses a less natural solvent (Alupului *et al*., 2012). The present study focused on the extraction of pectin from citrus peels using microwave at different combination and the characterization of extracted pectin was determined.

2. Materials and Methodology

2.1. Raw Material Preparation

Fresh orange peels were collected from the juice shops from campus of the Lovely Professional University. These peels were then stored, dried, ground & collected in air tight aluminium pouches at refrigerated temperature until used for further extraction process.

2.2 Procedure of Experiment

Orange peels was further used for extraction of pectin using following methods.

1. All sample peels were collected.
2. Unwanted things like dirt was removed from peels.
3. Peels were cut into smaller pieces and the white peel was removed with a knife.
4. Peels were dried in cabinet dryer at 60 °C for about 3 hours (till moisture was completely removed).
5. Dried peels obtained were further grinded and converted to powder.

Experimental plan

Parameters	Description
Sample	Orange peel
Sample: Solvent (distilled water)	1:30
Microwave power levels	540W
Time	2, 3, 4 minutes
Packaging material	HDPE

2.3 Microwave Assisted Extraction Process

Using extracting agent HCl (0.5 M), 5g of dry peel powder was mixed with it. Keeping the ratio dried peel/solvent = 1:30 g/ml extraction solution was prepared. Thereafter, to determine the MAE conditions practical was performed. Solution was placed in microwave oven. Parameters maintained were microwave power 540 watt for time of 2, 3, 4 minutes. The solution was irradiated in microwave and thereafter let it cool to room temperature. (Hosseini *et al*., 2016; Gharibzahedia *et al*., 2019) [20, 7]. The procedure is also depicted in the following flowsheet.

2.4 Determination of pectin yield & analysis of sample

2.4.1. Pectin yield

The pectin yield may be calculated from the subsequent equation (Duwee *et al*. 2022)

$$\text{Pectin Yield \%} = \frac{\text{Extracted pectin (g)}}{\text{Dried orange peel powder (g)}} \times 100$$

2.4.2 Characterization of pectin and first-class evaluation of pectin powder

The extracted pectin was characterised in terms of moisture content, methoxyl content material, equal weight, anhydrouronic acid, and degree of esterification.

2.4.2.1 Determination of moisture content

Hot air oven will be used for determination of moisture content from pectin .0.8 g of sample was weighed and then dried in the oven for 3 hours at 105 °C.

This method will be based on measuring weight loss of pectin due to evaporation of water. Moisture content of pectin will be determined by the formula given below (Hamid *et al*. 2022)

$$\text{Moisture content (\%)} = \frac{\text{(M1-M2)}}{\text{(M1 - M)}} \times 100$$

Where,

M= Weight of petri dish in grams (empty).

M1= Weight of petridish with sample in grams (before drying).

M2= Weight of petridish with sample in grams (after drying).

2.4.2.2 Determination of equivalent weight

The quantity of anhydrouronic acid present and the degree of esterification are determined by the use of equivalent weight. A 250 ml conical flask containing 0.5 g of sample and 5 ml of ethanol is used. 100 ml of distilled water and 1 g of salt chloride have been introduced with a purpose to sharpen the terminal point. At final, 6 drops of phenol red and titrating it in opposition to 0.1 N NaOH. Purple was used to indicate the titration point. This neutralised solution was stored so as to measure the quantity of methoxyl. (Rose *et al*., 2012)

$$\text{Equivalent weight} = \frac{\text{Sample weight in grams}}{\text{ml of alkali} \times \text{normality of alkali}} \times 100$$

