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Pectin Production from Biowaste (Fruits & Vegetables) by Crosscurrent Solid-Liquid Extraction Technique

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ABSTRACT

The impact of fruit and vegetable waste is becoming a significant concern for the environment. The biomaterial waste generated from fruit processing industries is very high. When discharged as processed waste, it also increases water pollution. 45% of the total industrial organic pollution originates from food processing industries. These generated wastes are suitable for the production of biochemicals. Pectin is one such biochemical that plays a vital role in reducing the burden on the environment. Pectin helps in the manufacturing of confectionaries. Vegetable waste like beetroot, carrot, beans can also act as a source for pectin production. This study depicts extracting Pectin from mixed fruit pomace waste. Mixed fruit (Orange, Pomegranate, Banana & grapes) pomace waste reacts with 0.1N HCl. This reaction uses a 2-stage crosscurrent solid-liquid extraction technique. For its nature, obtained pectin was tested as calcium pectate using methylated spirit. The filtrate from 2-stage crosscurrent leaching was further dried in an oven. RSM technique helps in optimizing parameters like drying time, temperature, pH, and concentration. The experimental setup generated Pectin gave an efficiency of 11.52% for 22.4g of dried mixed fruit pomace waste.

INTRODUCTION

The amount of discarded materials in various fruit processing industries varies with location and method of harvest. It generates approximately 45% of total industrial organic pollution (Lin et al. 2013, Joshi et al. 2012). Bioconversion of the carbohydrates present in the waste can act as feedstock for biomaterials and chemical intermediates. Besides this Pectin has good gelling properties, and its isolation for commercial production of Pectin started in the 20th century. Pectin is now manufactured to rigorous safety and quality standards in the United States, Europe, Latin America, and China.

Pectin (Endress & Mattes 2009, Thakur et al. 2009) is a high-molecular-weight carbohydrate polymer present in all plants. It contributes to the cell structure and covers many polymers according to their molecular weight, chemical configuration, the content of neutral sugars, and different plant types. The concentration of pectin content varies with the ripening of fruit. Commercial pectin extraction is performed mainly from citrus peel and apple pomace, and other sources include sugar beets, banana peel, mango peel, papaya peel, sunflower heads, and beetroots. Extraction of pectin can be performed by leaching, which is also called solid-liquid extraction (Wikandari et al. 2015). It is the process of extracting the substance from a solid by dissolving them in a liquid. The solid-liquid mixture is brought into contact with a solvent in which the desired substance is soluble, and the other components are insoluble. In leaching, the solvent is critical as it facilitates the ability to remove(solute) a given substance from a solid material. In this study, 2- stage crosscurrent leaching is performed to extract pectin from mixed fruit pomace using HCl as solvent. Many researchers worked on the extraction of pectin (calcium pectate) using different raw materials starting from fruit waste (pomace/peels) to vegetable waste (peels/pomace). Azad (2014) experimented on a wide variety of solvents like HCl, H_2SO_4, HNO_3, citric acid, acetic acid for the isolation of pectin extracted from lemon pomace during ripening. Pectin was extracted and characterized from the peels of lemons (Citrus Limon), grapefruit (Citrus Paradisi), and sweet orange (Citrus Sinensis). Collected fruit wastes were dried, extracted using 300 mL of 0.1 N HCl, and the results revealed that citrus peel contains higher pectin content - about 24.5%. Nakamura and Tobe (2015) used carrot as raw material to extract pectin with water at a temperature of below 100°C, pH from 2 to 5. The effect of nitric acid extraction variables on orange Pectin was studied by Aravantinos-Zafiris and Oreopoulou (1992). Banerjee et al. (2016) experimented on lemon juice for the extraction of pectin from mango peels. Extraction of Pectin from apple pomace by citric acid was studied by Canteri et al. (2005). Chandel et al. (2016) worked on the standardization of eco-friendly techniques for the extraction of pectin from

