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Qualitative Analysis of Pectin Extracted Ultrasonically from Sweet Lime Peel

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Abstract: In this study, ultrasound-assisted extraction of pectin from sweet lime peel was optimized by Box-Behnken design using a response surface methodology (RSM). The effect of independent factors such as solid-solvent ratio (1:10-1:30 g/ml), sonication time (15-35 min) and ultrasonic power intensity (48-80 W/cm^2) on the anhydrouronic acid concentration and degree of esterification was studied. The process factors had a significant effect on all responses of pectin extracted from the sweet lime peel. Under optimum conditions (solid-solvent ratio of 1:24.3 g/ml, sonication time of 18.4 min, ultrasonic power intensity of 80 W/cm^2), the highest degree of esterification (62.9%) and anhydrouronic acid content (64%) were obtained. The current study indicate that extracting pectin from sweet lime peel was an efficient strategy to minimize the current waste disposal problems.

Keywords: Pectin, Ultrasound assisted extraction, Anhydrouronic acid, Esterification

Agricultural waste management for economic, social, environmental and sustainable development is one of the major concerns. Agricultural waste comprises residues of raw agricultural products such as fruits and vegetable wastes (peels, pulp, seed, etc.), crop wastes (stalks, bagasse, prunings), dairy products, and meat and poultry wastes (bones, skin, feathers). From the agriculture phase up to industrial manufacturing, processing and distribution, and household consumption, each phase of the food life cycle involves food waste production. The required actions must be done to lessen agricultural waste and find the best viable approach to manage the trash that remains. Usually, agro wastes are thrown in landfills or discarded as animal feed. However, these methods provide a low economic and environmental value. The aim of the right waste management is not just to reduce the waste volumes, yet to utilize it in different effective ways. Waste valorization, the method of turning waste into more useful commodities is an efficient method for managing waste materials. The high-value products like antioxidants, pigments, protein, sugar, enzymes, films, nutraceuticals, etc can be extracted from food waste (Sindhu et al 2019). Bioactive compounds from fruits and vegetables can also be used as dietary supplements which can be responsible for most health impacts (Walia et al 2022).

Citrus is the most abundant horticultural produce, popularly grown worldwide. The citrus processing industries are responsible for the production of a significant amount of citrus waste, primarily in the form of citrus peel waste, which alone accounts for more than 50% of the wet fruit mass which has negative impacts on the environment. The citrus waste consists of valuable byproducts that can be utilized for the extraction of valuable compounds like carotenoids, flavonoids, dietary fibre, polyphenols, sugars, essential oils and ascorbic acid, along with some trace elements (Sharma et al 2017). Thus, the valorization of citrus processing waste for some value-added products has become a necessity. Sweet lime (*Citrus limetta*) is a citrus species belonging to the family of “Rutaceau”. Sweet lime is India's third most popular fruit. Sweet lime juice in India is a popular citrus drink, but the peel is discarded after the juice is extracted. Citrus peel is a good source of natural antioxidants, food flavours, dietary fibre and colorants, therefore the discarded peel can be used to extract a variety of value-added products. Peels of various citrus fruits can be extracted for phenols, flavonoids, and pectin, which can then be used in the food sector and give both economic and environmental benefits. Pectin is a heterogeneous carbohydrate consisting linear chain of α-(1,4) galacturonic acid residues that are partially esterified with methyl alcohol or acetic acid in a carboxylic acid, and is present in the intermediate lamellae and cell walls of many fruits and vegetables. It is used as stabilizing, gelling and thickening agent in food items such as confectionery, jams and jellies and fruit juice (de Oliveria et al 2016). Pectin might be extracted from a variety of citrus peels. It can also be utilised as a biodegradable surfactant and emulsifier, a chelating agent in detergents, a rheological modifier, and edible packaging (Liew et al 2019). It was observed that edible coating prepared from the polysaccharide extracted by food waste is a cost effective solution of waste management (Gupta et al 2020). Valorisation of citrus waste for pectin production is an efficient solution for waste management. Citrus waste, mainly in the form of citrus peels is one of the common problems faced in the juice processing sector and if these peels are left untreated, they may cause environmental pollution. The utilization of citrus waste to extract bioactive compounds and pectin is the best solution for this problem. Extraction is a crucial step in the separation of value-added products from raw materials. The development of novel extraction methods offer safer, compact, efficient, energy-saving and viable extraction processes. Green technologies such as supercritical fluid extraction, ultrasound extraction, microwave extraction, controlled pressure drop process and subcritical water extraction are typically employed in extraction methods that consume less solvent and energy (Sharma et al 2017). Ultrasound-assisted extraction is an effective non-thermal process than conventional heating methods for extraction due to low energy consumption, shortened treatment time, less solvent usage, increased safety of the operators, and increased yield (Chemat et al 2017). Ultrasound waves at a frequency of more than 20 kHz move through matter, generating cycles of shearing and compression that lead to the development of cavitation bubbles. The ultrasonic then cause cell rupture by collapsing the cavitation bubbles near cell walls, resulting in stronger and improved solvent entry into the cells and intensification of mass transfer (Tiwari 2015, Siddiqui and Chand 2022). The purpose of the present study is currently focused on implementing effective extraction technique which helps in the valorization of citrus peel, improving processing efficiency, increasing profitability and turning it into valuable products.

