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RESEARCHARTICLE

Microwave assisted extraction of pectin from dried hull of faba bean

Rafeeya Shams[1], Dorcus Masih[1], Ghousia Ashraf[1], Aamir Hussain Dar[2], Qurat ul eain Hyder Rizvi[3]

[1] Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, U.P, India
[2] Department of Food Technology Islamic University of Science and Technology Awantipora, India
[3] Eternal University, Baru sahib, Himachal Pradesh,India

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ABSTRACT

This study intended to estimate the potential uses of faba bean hull as pectin source for various commercial applications such as gelling, thickening, stabilization etc. The extraction was carried out at microwave power (160–640 W), time (3–12 min), pH (1.5) and solid: liquid ratio (1:20 g/ml). The optimal conditions of microwave assisted extraction to yield high pectin from faba bean hull powder (14.86 %) were found to be at pH 1.5, microwave power of 640 W, extraction time of 9 min, solvent: solid ratio of 1:20 g/ml respectively. The pectin extracted was characterized as high methoxylpectin with anhydrous acid content and methoxyl content of 51.38% and 4.89% respectively and degree of esterification 54.08%. The extracted pectin depicted equivalent weight, anhydrouronic acid content and methoxyl content of 746.26%, 51.38% and 4.89% respectively. As little information is present on extraction of pectin from faba bean hull. The present study revealed that faba bean hull is better and suitable pectin source and considered to be the potential raw material for various food processing applications.

Keywords: Faba bean hull, equivalent weight, methoxyl content, pectin, degree of esterification

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INTRODUCTION

Pectin being a natural hydrocolloid substance is present in primary cell walls of higher plants as calcium pectate. Pectin contains D-galacturonic acid, partly methylated galacturonic acid residues as well as neutral sugars namely D-galactose, L-arabinose L-rhamnose, and D-xylose, where the type and the amounts of various neutral sugars differ with the source of pectin (Noreen et al. 2017). The extraction method of pectin is basically a physicochemical technique in which molecules of pectin are hydrolyzed and solubilized from cell wall of tissues of plant, mainly focusing on cleaving the bonds of pectin and other components, without having detrimental effects on the pectin confirmation (Endress and Christensen 2009). The procedures employed in extraction of pectin depends on exposure of plant tissues to dilute acidic solutions at temperatures for definite period. Both physical and chemical properties of pectin are influenced by the conditions of extraction and plant source(Kjoniksen et al. 2005). Due to multifaceted gelling characteristics, pectin can make the structure in wide range of foods as well as pharmaceuticals (Tamaki et al. 2008). It is also found that pectin has various therapeutic uses like lowering hyperglycemia (Zhao et al. 2006), obesity (Kumar and Chauhan 2010), hyperlipidemia (Minzanova et al. 2018),diarrhea (Triplehorn and Millard 2002) and tumor rate (Glinsky and Raz 2009). The materials used for preparation of pectin are usually attained from by-products like citrus peels and pomace apple during food processing (Ruano et al. 2019).

* *For correspondence*: A. H. Dar (Email: daraamirft@gmail.com)

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Faba bean (*Vicia faba*) is an essential legume in the world, used as inexpensive protein source for a larger group of the population throughout the world (Barri and Shtaya 2013). Faba bean is among the most-older crops ranking 6th in legumecultivation throughout the world followed by soybean, peas beans, peanut, and chickpeas. They consist of considerable quantity of carbohydrates, proteins, B-complex vitamins as well as minerals. Their protein composition varies from 20% to 41%, depending on the cultivar. Seeds of faba bean consists of 51% to 68% of carbohydrate, the larger share of which is starch (41–53%). Faba bean seeds are also important sources of stachyose and verbascose. Both these oligosaccharides are responsible for the production of flatulence and the occurrence of such oligosaccharides in these beans is the limitation in its consumption. Księżak et al. (2017) stated that crude fiber present in faba beans range from 5.0% to 8.5%.

