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Research Paper

Ultrasound-Assisted Optimization of Pectin Extraction from Orange Peel Using Response Surface Methodology (RSM) and Artificial Neural Network (ANN)

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ABSTRACT

The objective of study was extraction of pectin from orange peel using ultrasound assisted extraction and response surface method and artificial neural network technique. The accuracy of the two models was studied to compare the performances of the two models to make decision for achievement of optimum process parameters during extraction of the pectin. The following findings are absorbed from the effects of extraction parameters. The pH solution was highly significant compared to ultrasound power. As well as interaction between ultrasound and pH solution were found to be strongly influenced the extraction yield of pectin. The optimal parameters for extraction were irradiation time of 22.5 min, pH of 1.5, and ultrasound power of 155W and liquid-solid ratio 22.5:1 mL/ g. Under these conditions, yield of pectin was 26.87% experimentally, while 26.74 and 26.93% of yield were predicted by response surface methodology and artificial neural network model respectively. The extracted pectin of orange peel was categorized as high methoxyl pectin, since it has 63.13% degree of esterification, which is above 50% affirmed by Fourier transform infrared spectroscopy detection. Both response surface methodology and artificial neural network model prediction was in good agreement with experimental data; however, the prediction of artificial neural network prediction was better than artificial neural network. Therefore, artificial neural network model is much more accurate in estimating the values of pectin yield and mean square error when compared with the response surface methodology.

Keywords: Pectin, Orange peels, Optimization, RSM and ANN, Ultrasound assisted Extraction

Industrial wastes and accumulation generated per year are direct consequence associated with universe population growth that brings the environmental pollution issues. Food processing industry wastes and agricultural wastes rich in reusable materials are used for the bioconversion to value added products like

additive product, biofuel and active biochemical materials (Salleh, Mahmoud, Karim, & Idris, 2011). One of, the largest product in worldwide per year are *citrus* fruits. Almost about fifty percent quantities of fruits are disposed as a waste by *citrus*-processing industries (Mahato *et al.* 2020; Sharma *et al.* 2017). Air quality, soil purity and sources of water around *citrus* processing industry, are directly consequences of problems, generated from these uncontrolled waste of this industry to the environmental (Zema *et al.* 2018). However, these products (*Citrus peels*) are used in several industries as polymethoxylated flavonoids that are applied as phytochemical, pharmaceuticals, food products (Kim *et al.* 2004) time, and HCl concentration of the washing solution were chosen as independent variables of the central composite design. The results showed a good fit with the second-order polynomial equation, which was statistically acceptable at P<0.05 level. Pectin loss was directly correlated with the increases in temperature, time, and HCl concentration during washing procedure.

Consequently, the higher the temperature and longer the time, the yield of narirutin extracted was greater, although higher HCl concentration had a negative effect on narirutin extraction yield. The present study indicated that the combination of washing temperature (62 °C. Conversion of these wastes to valuable product is considered as an alternate and common practices for recycling of byproduct and environmental management systems (Ng *et al.* 2020). One of the most source of raw materials largely produced in the worldwide are orange fruits used for extraction of complex compound known as pectin. Pectin is a complex polysaccharide substance contains one third of the mobileular wall of the plants. The larger amount of pectins are located within the middle part of lamella of cell wall and small amount of pectins are between the cell wall of plant (Abang *et al.* 2017). Pectin is used; as gelling, stabilizing and thickening agent jams and jellies confectionery in food as well as in pharmaceutical industry. In industrial level, pectin is divided as: Pectins with a degree of methylation (DM) above 50% (high methoxyl: HM) and pectins with a degree of methylation (DM) below 50% (low methoxyl: LM) (Hosseini, Khodaiyan, & Yarmand, 2016a). High methoxyl pectin makes gel between pH of 2–3.5 in the presence of concentrated co-solutes between 55–75%; while low methoxyl pectin forms gel in the absence of co-solutes, with the mixture of bivalent ions between pH of 2–6 (Chan & Choo, 2013). Therefore both types of pectins have dissimilar physical and chemical properties lead to dissimilar applications (Hosseini, Khodaiyan, & Yarmand, 2016b). In addition, properties of pectin primarily based on the plant source, the extraction technique chosen for separation as well as purity of final product. To increase extraction quality as well as the final yield of pectin, the use of an appropriate method is critical point (Berkani *et al.* 2020). Extraction of pectins from different plant sources haven been reported in several literatures. Anciently, extraction of pectins from *citrus* peels have done using hot acidified water that results destroying and make dissimilar its structure and instinctive character and usage of chemicals results wastewater which brings the higher environmental pollution (Hosseini *et al.* 2019a; Shivamathi *et al.* 2019). The use of several advanced techniques (including, enzymes, microwave as well as ultrasound assisted extraction (UAE)) can be used to maximize the final yield and its purity in addition minimizing; time of extraction, energy usage ,use of poisonous solvents and concentrated acid for product recovery (Marić *et al.* 2018). Due to the influence of ultrasound waves to improve the destroying of plant cell wall, increase the rate of mass transporting which bring up the better performance and quality of the product with a shorter time as well as lower consumption of solvents and energy, increased safety of the operators and in environmental problems, ultrasound assisted extraction is considered as alternative method (Hosseini *et al.* 2019; Marić *et al.* 2018). In overall, types of extraction variables involved and extraction mechanism will influence the efficiency of the yield.