apple pomace. Extraction of pectin was performed using an autoclave at a temperature of 121° for 60 min followed by precipitation using ethanol, resulting in an optimum pectin yield of 13.01%. Dranca & Oroian (2018) worked on the effect of acid type and particle size on the yield and purity of apple (Malus Domestica 'fălticeni') pomace pectin and the pectin yield (21.24%) and uronic acid content (93.90g.100g^{-1}) were obtained for citric acid extraction and particle sizes between 125 and 200 µm. Grassino et al. (2016) worked on ultrasound-assisted extraction and characterization of pectin from tomato waste. Using oxalic acid as solvent, a comparison of the pectin yields showed that extraction at 80°C for 24 h using the conventional method gave similar results as that of extraction by ultrasound-assisted extraction for 15 min. Under ideal conditions, Hay et al. (2017) extracted and characterized pectin from selected fruit peel waste, producing pectin yields of 11.31% and 18.5% for banana and mango peels, respectively. Temperature, extraction duration, and pH were all investigated, and the optimal values were found to be 82°C, 105 minutes, and pH of 2. Hosseini et al. (2016) and Jafari et al. (2017) used the Box-Behnken technique (Ferreira et al. 2007) for the optimization of microwave-assisted extraction of Pectin from sour orange peel and its physicochemical properties and the results revealed the optimum conditions were yield of Pectin 29.1%, at 1.5 pH, 700 W microwave power and irradiation time of 3 min. Jafari et al. (2017) optimized pectin extracted from carrot pomace using the CCD method. Kauser et al. (2015) compared conventional (water-hot acid) and non-conventional (ultrasonication) procedures for the extraction and chemical characterization of Pectin from mango cultivar "chaunsa" peel waste, finding that the non-conventional method yielded 15.8 g pectin from 100 g peel in 20 min, while the conventional method yielded 16.6 g pectin from 100 g peel in 90 min. As a result of the shorter extraction time, the traditional non-technique (ultrasonication) was found to be a more energy-efficient method. Madhav & Pushpalatha (2002) analyzed biochemical characteristics using pectin extracted from various wastes in particular gel grade of pectin was focused during the study. Maran & Priya (2015) and Maran et al. (2013, 2017) worked on ultrasound-assisted citric acid-mediated pectin extraction from the industrial waste of Musa balbisiana, and the mean experimental yield of Pectin of nearly 8.99% was observed. It was optimized using RSM. They also worked on ultrasound-assisted extraction of pectin from sisal waste. The experimental yield obtained was 29.32%, and the optimization was carried out by choosing the BBD technique in RSM. Popugaeva et al. (2018) and Papoutsis et al. (2016) worked on the impact of different solvents on the recovery of bioactive compounds and antioxidant properties from lemon (Citrus limon L.) pomace waste. Saberian et al. (2017) experimented on the extraction of pectin from orange juice waste assisted by ohmic heating, and optimization was performed by choosing CCD under RSM, and results revealed the optimum yield of Pectin to be 14.32%. Sharma et al. (2013) studied the extraction of pectin from "kinnow" peel and pomace using water acidified with HNO_3. Sudhakar & Maini (2000) worked on the isolation and characterization of mango peel pectins, and an optimum amount of 20.8% of pectin was extracted. Virk & Sogi (2004) collected fresh apple peel from the local preserve manufacturing unit and analyzed it for moisture, ash, acidity, crude fiber, and total sugars. The apple peel was extracted three times using HCl at different normalities and 1% citric acid. Citric acid was observed to be a better solvent in the extraction of pectin than HCl. Yang et al. (2018) experimented on the extraction of pectin from sisal waste by combined enzymatic and ultrasonic process, and research results showed that ultrasound extraction of sisal waste attained a much higher pectin yield of 31.1% than other methods like ultrasound followed by the enzyme (14.6%), combined enzymatic/ultrasonic (9.4%), ultrasonic extraction (11.9%) and acidic extraction (5.8%). Yuliarti et al. (2015) studied the extraction and characterization of pectin from gold kiwi fruit which revealed that enzyme extracted pectin gives a yield of 4.5% whereas acid and water extraction methods give 3.6-3.8%.

The literature survey observed that no experimental work was done using mixed fruit pomace waste. So, an interest was felt to use this mixed fruit pomace waste as raw material for the extraction of pectin.

MATERIALS AND METHODS

Feedstock includes **t**he mixed fruit pomace collected from nearby fruit shops. Chemicals used were HCl, NaOH, $CaCl_2$, CH_3COOH and methylated spirit.