Kassegn et al. (2018) showed the dietary fiber values of 15–30%, depending on the seed variety, despite hemicellulose being the main component (60%). Faba beans contains considerable amount of minerals, namely potassium, phosphorus, calcium, iron and sulphur, among which the calcium varies from 120 to 260 mg/100 g on dry basis and around 40–60% of the phosphorus is available as phytates (Ma et al. 2005). Over the past years, numerous ideal pectin extraction processes are being used viz. microwave-assisted extraction, pressurized solvent extraction, and super-critical fluid extraction to recuperate valuable extracts from waste materials. Among these extraction techniques, microwave-assisted extraction is commonly used as a potential method alternative to the conventional one, due to its distinct heating mechanism, reasonable cost, and better outputs under atmospheric situations (Eskilsson and Björklund 2000). MAE technique has lesser processing time, low solvent requirement, high extraction rates also provides good product at lower cost (Maran et al. 2014). Thus, the aim of this study was to examine the impact of process parameters of the microwave on the extraction of pectin from dried faba bean hull.

MATERIALS AND METHODS

Sample preparation

Faba bean hull (5 kg) was purchased from a nearby market of Allahabad U.P, dried in a Hot air oven (MicrotechMedcraft) at 60°C till constant weight was obtained, then grinded to obtain fine powder in a grinder (Bajaj Model Rex-500) and kept in sealed HDPE packs at refrigerated temperature until further analysis.

Microwave assisted extraction of pectin

Pectin extraction was carried out as per the process given by (Wang et al. 2007) using the microwave (LG, model MCI143CB) working at frequency of 2450 MHz with varying time as well as microwave power under varying microwave assisted extraction conditions. A 500 ml beaker consisting of a mixture of 10 g of dried faba bean hull powder with 200 ml of distilled water having pH value of 1.5 was kept in the microwave oven over a moving dish at varying microwave power ranges (160, 320, 480 and 640W) for different time periods (3, 6, 9 and 12 minutes).The pH of the solvent was set by using hydrochloric acid (1.0 M HCl solution). For selected time, the process of extraction was conducted followed by cooling the mixture in a beaker to ambient temperature (25°C). After which, the solution was filtered with the help of muslin cloth. Then the sample was separated from filtered cakes and this filtered cake was removed out of the filtrate. After filtration, centrifugation was done at 4000 rpm for 30 minutes with the help of centrifuge (REMI Model R-8CBL). Centrifugation was done to separate feed into two segments having lighter phase at top and dense phase at bottom. Precipitation of supernatant was done with 2 volumes of 95% (v/v) ethanol and was kept at optimal temperature (20ºC) and filtered after 4 hrs. The obtained precipitate was then washed thrice using 65, 85 and then 100 % ethanol to remove the mono and disaccharides (Koh, et al. 2014) followed by drying the extracted pectin in hot air oven (Microtech Medcraft) at 50 °C till constant weight was obtained. The dried pectin was then ground in mortar and pestle and kept in HDPE packets at refrigerator (4°C) for further analysis. The whole process was done in triplicates.

Pectin yield

The pectin yield (PY) was calculated using the formula given by Li et al. (2012)**.**

$$\text{Pectin yield(PY)} = \frac{M_p}{M} \times 100 \quad (1)$$

Where; PY = Pectin yield in percent (%), M_P = Weight of dried pectin (g), M = Weight of dried faba bean hull powder (g)

Characterization of pectin

Determination of moisture content: The moisture content was obtained by AOAC(1995) method. Theweight known amount of sample was taken in a pre-weighed dry and clean petri-plate, followed by drying at 103±2°C for 24 hrs or until the constant weight using a hot air over (made by Microtech Medcraft with the chamber size of 30x30x30 (HWD in cms). The percent moisture was obtained from the Eq. 2

$$\text{Moisture Content (\%)} = \frac{M_1 - M_2}{M_1 - M} \times 100 \quad (2)$$

Where; M= Weight of empty petri-dish (g), M1&M2= Weight of dish with sample before and after drying (g) respectively

Determination of ash content: Ash content was obtained using AOAC (1995) method, 1 gram of pectin sample was taken and ground in a clean, dry and tarred crucible. The sample was then ignited slowly, followed by heating for 3-4 hours at 600 ºC. The crucible was cooled to ambient temperature using desiccator and weighed again. The procedure was continued till constant weight was obtained

Ash content was measured by the given equation:

$$\text{\% Ash} = \frac{\text{Weight of Ash (g)}}{\text{Weight of sample (g)}} \times 100 \quad (3)$$

Equivalent weight : Equivalent weight was obtained using AOAC(1995) methods, 0.5 g sample was kept in a 250 ml conical flask. 5 ml ethanol, 1 g of NaCl (to increase the end point sharpness) and 100 ml of distilled water were added to it. After which, few drops of phenolphthalein indicator were added and titrated against 0.1 N NaOH till purple color was observed. This neutral solution was then used to determine the Methoxyl content.