For successful extraction and optimization, artificial neural network (ANN) and response surface methods (RSM) have been widely used in optimizing various process variables (Tesli *et al.* 2019; Ye *et al.* 2014). RSM is widely applied to optimize multivariate system to determine individual as well as combination influences of process parameters. However, RSM based models are only for a restricted range of input parameters and thus, impose a restriction on the use of RSM based models for non- linear behavior (Muthusamy, Manickam, Murugesan, Muthukumaran, & Pugazhendhi, 2019) "ISSN": "18790003", "PMID": "30414904", "abstract": In this work, Response Surface Methodology (RSM. Artificial neural network (ANN) has wide applications over RSM for non- linear multivariate modeling (Khayet, Cojocaru, & Essalhi, 2011) technology that includes different number of layers for selected number of parameters (Ghoreishi, Hedayati, & Mousavi, 2016). MATLAB® 2014a was used to build the ANN model with feed forward Multilayer back propagation (FMBP) to predict the response. Several investigations have been worked to extract pectin from orange peel using traditional as well as current methods. However, extraction of pectin from these peels was not investigated using combinations of current extraction method (UAE) and optimization (RSM and ANN*) methods. Therefore, the goal of this paper was ultrasound assisted extraction and optimization (response surface and artificial neural network) of pectin from orange peel.

MATERIALS AND METHODS

Materials

The orange peels were collected from juice producer located in Jimma. It was manually cleaned to remove impurity and dried for three days followed by an electric oven dry at 50 ℃ until similar weight of the peel achieved. The dried peels were washed, mixed in a mixer grinder and sieved to avoid non-finely particles. The result powder was stored in a dark and dry out place for subsequent requirement.

Chemicals and solvents: Totally analytical grade reagent chemicals and solvents [Citric acid, HCl, HS_2O_4, trifluoro acetic acid, NaOH, ethanol, 2, 2-Diphenyl-1-Picrylhydrazyl] were purchased from chemical purchasers.

Experimental Design; Response surface method (RSM) modeling

Experiment design was developed using Stat Ease Design Expert software version 11.1.0. Based on the design of experiment; central composite deign, the second order polynomial regression model was developed to predict the performance of variables. The extraction parameters selected according to previous literatures as shown in appendix; A table 1 with coded and actual level.

The second-order models generated by RSM are often used to determine the critical points and can be written in a general form as (Kleijnen, 2008):

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i X_i + \sum_{i=1}^{k-1} \sum_{j=1+1}^{k} \beta_{ij} X_i X_j + \varepsilon \qquad \dots(1)$$

Where, Y, x_i and x_j, β_0, β_i, β_i, β_{ii}, k and are anticipated response, input factors, constant term, linear coefficient, quadratic coefficient, interaction term, number of factors and random error respectively.