MATERIAL AND METHODS

Sweet lime peels (10 kg) were acquired from the local market of Pantnagar, Uttarakhand. Sweet lime peels were separated, rinsed, and dried for 48 hours in a tray dryer at 60°C. 2-3 kg of dried sweet peel was ground and sieved into a fine powder of 300 microns. Prior to the studies, the obtained sweet lime peel powder was stored in zip-lock plastic bags. Chemicals of analytical grade were used in the research. To generate an acidic medium with a pH of 1.8 for pectin extraction, citric acid was combined with deionized water.

Extraction of pectin by ultrasonication: The ultrasonication procedure was done for pectin extraction from the peels of sweet lime as mentioned by Siddiqui et al (2021).The extracted pectin in powdered form was then further used for the qualitative analysis (Table 1).

Qualitative Analysis of Pectin

Estimation of total anhydrouronic acid content: The anhydrouronic acid content of pectin was calculated by using equivalent weight and methoxyl content values. Anhydrouronic acid content was calculated (Ranganna 1951):

$$\mathrm{AUA}(\%) = \frac{176 \times 0.1\mathrm{z} \times 100}{\mathrm{w} \times 1000} + \frac{176 \times 0.1\mathrm{y} \times 100}{\mathrm{w} \times 1000}$$

Where, Molecular unit of AUA (1 unit) = 176 g, z = ml (titre) of NaOH from equivalent weight determination, y = ml (titre) of NaOH from methoxyl content determination and w = weight of the sample

Estimation of degree of esterification: Degree of esterification of pectin was measured on the basis of methoxyl and AUA content (Owens et al 1952) and calculated by the expressiongiven below:

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

Where, %MeO = % Methoxyl content and %AUA = Anhydrouronic Acid Content

Statistical analysis: Box Behnken Design of Response Surface Methodology having three factors, three levels (-1, 0, 1) was used to evaluate and optimize the effect of independent variables on the quality of extracted pectin (Table 1). The independent variables considered were solid-solvent ratio (1:10, 1:20 and 1:30 g/ml), sonication time (15, 25 and 35 min) and ultrasound power intensity (48, 64 and 80 W/cm^2). Design Expert software 10.0.1 was used that designed a total of seventeen experiments with five centre points. It was used for the regression analysis of collected data to ascertain how independent and dependent variables relate to one another. The second-order polynomial regression model was applied and the equation is presented as:

$$\mathrm{Y} = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^n + \sum_{i=1}^{n} \sum_{i=1}^{n} \beta_{ii} X_i X_j \quad (1)$$

Where, β_0, β_i, β_{ii} = regression coefficients, X_i and X_j = independent parameters (where, i = 1, 2, …n and j = 1,2 …n), n = number of independent parameters (n=3) and Y = predicted response

The model developed for each determination was then assessed for significance and lack-of-fit, the response surface graphs were plotted after removal of non-significant variables.