Trial Experiment (Using Orange Peel)

A trial run was conducted using orange peel as raw material. The collected peel was then sun-dried and chopped. Around 50g of powdered orange peel was extracted with 300 mL of 0.1 N HCl, boiled for 30 min, and filtered under suction. After filtration, the raffinate is again extracted with 300 mL of 0.1N HCl and boiled for 30 min. The filtrate was neutralized with 1 N of NaOH using phenolphthalein indicator and is further allowed to stand for 24 h. 50 mL of 1 N acetic acid was added, and after 5 min, 25 mL of 1 N calcium chloride was added, stirred, and kept still for an hour. The contents were then boiled for 2 min, filtered through a pre-weighed Whatman No1 filter paper. The obtained precipitate was then washed with water until the filtrate was chloride-free, the filter cake was dried for 7.5 h. The percentage of pectin

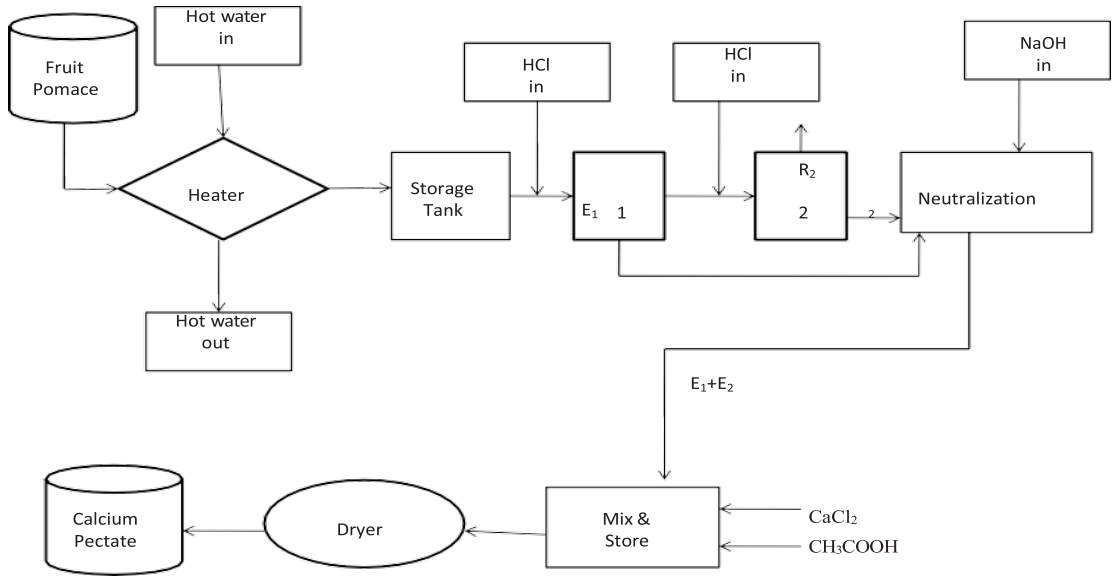

Fig. 1: Flowsheet representing the extraction of pectin (peel/pomace). Here R_1, R_2 refers to raffinate from stages 1 and 2; E_1, E_2 are extracted from stages 1, 2, respectively.

extracted utilizing a two-stage cross current extraction procedure (leaching) with the solvent HCl is just 2%.

Experimentation (Using Mixed Fruit Pomace)

Mixed fruit pomace taken as feed (orange, pomegranate, banana, grape pomace) was dried and chopped. About 22.4 g of powdered mixed fruit pomace waste was extracted with 60 mL of 0.1 N HCl (1st stage of extraction), which was then boiled for 30 min and filtered under suction. After filtration, the raffinate was sent for 2nd stage of extraction with 60 mL of 0.1 N HCl and again boiled for 30min. The acid present in the filtrate was neutralized with 0.1 N of NaOH using phenolphthalein indicator and was kept still for 24 h. To this, 50 mL of 1 N acetic acid was added, followed by 25 mL of 1 N calcium chloride, stirred, and allowed to stand for 1 h. The contents were then boiled for 2 min, filtered, and the obtained precipitate was washed with water. The filter cake was then dried for 3 h. The entire process of extraction of pectin is indicated in Fig. 1. Percentage of pectin from the 2- stage cross current extraction process (Leaching) using HCl is 11.29%. This process is optimized using response surface methodology (RSM).