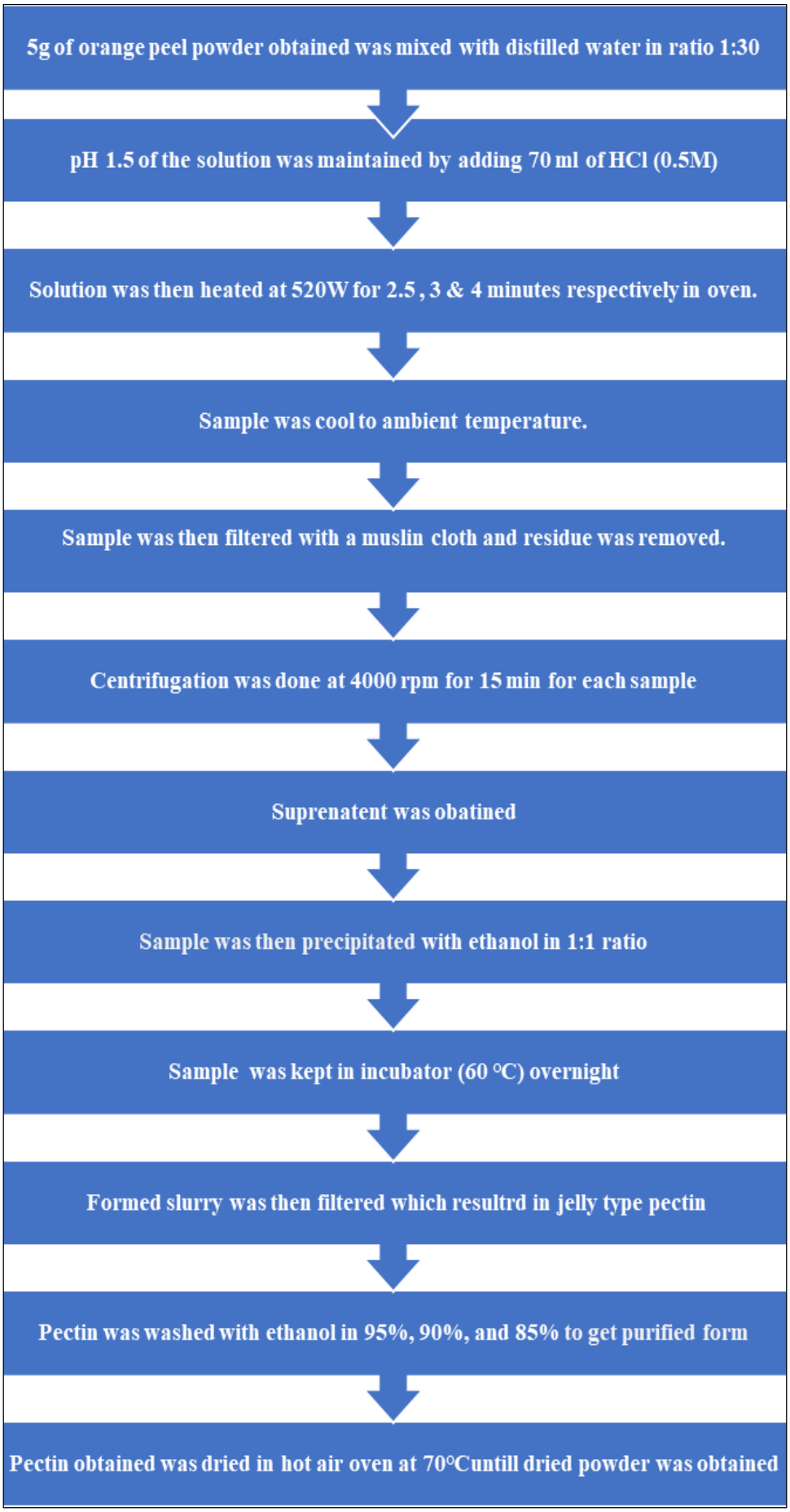

Fig 1: Microwave Assisted Extraction Process

Fig 2: Raw materials and extracted pectin

2.4.2.3 Determination of Methoxyl Content (MeO)

After saponifying the pectin and titrating the released carboxyl groups, the methoxy concentration was verified. After determining the neutral solution's equivalent weight, 25 ml of sodium hydroxide (0.25 N) was introduced. After vigorously stirring, the mixture was allowed to stand atroomtemperature for 30 minutes. After 30 minutes, 25 ml of 0.25 N hydrochloric acid was added, and the end point was measured against 0.1 N NaOH using an equivalent weight titration (Rose *et al*. 2021).