Equivalent weight was determined by the given formula:

$$\text{Equivalent Weight} = \frac{\text{Weight of sample (g)} \times 1000}{\text{mL of alkali} \times \text{Normality of alkali}} \quad (4)$$

Methoxyl content*:* Methoxyl Content was obtained following the standard method of AOAC(1995). The neutralized solution obtained from equivalent weight formula was taken and 25 ml of sodium hydroxide (0.25 N) was added to it. The solution was thoroughly agitated and left undisturbed at ambient temperature for 30 min. After which, 25 ml of 0.25 N HCl was added and titrated against 0.1 N NaOH to the similar end point as that of equivalent weight titration.

Methoxyl content was measured using the equation:

$$\text{Methoxyl content \%} = \frac{\text{mL of alkali} \times \text{Normality of alkali} \times 3.1}{\text{Weight of samle}} \quad (5)$$

Total anhydrouronic acid content*:* Anhydrouronic Acid Content was determined by making use of the equivalent weight and methoxyl content value of titre obtained. Total AUA of pectin was measured using the equation (Yadav et al. 2017)

$$\%\ \text{of AUA} = \frac{176 \times 0.1z \times 100}{w \times 1000} + \frac{176 \times 0.1y \times 100}{w \times 1000} \quad (6)$$

When molecular unit of AUA (1unit) = 176

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

Degree of esterification: The DE of pectin was obtained based on methoxyl and AUA content (Aina 2012) and obtained by equation as:

$$\%DE = \frac{176 \times \%\ MeO}{31 \times \%\ AUA} \times 100 \quad (7)$$

Where; % MeO = Methoxylcontent, % AUA = Anhydrouronic Acid Content

Statistical analysis

Statistical analysis was carried out using the data obtained from four levels of power level for four levels of time periods i.e. from 16 treatments and 3 replications and was analyzed statistically by Analysis of Variance technique - ANOVA Two way classification with 'm' observations per cell using SPSS 16.0 The significant effect of treatments were determined with the help of 'F' (variance ratio). Calculated F values were compared with that of tabulated F values at 5% level of significance.

RESULTS AND DISCUSSION

Effect of microwave processing power levels on pectin extraction yield

The extraction of pectin showed an increasing trend with rise in microwave power levels from 160 - 640 W. The rise in microwave energy enhances the diffusion of solvent within the plant matrix and reaches adequately to material by the molecular interaction with electromagnetic field, causing faster energy transfer to the matrix and solvent and the dissolution of compounds to be extracted **(**Yan et al. 2010**).** Water has the potential to absorb microwave energy due to its polarity leading to effective heating. However, the microwave radiation caused rupturing of cells due to sudden rise in pressure as well as temperature within the plant sample cell promoting sample surface destruction and hence the release of pectin within the plant matrix into the solvent **(**Zhang et al.2008**)** and enhanced the yield of extraction.

Table 1: Different variable considered during microwave extraction

Model Parameter	160W	320W	480W	640W
Linear				
M	0.25367	1.09567	1.32211	Not fit
C	-0.51833	-1.45167	-2.26833	Not fit
R^2	0.89048	0.93305	0.94333	Not fit
Power				
A	0.25404	0.97487	1.03597	1.04108
R^2	0.33067	0.92466	0.92807	0.20945
Exponential				
K	0.065	0.210	0.223	Not fit
R^2	0.50153	0.81307	0.83093	Not fit

Effect of time on pectin extraction yield

In this study, the extraction was done at varying time intervals (3, 6, 9, 12 min) so as to choose a specific time to increase the pectin yield. The microwave assisted extraction method depicted that there was a linear relationship between the time and extraction yield. The more exposure time causes increase in extraction yield, while as further increase in time i.e. above 9 minutes, showed no improvement in the results, but in turn caused reduction in the extraction yield. From such results, it was

found that yield of extraction raised significantly and reached maximum at 9 min and then reduced slowly. This process may describe the fact of thermal accumulation into extraction solution because of microwave energy absorption that increased the dissolution of pectin into solution till 9 min and then reduced the yield up to 12mins. Prolonged time periods may cause degradation of pectin chain molecules, hence, influencing the rate of pectin extraction **(**Maran et al. 2013**).**