Test for Pectin

After completing the 2-stage cross current extraction process, the filter cake was dried for three h and tested by adding 7-8 mL of methylated spirit. Rigorous swirling or gentle shaking was performed till the clots were formed, as shown in Fig. 2. which indicates the gelling property of pectin in the extract.

RESULTS AND DISCUSSION

Using HCl as a solvent, the percentage extraction of pectin from orange peel is 2%, and for mixed fruit pomace waste, it is 11.29%. Parameter studies were performed to find out the optimum conditions for the extraction of pectin using HCl from mixed fruit pomace waste. Parameters like drying time, drying temperature, pH of the solution, and concentration of HCl were varied.

Fig. 2: Gelling property of pectin.

Effect of Drying Time

The extracted calcium pectate is kept in a hot air oven for drying. The weight of calcium pectate was noted for every hour, and the drying operation was performed for five hours. Optimum drying time was found to be 3 h with 2.53 g of calcium pectate, beyond which no appreciable change in weight of calcium pectate was observed.

It can be inferred that 3 h of drying dried all the calcium pectate present in the solution. The effect of drying time on the weight of calcium pectate is shown in Fig. 3.

Effect of Temperature

Calcium pectate obtained from the 2nd stage of extraction was kept for drying in a hot air oven. The product sample was kept for 3h (optimum drying time) at varying temperatures starting from 50° to 90° (at 10°-time intervals), and the weight of calcium pectate was noted at each temperature. With the increase in temperature, a decrease in trend curve was observed till 80°, after which the weight of calcium pectate remained constant, as shown in Fig. 4. At 80°, all the moisture and volatiles were removed from the solution, and hence the optimum temperature is considered as 80°.

Effect of pH of the Solution

During the extraction process, the pH of the solution is varied, and the optimum pH was found to be 3.09. As shown in Fig. 5, the pH of the solution was varied from 3.09 to 9.1, and the maximum weight of calcium pectate was 2.53g (at 3.09 pH value) which shows that the extraction is favorable under acidic conditions.

Effect of Concentration of HCl (Solvent)

During experimentation, solvent concentration varied from 0.05 N to 0.2 N (at 0.05 N regular intervals), and the same experimental procedure and drying phenomenon were repeated. The majority of the available calcium pectate present in the sample solution is extracted using 0.1 N HCl solvent for a given weight of the sample. So, the optimum concentration was found to be 0.1 N with calcium pectate weighing around 2.53 g. The trend curve is shown in Fig. 6.

Optimization of The Selected Parameters by Design of Experiments (DOE)

In the present study, % of Y is the function of drying time(X1), pH(X2), temperature(X3), the concentration of HCl (X4), and weight (X5) response based on experimental runs and predicted values proposed by BBD design. 3-factor levels are indicated in Table 1. Multiple regression analysis of the experimental data resulted in the following equation:

$$Y = 3105.5 - 3.8X_1 - 1764.8X_2 - 0.439X_3 + 391X_4 + 2.69X_5 - 0.00X_1^2 + 255.42X_2^2 - 0.00644X_3^2 - 282X_4^2 + 2.302X_5^2$$

$$+ 2.74X_1X_2 - 0.027X_1X_3 - 63.3X_1X_4 - 0.76X_1X_5 - 0.178X_2X_3 - 142.4X_2X_4 - 1.76X_2X_5 + 7.54X_3X_4 - 0.1081X_3X_5 - 53.6X_4X_5$$

Where Y is the percentage represented in terms of weight of calcium pectate

The correlation coefficient is a measure of the model's variability to response (R2). The model is inviolable since R2→1. R2 = 0.9986 in this investigation, with an error of 0.0014, indicates that the model is statistically significant at a 95% confidence level.

Contour Plots

Contour plots are three-dimensional plots in which a 3-D surface is projected on a 2-D plane. These plots represent two predictor variables X, Y on the y-axis, and response variable Z as contours. Contour lines can be curved, straight, or a mixture of both. The contour plots show the interactive

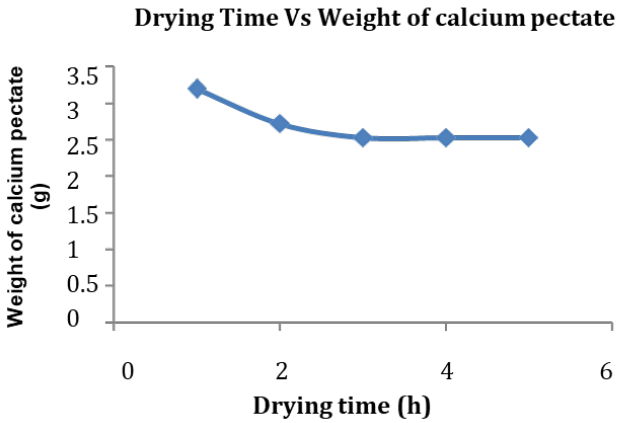

Fig. 3: Effect of drying time.