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

2.4.2.4 Determination of Anhydrouronic Acid Content (AUA)

To assess the purity and level of esterification as well as the physical qualities, an estimation of the AUA content is necessary.

Total AUA of pectin was calculated using the subsequent formula using the equivalent weight and methoxyl content value of the titrate employed (Mahmud *et al*. 2020) [12]

$$\text{AUA \%} = \frac{176 \times 0.1z \times 100}{w \times 1000} + \frac{176 \times 0.1\,y \times 100}{w \times 1000}$$

When molecular unit of AUA (1 unit) = 176.

Where,
z = ml (titre) of NaOH from equivalent weight determination.
y = ml (titre) of NaOH from methoxyl content determination.
w = weight of sample.

2.4.2.5 Determination of Degree of Esterification (DE)
Methoxyl content (MeO) and total anhydrouronic acid content (AUA) measurements were used to compute the DE of pectin using the formula (Hamid *et al*. 2022)

$$DE\ \% = \frac{176 \times MeO\%}{31 \times AUA\%} \times 100$$

Where,
176 & 31 are the formula weights of AUA & MeO respectively.
MeO = Methoxyl Content.
AUA= Anhydrouronic acid content.

3. Result and Discussion

3.1 Pectin Yield
For treatments T1, T2, and T3, the sample's pectin yield was determined to be 12.1%, 19%, and 16%, respectively. The treatment T2 yield of pectin was the highest (540W for 3 mins). Spanish grape fruit peels had a similar yield of 30.7%, as determined by Iranzo *et al*. (2012) while Indian citrus peels were reported to have a pectin output of 15-17%. Pectic polysaccharide extraction from waste mango peels, Citrullus lanatus fruit rinds, *Carcia papaya* L. peel, and pectin extraction from waste orange peels have all been found to produce similar side effects (Maran *et al*., 2015) [10].
Under the similar circumstances, MAE was able to produce pectin from kiwi peel because a higher power level of microwave produced more irradiation energy, which allowed the extraction solvent to penetrate the pectin source. The released pectin from the plant material to the solvent increased as a result of the rupture of the cell material. In addition, as the irradiation period was extended, a greater amount of heat was accumulated in the extraction solution, which accelerated pectin dissolution.

3.2 Moisture content
For treatments T1, T2 and T3, the sample's moisture content was determined to be 7%, 6%, and 4%, respectively. The treatment T2 yield of pectin was the highest. (3 minutes at 540W) Similar result was observed by (Azad *et al*. 2014). At power level 480 W and time 12 min, the moisture content was at its greatest, at 10.14%, and at power level 480 W and time 9 min, it was at 9.63% and at power level 320 W and time 3 min, it was at 5.06%. Pectin powder should have a lower water content from a safety standpoint in order to improve storage and prevent the deterioration of pectin quality caused by the production of pectinase as a result of microbial growth (Ismail *et al*., 2012).

3.3 Equivalent weight
For treatments T1, T2, and T3, the equivalent weight of the sample was determined to be 333.54 g/mol, 263.15 g/mol, and 200 g/mol, respectively. These outcomes were comparable to pectin obtained from orange peels, which varied from 318.50 to 378.80. (Hend *et al*., 2015) [8]. Although Roy *et al*. (2018) [17] indicated that the equivalent weight of the present study ranged from 540.0411.89 to 711.3313.77, Bagde *et al*. (2016) reported that it was greater than lemon (100) and orange (86.87) pectin (2017). EW was considerably influenced by fruit maturity, with overripe fruits showing lower equivalent weight (3683) than mature fruits (1632137). (Azad *et al*., 2014). EW for watermelon rind pectin was considerably reduced by increasing microwave power and prolonging the irradiation period. Additionally, citric acid might influence lowering EW. Lower EW of extracted pectin was caused by higher partial degradation, and the amount of free acid may also have an impact on equivalent weight content (Ram-Li and Nazaruddin, 2011) [16].