Effect of solid–liquid ratio on pectin extraction

It was evident from previous studies that the pectin yield was increased up to the solid-to-liquid ratio of 1:20 beyond which there was decrease in the pectin yield. Hence no changes were done in solid-to-liquid ratio. The water has the potential to absorb microwave energy and results in increase in swelling of plant matrix, that rises the surface area contact between the solvent (water) and plant matrix, leading to the increase in pectin extraction yield up to solid–liquid ratio of 1:20. But, increased solvent may reduce the microwave adsorption of components, as additional energy will be absorbed by the solvent. Thus, the rupturing of the cell wall material and mass transmission may be destructively affected **(**Li et al. 2010**)** and reduced the pectin yield. The highest pectin yield was obtained by microwave assisted extraction (14.86%) at power level 640W and time 9min followed by microwave assisted extraction at (12 min at 480 watt) (13.18%) at power level 480W and time 12min and least was obtained at (6 min at 160 watt) (1.4%) at power level 160W and time 6min.

Equivalent weight of pectin extracted

The equivalent weight of isolated pectin was observed in the range of 700 to 1008.3 at 160 W, 515.46 to 854.1 at 320 W, 521 to 952.38 at 480 W, and 550 to 746.6 at 640 W as shown in **Table 2** this study was favored by Shah et al. (2013). The equivalent weight of extracted pectin was observed in the range of 515.46 to1008.3 against 493 for commercial pectin. From literature, equivalent weight varies with the process and the product nature used in extraction. The highest Equivalent weight of 1008.3 was found at power level of 160 W and time 6 min followed by 952.38 at power level 480 W and time 3 min and least (515.46) was obtained at power level of 320 W and time 9 min. This study shows that the extraction of pectin for longer period causing pectin degradation is due to greater un-esterified galacturonate acid (IPPA 2014). The high equivalent weight found in present study might be because of the nature of extraction process, lower partial degradation of pectin, or it may also depend on the proportion of free acid (Kumar and Chauhan, 2010). Equivalent weight of pectin signifies anhydrouronic acid content as well as degree of esterification. Pectin with more equivalent weight has high gel forming capacity whereas the low equivalent weight pectin causes pectin degradation partially. The high or low equivalent weight depends on the quantity of free acids (Ramli and Asmawati 2011)**.**

Table 2: Physicochemical analysis of pectin

Time (min)	160 W	320 W	480 W	640 W
Equivalent weight (%)				
3	No pectin extracted	854.10±0.11d	952.38±0.13d	633.33±0.09b
6	1008.30±0.3c	731.50±0.11c	757.57±0.09c	550.00±0.11a
9	772.70±0.5b	515.46±0.12a	714.28±0.09b	746.26±0.09d
12	700.20±0.12a	526.31±0.11b	521.51±0.11a	718.75±0.12c
Methoxyl content (%)				
3	No pectin extracted	10.19±0.06	10.38±0.04	6.68±0.04
6	10.23±0.04	7.79±0.04	4.46±0.03	7.35±0.03
9	12.38±0.07	6.06±0.04	4.65±0.03	4.89±0.02
12	12.79±0.05	4.33±0.03	6.16±0.03	4.91±0.03
Anhydrouronic acid content (%)				
3	No pectin extracted	78.90±0.15	77.44±0.19	65.67±0.11
6	75.63±0.21	68.36±0.14	48.57±0.14	73.60±0.11
9	93.10±0.20	68.60±0.17	51.04±0.11	51.38±0.12
12	98.00±0.28	58.08±0.13	68.76±0.13	52.41±0.13