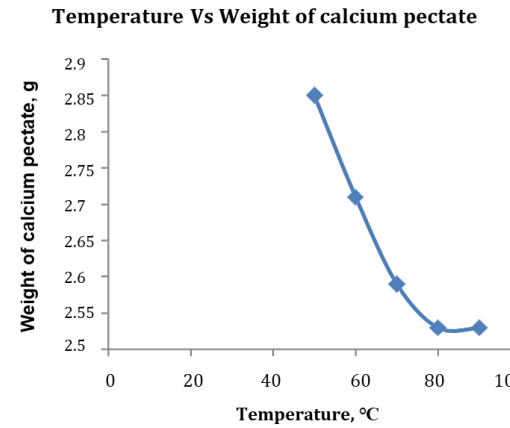

Fig. 4: Effect of temperature.

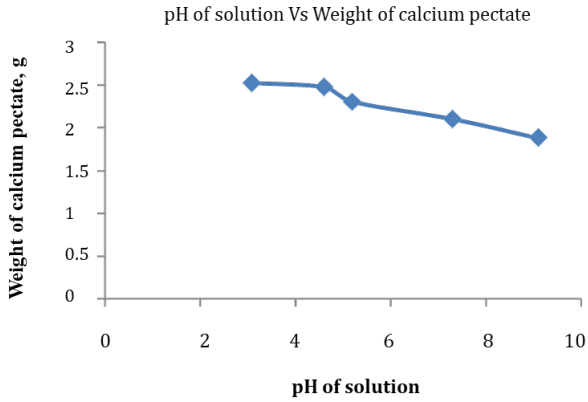

Fig. 5: Effect of pH of the solution.

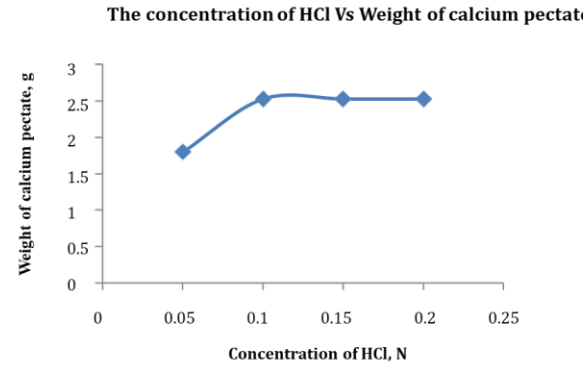

Fig. 6: Effect of Concentration of HCl.

effect of every parameter with the weight of calcium pectate. The contour plots representing the optimization study of the extraction of calcium pectate with mixed fruit pomace are shown in Fig. 7.