Using Eq.(1), if n is the number of repetitions at the cube's center and R is the total number of run required for four independent variables (irradiation time, pH, Ultrasound power and liquid-solid ratio) is given by Eq.(2):

$$R = 2^k + (k*2) + n \qquad \dots(2)$$

Artificial neural network (ANN) modeling

MATLAB® 2014a was used for the formulation of Artificial neural network model using feed forward multilayer network contains three primary layers knwon as input (used hyperbolic tangent sigmoid transfer function), hidden and output layers (used pure-linear transfer function) to anticipate the yield (Nazerian, Kamyabb, Shamsianb, Dahmardehb, & Kooshaa, 2018) as indicated in Eq.(4). The developed artificial neural network model constitutes four inputs (irradiation time, pH solution, ultrasound power and liquid-solid ratio) and one output (pectin). The Marquardt–Levenberg back-propagation (MLBP) algorithm was selected for training. The input and output data detected from the actual values are categorized into different parts; (60% - eighteen samples) for training (20% - six samples) for testing and (20% - six samples) for validation. To find out best training efficiency and reduce the effect of larger values in input and output data, input and output were normalized between -1 and 1 (Muthusamy *et al.* 2019) "ISSN": "18790003", "PMID": "30414904", "abstract": In this work, Response Surface Methodology (RSM. The normalized data was forwarded to the artificial neural network model in feed forward multilayer back propagation. The mean square error values between the output neurons and the observational outputs were determined and backward propagated via the network. Then, the individual weights of the neuron, corrected by the algorithm. For cross-validation of parameters, by MATLAB, ANN Tool was developed. After the ANN tool memorizes the data from the training, the cross-validation was applied to prevent over fit of the training. By repetition in testing a number of NN, the best number of neurons in the hidden layer was determined when the mean square error (MSE) value of the output reached its minimum value. For determination of the number of neurons required in the hidden layer, addition of the weighted inputs and the related bias are important data towards for nonlinear situation (Cheok *et al.* 2012) solid to solvent ratio, and methanol concentration for extracting total phenolic content (TPC; Madadlou *et al.* 2009) one hidden layer and an output layer with one neuron optimized using response surface methodology (RSM

$$F(x) = tansig(x) = \frac{1-e^x}{1+e^x} \qquad \dots(4)$$

Comparison of ANN & RSM performance

The coefficient of determination; R^2, Root Mean Squared Error; RMSE, mean average error; MAE, standard error of prediction; SEP, and absolute average deviation; AAD were determined to check the accuracy and predictive ability of ANN and RSM using Eq. (4):

$$R^2 = 1 - \sum_{i=1}^{r} \left[\frac{\left(y_p - y_e\right)^2}{\left(y_p - m\right)^2} \right] \qquad \dots(4)$$

$$MSE = 1 - \frac{1}{r}\sum\nolimits_{i=1}^{r}\left(y_p - y_e\right)^2 \qquad \text{...(5)}$$

$$RMSE = MSE^{1/2} \qquad \text{...(6)}$$

$$SRP = \frac{RMSE}{m} \qquad \text{...(7)}$$

$$AAD = \frac{100}{r}\sum\nolimits_{i=1}^{r}\left(\frac{y_e - y_p}{y_{\exp}}\right) \qquad \text{...(8)}$$

$$MAE = \sum\nolimits_{i=1}^{r}\left[\frac{y_e - y_p}{y_e}\right] \qquad \text{...(9)}$$

Where; r = number of run, y_p = predicted values from model, y_e = experimental values and m = average experimental values

Extraction of Pectin

By using previous study as reference with modification and it should also be declared that the degrees of parameters were selected depend on previous study (Hosseini *et al.* 2019; Lin *et al.* 2018) there is a lack of studies on the extraction of pectin by ultrasound assisted extraction (UAE. In all runs, fifteen gram (15g) of the peel powder was mixed with pH of (13) citric acid solution in 250ml flask. It was then sonicated for (1530 min), in the range of (60120W), ultrasound power, thereafter the mixture was maintained at temperature of 25^0C for a certain time and percolated. The percolate carrying pectin was chilled down, for 25 min and centrifuged at 6500 rpm. Ethanol of 96% was utilized, to precipitate the supernatant and impurities, and allowed for one hour for pectin flotation. The floating result was then classified by filtration process and the end result was dried in drier at 50°C to remove moisture. The resulting dried pectin was milled to powdered pectin and stored for analysis and its yield was determined using to Eq. (2) and indicated in table 2.

$$\text{Yield (\%)} = \frac{\text{Amount of dried pectin extracted in gram (g)}}{\text{Amount of sample taken upfor extraction in gram (g)}} * \times 100 \qquad \text{...(10)}$$

FT-IR spectroscopy

The produced pectin contents were analyzed by using prinks Elmer spectrum 65 FT-IR technique with the help of IR correlation. The wavenumber region for the analysis was 4000-400 cm^{-1} (in the mid-infrared range) and IR spectrum was reported by % transmittance.