3.4. Methoxyl content
The Methoxyl Content of the sample was obtained to be 7.44%, 4.96% and 3.72% for treatment T1, T2 and T3 respectively. Khan *et al*. (2015) [8] used the aqueous extraction method to extract pectin from sweet oranges and reported a yield of 21% under the extraction conditions of 70 °C temperature, 2.5 pH, and 30 min of incubation. Methoxyl concentration in the isolated pectin was close to 70%. Similar to this, Aina *et al*. (2012) [3] found that orange peel had a low methoxyl content (5.79%) and an equal weight of 534. Luzio (2008) used a closed vessel reactor heated with microwave irradiation to extract pectin from orange peel (albedo). At 110 °C for 2 minutes and pH 1.7, the greatest yield was 17%. Methoxylation level for the same was 50.3%.

3.5 Anhydrouronic acid content
For treatments T1, T2 and T3, the anhydrouronic acid concentration of the sample was determined to be 130.24%, 95.04%, and 73.92%, respectively. Food and agriculture organization of the United Nations (FAO) stated that pectin ought to have more than 65% AUA. Ismail *et al*. (2012) hypothesised that a lower AUA concentration could be caused by insufficiently pure pectin extraction. This could be as a result of the extract's protein, sugar, and starch content. It is possible to detect that the AUA content of CP is larger but the DE % is lower, indicating that commercial pectin has undergone greater deesterification as a result of the higher level of degradation of neutral sugar (Fishman *et al*., 2008).

3.6 Degree of esterification
For treatments T1, T2 and T3, the sample's degree of esterification was determined to be 32.43%, 29.62%, and 28.8%, respectively. Treatment T2 had the highest degree of esterification. (3 minutes at 540W). According to earlier reports, pectin extracted by the UAE has a higher degree of esterification than pectin extracted by the MAE; the percentage of esterification of pectin extracted from tomato peel for the UAE was 66.43% and the percentage for the MAE was 59.76. (Sengar *et al*., 2020) [12]. Since the MAE's extraction of pectin from dragon fruit valued from 45.82 to 46.95% esterification, it was categorised as LMP (Rahmati *et al*. 2019) [15]. When sisal pectin was extracted using UAE, its esterification level was 44.35%, indicating that it is LMP (Yang *et al*. 2018) [22].
Hosseini *et al*. (2016) [20] investigated the MAE of pectin from bitter orange peel (SOPP). Since the SOPP isolated for this investigation displayed an esterification level of 1.7-37.5%, it can be categorised as LMP. They came to the conclusion that because these conditions increase the de-esterification of polygalacturonic chains, pectin level extracted under extremely

harsh conditions (high power, long irradiation interval) had a low degree of esterification.

Treatments / Parameters	T1 (540:2)	T2 (540:3)	T3 (540:3)
Pectin yield (%)	12.1	19	16.5
Moisture content (%)	7%	6%	4%
Equivalent Weight	333.54	263.15	200
Methoxyl Content (%)	7.44	4.96	3.72
Anhydrouronic Acid Content (%)	130.24	95.04	73.92
Degree of Esterification (%)	27.43	29.62	28.8

4. Conclusion

Pectin is a significant hydrocolloid which is frequently utilized in the food, cosmetic, and drug industries. In the current work, pectin from orange peels was successfully extracted utilizing a low-cost, high-effectiveness approach called microwave-assisted extraction method (MAE). The extraction conditions had a substantial impact on the yield and pectin characteristics. Based on DE value and anhydrouronic acid content, all of the pectin extracted was characterized as low methoxy pectin (LMP) and extremely pure pectin, respectively. However, the physical composition of pectin did not significantly alter. Thus, the commercial pectin extraction from waste orange peels by the MAE method raises economic interest. It is necessary to conduct more research on the mechanisms underlying various approaches and the optimization of pectin from diverse species.

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