RESULTS AND DISCUSSION

Degree of esterification and anhydrouronic acid content: The higher anhydrouronic acid content indicates the maximum purity of pectin, which is recommended to be at least 65% (Ramachandran et al 2017). However, the anhydrouronic acid content of the pectin obtained under all of the extraction conditions ranged from 62.4 to 70.3%. The maximum anhydrouronic acid content of 70.3% for the pectin was in the experiment 17. It was due to the presence of more galacturonic acid residues which is the major uronic acid in the pectin structure (Kurita et al 2008). However, the minimum anhydrouronic acid of 62.4% was obtained in the experiment 4. This decrease in anhydrouronic acid content was due to the higher sonication time. The prolonged ultrasound treatment leads to lower anhydrouronic acid content. The result was in accordance with the previous study (Grassino et al 2016). The degree of esterification of the pectin ranged from 55.8 to 64.6%. The highest degree of esterification of 64.6% for the pectin was obtained in experiment 4. This might be because of the higher levels of ultrasound power intensity and sonication time due to which enhanced cavitation effects yield the maximum amount of pectin that have a higher amount of methyl ester groups. However, the minimum degree of esterification of 55.8% for the pectin was in experiment 17. This decrease might be due to depolymerization of pectin chains at a higher solid-solvent ratio which in turn decreased degree of esterification. The result showed that degree of esterification was higher than 50% which showed the presence of high methyl ester pectin.

Optimization of degree of esterification and anhydrouronic acid content: The second-order polynomial regression model was statistically significant in both the responses showing that the extraction factors had a considerable impact on the responses (Table 2). Furthermore, the non-significant lack of fit value for regression models demonstrated that the regression model equation was acceptable to reflect the anhydrouronic acid content and esterification degree. In addition, the coefficient of determination (R^2) for a degree of esterification and anhydrouronic acid content was 0.9899 and 0.9916 respectively, thus the regression model could account for 99.16 and 98.99% of data respectively. The gap between the predicted and adjusted coefficient of determination should be less than 0.2, the appropriate precision should be larger than 4, and the coefficient of variation should not exceed 10% to improve the model's appropriateness. In this situation, the "Pred R^2" of 0.9368 and 0.8861 for anhydrouronic acid concentration and degree of esterification were in reasonable agreement with the Adj R^2 of 0.9808 and 0.9768, respectively. For anhydrouronic acid concentration and

Table 1. Box-Behnken design (BBD) with the observed values for anhydrouronic acid content and degree of esterification

Factors	Unit	Level of factors		
		-1	0	1
Solid-solvent ratio (X_1)	g/ml	1:18	1:24	1:30
Ultrasound power intensity (X_2)	W/cm^2	48	64	80
Sonication time (X_3)	min	15	25	35

Exp. No.	Solid-solvent ratio (X_1, g/ml)	Ultrasound power intensity (X_2, W/cm^2)	Sonication time (X_3, min)	Anhydrouronic acid content (%)	Degree of esterification (%)
1	1:24	48	35	63.5	61.7
2	1:30	64	35	62.6	62.6
3	1:24	64	25	66.0	60.2
4	1:24	80	35	62.4	64.6
5	1:24	64	25	65.2	60.9
6	1:24	48	15	65.2	60.1
7	1:24	64	25	66.0	60.3
8	1:24	80	15	63.2	63.8
9	1:24	64	25	65.9	60.3
10	1:18	80	25	70.0	56.8
11	1:30	64	15	68.8	56.1
12	1:24	64	25	66.0	60.2
13	1:18	64	35	68.0	56.8
14	1:30	80	25	63.8	62.3
15	1:18	64	15	64.8	59.6
16	1:18	48	25	66.9	56.8
17	1:30	48	25	70.3	55.8

degree of esterification, adequate precision values of 31.843 and 29.521, as well as a coefficient of variation of 0.68 per cent, were determined, confirming the accuracy adequacy of the model. The observed and projected values were strongly correlated, as indicated by the coefficient of determination (R^2) and adjusted determination coefficient (Adj R^2) values that were both near 1.