Table 1: central composite design parameters level for pectin extraction

Parameters	Unit	Minimum	maximum	Levels		
A: Irradiation time	min	15	30	10(-1)	25(0)	40(+1)
B: pH solution	–	2	3	2(-1)	2.5(0)	3(+1)
C: Ultrasound power	w	60	140	60(-1)	100(0)	140(+1)
D: Liquid-solid ratio	mL/g	15:1	25:1	15:1(-1)	20:1(0)	25:1(+1)

Table 2: Independent variables with experimental and response with RSM and ANN

Run Order	Factors				Actual %	RSM Predicted %	ANN Predicted %
	Irradiation time min	pH solution	Ultrasound power (W)	L-S-R (ml/g)			
1	30	2	120	30	20.01	20.10	20.4683
2	15	3	120	15	9.90	9.91	9.899
3	22.5	2.5	85	22.5	23.23	23.17	23.21
4	15	3	50	30	11.30	11.33	11.2922
5	30	3	120	30	10.30	10.30	10.301
6	30	2	50	15	16.30	16.53	16.54
7	22.5	2.5	85	7.5	16.70	16.44	16.8996
8	37.5	2.5	85	22.5	7.30	7.14	7.361
9	30	2	120	15	22.57	22.61	22.5638
10	7.5	2.5	85	22.5	8.50	8.25	8.4994
11	22.5	2.5	15	22.5	15.80	15.52	15.783
12	22.5	2.5	85	37.5	16.70	16.55	17.098
13	22.5	2.5	85	22.5	23.40	23.17	23.21
14	30	2	50	30	15.01	14.94	13.5319
15	22.5	1.5	155	22.5	26.87	26.74	26.93
16	22.5	3.5	85	22.5	5.75	5.47	6.1308
17	15	2	50	30	15.00	15.19	14.9644
18	30	3	50	30	10.00	10.29	10.2657
19	15	3	50	15	8.31	8.70	9.5966
20	30	3	50	15	8.10	8.10	8.2907
21	22.5	2.5	85	22.5	23.10	23.17	23.21
22	15	2	120	30	20.54	20.61	21.0478
23	15	2	120	15	22.50	22.69	22.75164
24	22.5	2.5	85	22.5	23.00	23.17	23.21
25	15	2	50	15	16.40	16.34	16.4342
26	30	3	120	15	8.88	9.04	8.938
27	22.5	2.5	85	22.5	22.99	23.17	23.21
28	22.5	2.5	85	22.5	23.40	23.17	23.21
29	22.5	2.5	85	22.5	23.23	23.17	23.21
30	15	3	120	30	11.50	11.61	11.4755

Product (Pectin) characterization

The pectin extracted at the optimum processing conditions (the highest yield) was analyzed by determining the following properties.

Esterification Capacity/degree of esterification (EC): It was calculated using the titrimetric method using potassium hydroxide (KOH), instead of sodium hydroxide. Twenty-five (25g) of dried product was moistened with standard ethanol and dissolved in 25 ml of deionized water at forty degree Celsius (40 ^{0}C). Following full dissolution of product, five pearls of indicators were added to the mixture. The mixture was thereafter volumetrically analyzed with 0.4 M KOH, and the amount of the KOH solution used for color change was recorded as volume (V_1). Subsequently, 15 ml of 0.4 M KOH was added, and the solution was shaken powerfully and allowed to cool down for 10 min. In addition, 15 ml of 0.6 M hydrochloric acid was added and the solution was shaken until the disappearance of pink color. The mixture was volumetrically analyzed with 0.4 M KOH for the end step and the consumed amount was collected as volume (V_2) and the esterification capacity was determined using Eq. (11):

$$EC(\%) = \frac{V_2}{V_1 + V_2} * 100 \qquad \dots(11)$$

EC = esterification capacity of dried product, V_1 = initial titer (ml), and V_2 = final titer (ml)

Equivalent weight (EW): This was calculated as method reported by (Mohamed, 2016); 5 g of dried product was put in a 250 ml conical flask, and 15ml ethanol was added. One gram of NaCl and 100 ml of deionized water were added. Finally, five drops of phenol red indicator was added and volumetrically analyzed with 0.1 M KOH. The end of volumetric analysis was represented by pink color and the equivalent weight was calculated according to Eq. (12):

$$EW(\%) = \frac{m_s * 1000}{V_a * K_n} \qquad \dots(12)$$

EW= Molecular mass of dried product, VA = volume of alkali (ml), and K_{en}= normality of potassium.