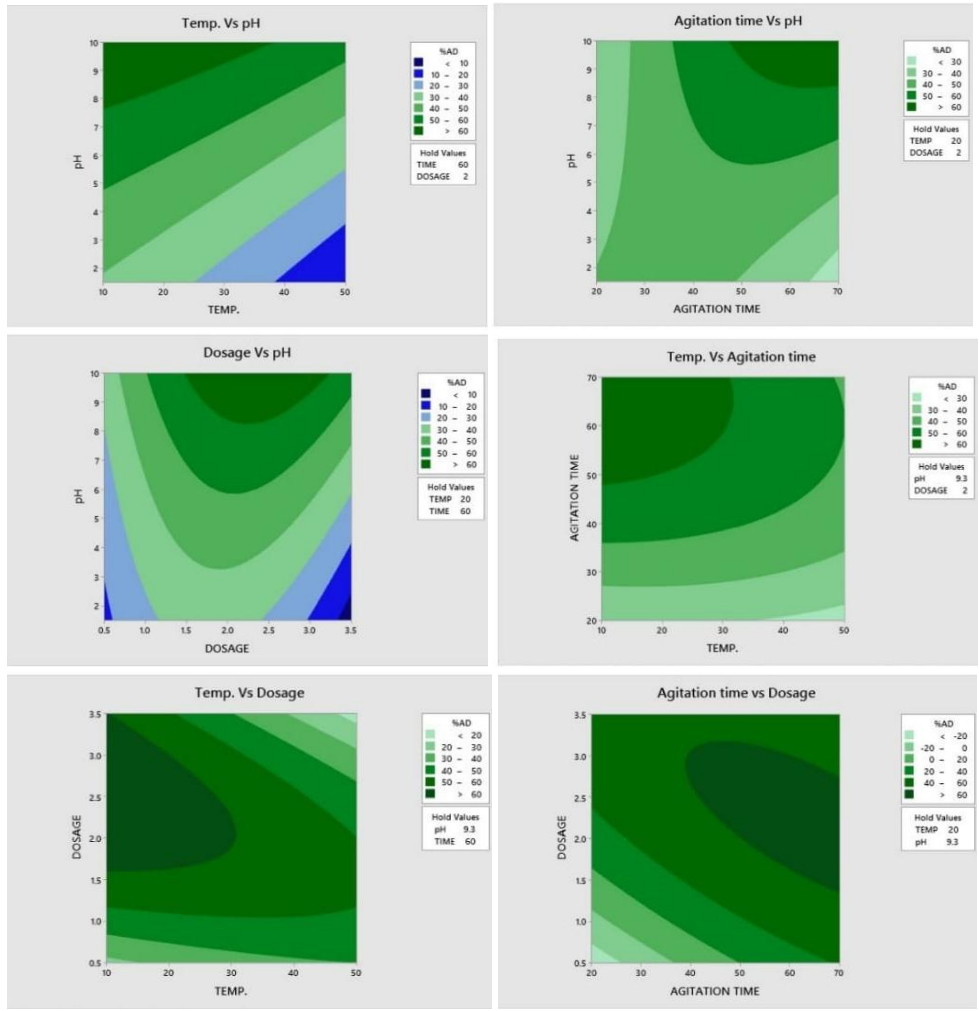

Fig. 7: Contour plots representing the optimization study of the extraction of calcium pectate with mixed fruit pomace.

Table 1: 3-factor levels in BBD method.

Parameters	Minimum	Average	Maximum
Temperature[°C]	46.29	63.145	80
Weight [g]	1.677	3.257	4.8375
pH	3.0	3.5	4.0
Time [h]	2.311	3.5	4.689
Concentration of HCl [N]	0.0155	0.075	0.1345

Table 2: Comparison of optimum values by experiment and RSM.

Parameters	Experimental	RSM
Drying time	3 h	3.23 h
Weight of calcium pectate	2.53g	2.58 g
pH of solution	3.09	3.0
Temperature	80ºC	80ºC
Concentration of HCl	0.1N	0.1345N

The influence of several factors and their interactions on pectin yield is illustrated in Fig. 7. The optimum conditions observed from DOE are drying time of 3.23 h, pH of the solution to be maintained at 3, drying temperature at 80°, and concentration of HCl of 0.1345 N for an optimum weight of calcium pectate of 2.58 g. A comparison of the values obtained by experimentation and RSM study is shown in Table 2.

CONCLUSION

Around 2.53 g (11.29%) of calcium pectate was extracted from 22.4 g of mixed fruit pomace waste. In this 2-stage extraction process, various process parameters have shown a marked effect on the percentage extraction of Pectin (calcium pectate). Gelling nature of calcium pectate was confirmed by methylated spirit. Besides the percentage extraction of pectin, solid organic waste was used as a potential source for the extraction, which in turn helps in the reduction of environmental pollution (in particular water pollution) to a greater extent. As per the experimentation, the drying time of 3 h, drying temperature of 80°, pH of solution maintained at 3.09, and 0.1 N concentration of HCl resulted in an optimum extraction of 11.29% calcium pectate. An optimization study through RSM by DOE reveals that the optimum conditions for the parameters were drying time of 3.23 h, the temperature at 80°, pH of the solution to be 3.0, and at 0.1345 N of solvent concentration, which yielded 11.52% of Pectin. The experimental values obtained were well matched with optimized parameters by RSM to a correlation coefficient of 0.9986.

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