The surface models for the degree of esterification and anhydrouronic acid content of extracted pectin using ultrasonic aided extraction technique were developedbased on these findings and were given as below:

Anhydrouronic acid content (%) =
$$65.69 - 0.53\,X_1 - 0.82\,X_2 - 0.67\,X_3 - 2.38\,X_1X_2 - 2.32\,X_1X_3 + 2.18\,X1^2 - 1.98X3^2 \quad (2)$$

Degree of Esterification (%) =
$$65.39 - 0.84\,X_1 - 1.64\,X_2 - 0.74\,X_3 - 1.65\,X_1X_2 - 2.29\,X_1X_3 + 3.12\,X1^2 + 0.65X2^2 + 1.49X3^2 \quad (3)$$

All the independent variables viz solid-solvent ratio (X_1), ultrasonic power intensity (X_2) and sonication time (X_3) had a significant effect on both responses. Linear coefficients (X_1, X_2 and X_3) of the model were significant at a 1% level of significance ($p < 0.01$). Quadratic coefficients (X_1^2 and X_3^2) and interaction coefficients (X_1X_2 and X_1X_3) of the regression model of anhydrouronic acid content were significant. However, the quadratic coefficient (X_2^2) and interaction coefficient (X_2X_3) of the regression model of anhydrouronic acid content were non-significant. Similarly, for the regression model of degree of esterification, quadratic coefficient (X_1^2, X_2^2, X_3^2) and interaction coefficients (X_1X_2 and X_1X_3) were significant. However, the interaction coefficient (X_2X_3) of the regression model of the degree of esterification was non-significant.

Response surface graphs of anhydrouronic acid content and degree of esterification: The effects of all three processes on the degree of esterification and anhydrouronic acid content were examined and illustrated by three-dimensional (3D) response surface plots (Fig. 1). Figure 1A depicts the interactive effects of ultrasound power intensity and solid-solvent ratio on anhydrouronic acid content at an optimum level of sonication time (18.4 min) and with the increment in solid-solvent ratio, anhydrouronic acid content increased. However, higher anhydrouronic acid content was observed at the lower level of ultrasound power intensity and a higher level of solid-solvent ratio. This increase in anhydrouronic acid content has been attributed to the hydrolysis of protopectin (Nazaruddin 2011). It was observed that with higher solid-solvent ratio more protopectin gets hydrolyzed to soluble pectic substances and thus increased galacturonic acid residues. At lower level of solid-solvent ratio (1:18 g/ml), with the increase in sonication time from 15 to 35 min, the anhydrouronic acid content increased and reached the maximum at the higher levels of sonication time (Fig. 1B). With the further increase in solid-solvent ratio, there was a slight increase in the anhydrouronic acid content over the entire range of sonication time. The higher levels of both the variables resulted in decrease of anhydrouronic acid content. This decrease may be because of some other constituents that might be extracted along with pectin at the higher levels of both the variables which reduced the purity of pectin and hence decreased the anhydrouronic acid content.