Methoxyl content (MC): MC was carried out using the Ranganna's method as reported by (Fakayode & Abobi, 2018). When the neutral solution was accumulated from determination of equivalent weight, 20 ml of KOH was added. The mixed solution was stirred thoroughly and kept at 25^{0}C for 25 min. Then after, 25 min, 20 ml of 0.3 M HCl introduced and volumetric analysis started with 0.1M KOH. The content of methoxyl was calculated using Eq. (13):

$$MC(\%) = \frac{V_a * N_a * 3.1}{m_s} \qquad \dots(13)$$

MC = methoxyl content of dried product

Total anhydrouronic acid content (TAAC): This was carried out using the method adopted by Mohamed, (2016) and determined using Eq. (14):

$$TAAC\ (\%) = 1.76 * \frac{(a+b)}{m_s} \qquad \dots(14)$$

TAAC = total anhydrouronic acid content of dried product a = volume of KOH from equivalent weight determination (ml), and b = volume of KOH from methoxyl content finding (ml) and m_s = mass of sample (g)

Acetyl value (AV);- This was carried out using the Ranganna's method as reported by (Kliemann *et al.* 2009); 5 g of dried product was dissolved in 0.2 M KOH solution by stirring and allowed to stand overnight. The contents were diluted to 40 ml with deionized water and an aliquot (15 ml) was located into the distillation equipment. Magnesium sulfate-sulfuric acid mixture (15 ml) was also shifted to distillation equipment and distilled. About 100 ml of distillate was collected and the distillate was titrated with 0.4 M KOH using phenol red indicator. A blank distillation using 15 ml of the Magnesium sulfate-sulfuric acid solution was carried out, and the distillate was volumetrically analyzed. The acetyl content was calculated using Eq. (15):

$$ACV(\%) = \frac{V_a * N_a * 4.3}{m_s} \qquad \ldots(15)$$

ACV = acetyl value of dried product

RESULTS AND DISCUSSION

Validation of RSM model optimization for experimental data analysis

A very good correspondence between the observational and anticipated values for pectin yield was obtained from the check bit plot between the anticipated and the observational values as shown in Fig. 2. The anticipated values and observed values are in reasonable agreement, since there is closeness between the both results for pectin yield. Referencing table 2, the expected optimum pectin yield was 26.74% at time of 22.5 min, pH of 1.5, ultrasound power of 155W, and liquid-solid ratio of 22.5:1 mL/g using Eq. (10). Under these conditions, the actual value of pectin was 26.87%, which in corresponding with those expected by computation.

The UAE yield of pectin was less than the result reported by, Hosseini *et al.* (2019) from microwave-assisted extraction (28.07 ± 0.67 %) and higher than, Hosseini *et al.* (2016) aqueous extraction (17.95 ± 0.3%) in their optimum extraction conditions. The variation could be due to; plant source, the extraction technique taken for separation (Hosseini *et al.* 2016b). However, the result indicates that the UAE method is an efficient technique since; this it has minimum consumption of time and energy and in addition, that the extracted yield of pectin in this research was better than result achieved by Wikiera *et al.* (2016) 19.8% of pectin yield from apple pomace by Endo-xylenes and endo-cellulose-assisted method. Shivamathi *et al.* (2019) has reported the achievement of 8.93% yield of pectin from custard apple peel at optimized conditions. This implies that the model chosen for anticipation of the pectin yield from orange peel was adequately.