Methoxyl content of pectin powder

The Methoxyl content of isolated pectin was found in the range of 4.34 to 12.8 % against 3 % for commercial pectin. The Methoxyl content of pectin obtained from faba bean hull powder using different power levels and times was obtained to be in the range of 10.24 to 12.8 % at 160 W, 4.34 to 10.2 % at 320 W, 4.46 to 10.38 % at 480 W, and 4.89 to 7.32 % at 640 W as shown in **Table 2.** Azad et al. (2014) has also reported similar results**.** The pectin extracted in present study was characterized as high methoxyl pectin depending on power level used, Methoxyl content value reduced with rise in irradiation time. Commercial pectin which contains Methoxy content 6 % or above is referred as high Methoxyl content and less than 6 % referred as low Methoxyl content. Moreover, the methoxyl content shows dissolution of pectin in water anditsgel forming capability (Castillo-Israel et al. 2015). At commercial level, a high methoxyl pectin (usually 8-11 %) forms gel at a more sugar content (>65% sugar), where as a less methoxyl pectin (below 7 %) forms gel at a lower sugar content (Broomes and Badrie 2010). The highest Methoxyl content was obtained at 10.38 % at power level 480 W and time 3 min followed by 10.24 % at power level 160 W and time 6 min and least of 4.34% was obtained at power level 160 W and time 12 min. Methoxyl number shows free esterified carboxyl group in the pectin and affects the gel formation. Methoxyl content is an essential parameter to determine the formation of gel capability and in influencing the setting time of pectin. Variation in methoxyl content is based on the nature of product and method used in extraction (Constenla and Lozano 2003).

Anhydrouronic Acid (AUA) content of pectin powder

The AUA content of isolated pectin was observed in the range of 75.63 to 98 % at 160 W, 58.08 to 78.9 % at 320 W, 48.57 to 77.44 % at 480 W, and 51.38 to 73.6 % at 640 W as shown in **Table 2,** this study was supported by Kumar and Chauhan (2010). The AUA represents the purity of the pectin and it must be more than than 65 % (Azad et al.2014)**.** Lower values of AUA indicates that the extracted pectin may contain greater quantity of protein, sugars and starch in the precipitated pectin **(**Ismail et al.2012**).** The highest Anhydrouronic acid content was obtained at (98%) at power level 160 W and time 12min followed by T3 (93.1%) at power level 160W and time 9min and least was obtained at T10 (48.57%) at power level 480W and time 6 min.

Table. 3. Physicochemical analysis of pectin

Time (min)	160W	320W	480W	640W
Degree of Esterification				
3	No pectin extracted	73.3 ± 0.26^{dB}	76.09 ± 0.06^{cC}	57.75 ± 0.19^{cA}
6	76.85 ± 0.12^{Cd}	64.70 ± 0.20^{cC}	52.15 ± 0.16^{bA}	56.46 ± 0.21^{bB}
9	75.62 ± 0.37^{bC}	50.23 ± 0.21^{bA}	51.72 ± 0.19^{aA}	54.08 ± 0.07^{aB}
12	74.15 ± 0.09^{aC}	42.42 ± 0.19^{aA}	50.86 ± 0.11^{aB}	53.18 ± 0.09^{aB}
Moisture Content				
3	No pectin extracted	5.06 ± 0.05^{aA}	8.90 ± 0.070^{aC}	6.54 ± 0.10^{aB}
6	5.91 ± 0.06^{aA}	5.10 ± 0.08^{aA}	9.32 ± 0.15^{bC}	6.83 ± 0.11^{aB}
9	6.22 ± 0.08^{aB}	5.18 ± 0.10^{aA}	9.64 ± 0.12^{bD}	7.63 ± 0.09^{bC}
12	6.43 ± 0.08^{bB}	5.20 ± 0.08^{aA}	10.14 ± 0.12^{cD}	7.31 ± 0.14^{cC}
Ash Content				
3	No pectin extracted	4.51 ± 0.20^{aA}	5.41 ± 0.05^{aB}	3.91 ± 0.03^{aA}
6	2.800 ± 0.16^{aA}	4.70 ± 0.10^{aB}	5.73 ± 0.06^{aC}	4.35 ± 0.05^{bB}
9	2.937 ± 0.05^{aA}	4.91 ± 0.05^{bB}	6.05 ± 0.06^{bC}	4.87 ± 0.11^{cB}
12	3.237 ± 0.17^{bA}	5.26 ± 0.09^{cB}	6.60 ± 0.08^{cC}	4.67 ± 0.07^{bB}