Degree of esterification is also another crucial consideration to determine the quality characteristics of the pectin extracted from sweet lime peel powder. The percentage degree of esterification (% DE) of pectin indicates whether the pectin extracted has high methyl ester pectin (HM pectin- DE>50%) or low methyl ester pectin (LM pectin- DE<50%). This is important in determining the type of gel. The combined effects of solid-solvent ratio and ultrasound power intensity on degree of esterification at the optimum level of sonication time (18.4 min) (Fig. 1C). Degree of esterification increased with the increase in ultrasound power intensity (from 48 to 80 W/cm^2) and solid-solvent ratio (from 1:18 to 1:26 g/ml), overall higher degree of esterification is obtained at the central level of solid-solvent

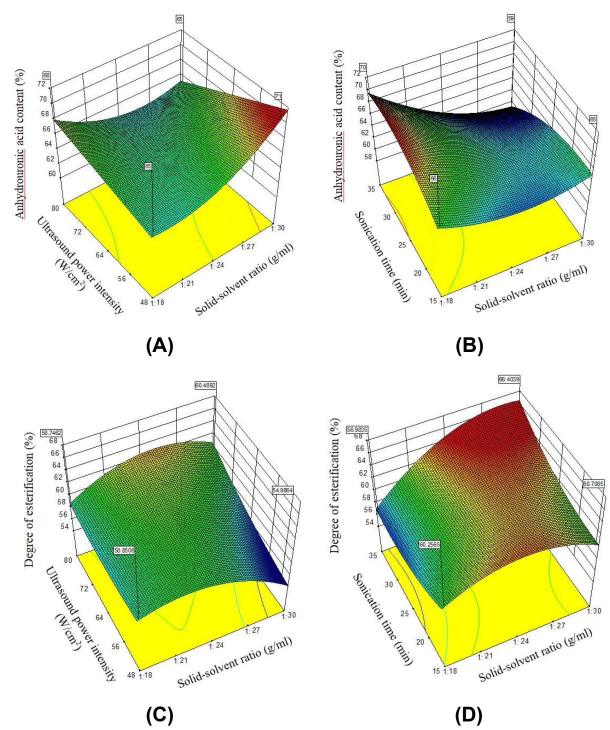

Fig. 1. Three dimensional plots (3D) showing the effect of processing variables on anhydrouronic acid content and degree of esterification

Table 2. Statistical analysis of regression model of responses

Source	Responses	
	Anhydrouronic acid content	Degree of esterification
Model	Significant	Significant
R^2	0.9916	0.9899
Adj R^2	0.9808	0.9768
Pred R^2	0.9368	0.8861
Adeq Precision	31.843	29.521
C.V. %	0.50	0.68

ratio and higher levels of ultrasound power intensity. The increase in degree of esterification was due to the sufficient solubilization of pectin at this level of solid-solvent ratio. However, at the higher solid-solvent ratio degree of esterification was decreased. This decrease was due to the dissolution of pectic substances at a higher level of solid-solvent ratio which in turn reduced the amount of esterified carboxyl group. Thus, degree of esterification was decreased.At the interactive level, the 3D surface plot shows the combined effects of sonication time and solid-solvent ratio on the degree of esterification at optimum level of ultrasound power intensity (80 W/cm^2) (Fig. 1D). The degree of esterification increases with increment in both solid-solvent ratio and sonication time. A higher degree of esterification was observed at the central level of solid-solvent ratio when the sonication time was increased from 15 to 35 min. This shows the presence of a more esterified carboxyl group under these conditions. When the sonication time increased it offers more reaction time opportunities to solvent to extract more and more pectin.

CONCLUSION

The range of anhydrouronic acid content of extracted pectin (62.4-70.3%) is close to the commercial standards (>65%). The degree of esterification varies from 55.8 to 64.6%, which shows the presence of high methyl ester (HM) pectin (DE>50%). The anhydrouronic acid content decreases with increasing values of all the independent variables. However, with the increment in the values of independent variables, the degree of esterification increases. The maximal anhydrouronic acid concentration and degree of esterification under ideal conditions were 64 percent and 62.9 percent, respectively. The present study led to the valorization of sweet lime peel for pectin as an efficient way of reducing the present waste and waste disposal problems encountered. This could provide an additional boost to the economic sector through valorization of food wastes. This research has therefore, been proved to be an efficient approach to extract valuable product from agro waste.

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