The regression coefficient (interms of coded), variance analysis of the expected model and all colleration coefficients(R^2, R^2 adj and R^2 pre) have been used to test the goodness of the model (Zhang, Chen, Mao, Guo, & Dai, 2014) "ISSN": "18790003", "abstract": The central composite design (CCD listed in table 3. The analysis results proved that the relationship between factors and the response of the expected

model was well-correlated. Residual is the difference between the actual and the fitted value of a model and it is used to predict the adequacy of the model. Small residual mean's; model anticipated is accurate (Samavati, 2013) based on a five level, four variable central composite rotatable design (CCRD. The statistical effluences of all the terms of the model were tested by the F and P-value. The corresponding variables would be more significant if the F-value became higher and the P-value became smaller (Zhang *et al.* 2014) "ISSN": "18790003", "abstract": The central composite design (CCD. In this study, results showed that, high F-value and p value of developed model were below 0.05, which in turn show the fitness of model was strongly significant (Prakash *et al.* 2013). The results also proved that, the proposed regression model for pectin yield was satisfactory high with all squared R values table 3, which exhibited a closeness between the actual and the anticipated values by the model.

Table 3: ANOVA for Quadratic model

Source	Sum of Squares	DF	Mean Square	F-value	p-value
Model	1160.73	14	82.91	1305.28	0.0001***
A-irradiation time	1.86	1	1.86	29.27	0.0001***
B-pH solution	362.96	1	362.96	5714.31	0.0001***
C-Ultrasound power	48.42	1	48.42	762.26	0.0001***
D-L-S-R	0.0204	1	0.0204	0.3214	0.5791*
AB	0.6320	1	0.6320	9.95	0.0065**
AC	0.0729	1	0.0729	1.15	0.3010*
AD	0.1936	1	0.1936	3.05	0.1013*
BC	34.80	1	34.80	547.81	0.0001***
BD	14.29	1	14.29	224.95	0.0001***
CD	0.8556	1	0.8556	13.47	0.0023**
A²	414.24	1	414.24	6521.56	0.0001***
B²	91.14	1	91.14	1434.84	0.0001***
C²	22.57	1	22.57	355.40	0.0001***
D²	77.15	1	77.15	1214.57	0.0001***
Residual	0.9528	15	0.0635		
Lack of Fit	0.7772	9	0.0864	2.95	0.1004*
Pure Error	0.1755	6	0.0293		
Total	1161.68	29			
R²			0.9678		
AdJ.R²			0.9475		
Pred. R²			0.9575		
Ade Pr.			26.3910		
Std. Dev.			1.14		
Mean			15.78		
C.V. %			7.23		

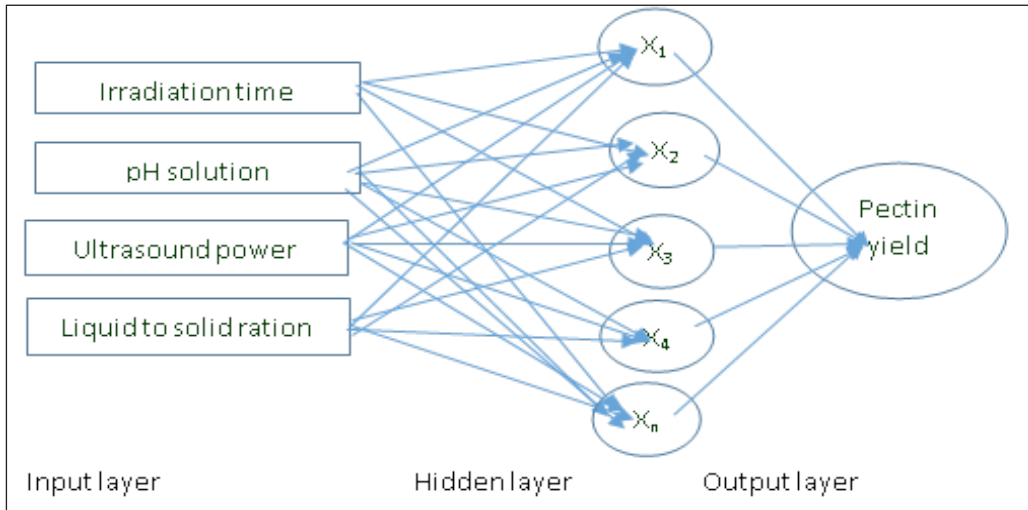Highly significant; ***, significant; **, not significant; *