Time= small alphabet, Power level = capital alphabet

Moisture content

Moisture content decreased with increase in extraction time and microwave power levels. The extraction process of 3 to 12 min at constant microwave power as well as with the increase in microwave power level from 160 to 640 W decreased the moisture content. Moisture content influences the stability of pectin, where high moisture content reduces the pectin quality and causes proneness to microbial load (IPPA 2014). The moisture content of pectin obtained from faba bean hull powder using different power levels and time was observed to be in the range of 5.06-10.14% as shown in **Table 3**. Similar result was observed by **(**Azad et al. 2014**).** The highest moisture content 10.14 % was obtained at power level 480 W and time 12 min followed by 9.63 % at power level 480 W and time 9 min and least (5.06 %) was obtained at power level 320 W and time 3 min.From safety point of view, pectin powder should contain less water content for enhanced storage and avert the degradation of pectin quality due to formation of pectinase due to microbial growth (Ismail et al. 2012).

Ash content

Ash content designates purity level of pectin, where high pectin purity is depicted by less ash content. The ash content in pectin powder is affected by extraction methods and the occurrence of inorganic matter residues present in faba bean hull (Kalapathy and Proctor 2001). The ash content of isolated pectin from faba bean hull powder using different power levels and times was observed to be in the range of 2.81-6.6 % as shown in **Table 3.** As reported from literature ash content may vary with the process and nature of the product used in the extraction. A quantity of 10 % ash content is referred as good quality pectin from the gel formation point of view. Hence the pectin extracted in present study can be regarded as of good quality (Ismail et al. 2012; Azad et al. 2014). Ash content showed increment risewith rise in extraction period. High ash content was achieved at microwave power level from 160 to 640 Watt. Higher power levels and extraction period caused more pectin ash content (Sarah et al. 2018). The highest ash content was found 6.6 % at power level 480 W and time 12 min followed by 6.05 % at power level 480W and time 9 min and least (2.81%) was obtained at power level 160 W and time 6 min.

Degree of esterification of pectin powder

Galacturonate levels in pectin molecules have a significant role in defining the functional characteristics of the pectin and influencing the texture and structure of the gel formation process (Wachida and Yunianta 2005). Galacturonic acid is the mostessential parameter for characterizing pectin other than that of neutral sugar content, molecular weight distribution and degree of esterification (Canteri et al. 2012). Higher galacturonic acid and low ash content depicts the pectin purity (Liang et al. 2012). The Degree of Esterification (DE) is basically the ratio of the esterified Galactouronic acid groups to the total Galactouronic acid groups present. The DE of pectin extracted from faba bean hull powder using different power levels and times was found to be in the range of 74.15 to 76.86% at 160 W, 42.42 to 73.3% at 320W, 50.86 to 76.09% at 480W, and 53.18 to 56.46% at 640W as shown in **Table 3,** this study was supported by Sundar Raj et al. (2012). The DEfor isolated pectin was observed to be in the range of 42.42 to76.86 % against 89.64% for commercial pectin. Depending on DE, pectin is classified as low methoxyl pectin whose DE is less than 50%. DE decreases with maturity. High Methoxyl Pectin (Degree of methylation above 50%) makes gels via hydrophobic interactions as well as hydrogen bonds under optimal conditions i.e., pH≤3.5 and higher sugar content (>55%) (Kastner et al. 2014). Also, the gelation phenomena of Low Methoxyl Pectin (Degree of Methylation <50%) is due to the cross-linking of HG chains through Ca2+ bridges (calcium ions) to the pectin solution (Han et al. 2017).The pectin extracted from faba bean hull powder could be classified as high Methoxyl pectin as it has a percentage of DE which is more than 50%.The highest DE (76.86%) was obtained at 160 W and time 6 min followed by 75.61 % at power level 160 W and in 9 min and minimum of 42.42% was obtained at 320 W in12 min. Hence, microwave assisted extraction proves to be a significant extraction process in maintaining quality of pectin.

CONCLUSION

This study suggested that the microwave assisted extraction of pectin from faba bean hull showed better results in terms of pectin yield as compared to traditional methods. The study indicated that the pectin extraction at low power levels gave good yield. The results also infer that the extracted pectin was of better quality and of high purity, which is ascribed to its higher degree of esterification (greater than 50%).

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