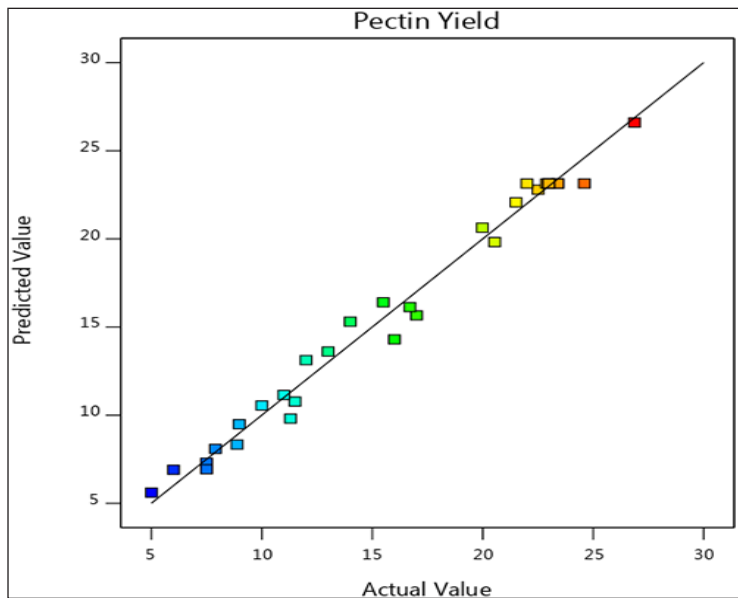**Fig. 1:** Artificial neural network for input, hidden and output layers $(4–X_n–1)$

Fig. 2: Experimental value versus RSM expected of pectin yield (%)

The Pred. R^2 of 0.9575 is in reasonable estimate with the Adj. R^2 of 0.9475; i.e. the distinction is much less than 0.2. The adequate precision ratio of 58.439 shows an adequate signal and this model can be used to navigate the design space since, adequate precision is used to find out the signal to noise ratio and a ratio greater than 4 is desirable. Meanwhile, the very small value of coefficient of variation (CV:7.23%) clearly shown a very strong degree of precision and a great deal of reliability of the experimental values

(Prakash *et al.* (2014). The F-value of 2.95 the lack of fit for this estimation shows that, the model is suitable for anticipation of optimum conditions to extract pectin from orange peel using UAE. The proposed quadratic polynomial model is therefore, perfectly suitable for anticipation of the relationship between independent and dependent variables. The dependent and independent variables are related by the following second-order polynomial equation interms of coded terms by applying multiple regression analysis on the experimental data.

Final Equation in Terms of Coded Factors

$$\text{Pectin Yield} = +23.17 - 0.28A - 4.36B + 1.59C + 0.03D - 0.2AB - 0.07AC - 0.11AD$$
$$- 1.28BC + 0.95BD - 0.23CD - 3.87A^2 - 2.25B^2 - 1.12C^2 - 1.67D^2 \qquad \ldots(16)$$

The importance of the independent variables and their effects could be indicated by the magnitude and sign of the coefficients (Zhao-Hui *et al.* 2011). It could be seen from table 3 and Eq. (16), all the liner, the quadratic and the interaction coefficients (BC, BD, CD) were statistically significant.

Effect of extraction variables on the pectin yield

The result in table 3 and Eq. (16) separately showed that all of the applied factors were highly effective on extraction yield of pectin except liquid-solid ratio not significant. Citric acid pH solution should be considered more than the other factors, since it was the most influential on extraction process. Similarly the interaction between pH, solution versus ultrasound power and pH solution versus liquid-solid ratio had a high influence on the yield of extraction. Apart from the liquid-solid ratio and pH solution, the interaction between pH and ultrasound power had highly influenced the yield of pectin. The 3D plots were demonstrated below for more exploration in the relationship between response and parameters. At the least pH solution and highest ultrasound power, the maximum extraction yield was attained. This means, high acidic media, brought the pectin leakage from the plant substance and in consequence, increased the extraction efficiency (Hosseini *et al.* 2019b and Dranca, 2019). Another efficient variable that had a direct influence on the extraction was ultrasound power. Fig. 3a reveals that ultrasound power was better efficient in the study, enhancing the ultrasound power; the extraction efficiency was also enhanced. This result may relate to the cavitation consequences of ultrasound waves and this cavitation bring up the solvent insight into intercellular substance of plant and thereby upgrades the pectin escapees leads to increase the extraction efficiency (Wikiera *et al.* 2016).

Irradiation time is one of the important process parameters that affect the yield of pectin significantly compared to L-S-R in this study. Due to creation of the cavitation bubble by ultrasound waves that supports the disruption of plant cell wall to improve the extraction efficiency of pectin and produce swelling and hydrate the plant material, in the initial stage, the extraction efficiency was increased up to 22.5 min and the reduction was observed above this value (Shivamathi *et al.* 2019).

Yield of pectin did not highly influenced with liquid-solid ratio compared to other parameters; an observation that was confirmed by the results of the analysis of variance (p > 0.05) and Eq. (16). It can be assumed that varying the range of study above a SLR of 1:22.5 g/mL might be lead to a different conclusion regarding the involvement of this working parameter in the cavitation effect occurring in an extraction assisted by ultrasound method and consequently its impact on the efficiency of extraction (Dranca, 2019).
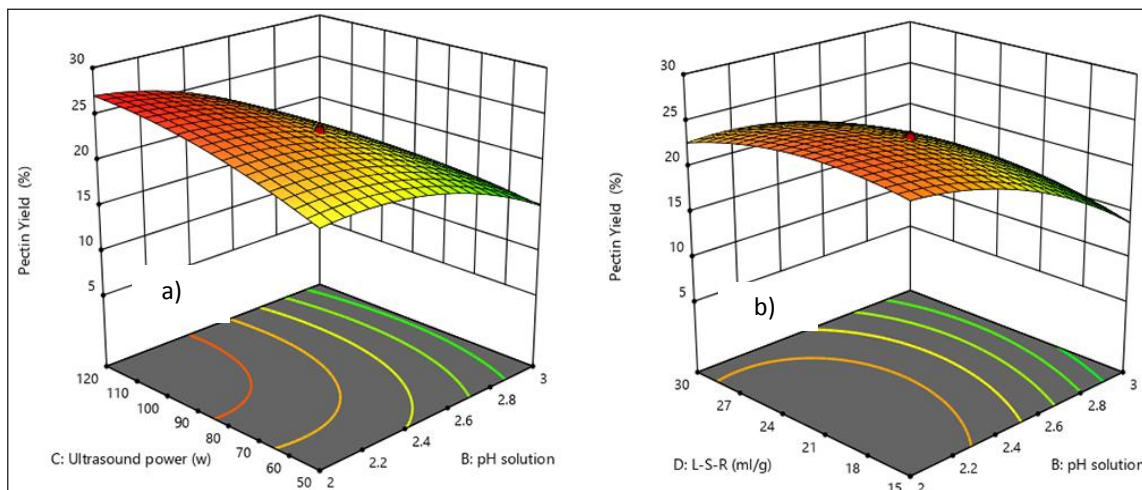
Fig. 3: Ultrasound versus pH (a) and Liquid-solid ratio versus pH on the yield of pectin (b)

ANN-based modeling

The ANN anticipation has been carried out successfully using information shown in indicated in table 1. The ANN-based model was done by; choice of the appropriate training algorithm, determination of optimum value of neuron and validation of the model. Eighteen (80% –18) samples of the data were used for training, six (20%–6) samples for testing and six (20%–6) samples for validation. Using the available actual value, Levenberg–Marquardt (LM) ANN fitting tool and TANSIG Transfer Function 4– X_n–1 (number of input layer, neurons in hidden layer and output layer) model were implemented. Prior training, the network inputs and target were normalized by assigning the maximum (+1) and minimum (–1) levels. Statistical parameters like R; regression coefficient, RMSE; root-mean square error, MAE; mean average error, SEP; standard error of prediction and AAD; absolute average deviation were assumed to determining the higher predictive power of the model techniques. The statistical values of ANN and RSM models are decided using Eq. (4–9) and shown in table 4.

Table 4. Relative statistical indicators values of RSM and ANN model

Variables	RSM	ANN
R^2	0.9678	0.9990
RMSE	0.627368	0.568154
SEP	0.007201	0.006576
MAE	0.521	0.352
AAD	0.547091	0.37780

The degree of association or relationship among the parameters in the question was specified by correlation coefficient (R). A unit (1) value of coefficient of correlation implies perfect relationship between variables while zero (0) value is believed to be the absence of linear relationship the parameters. The best solution was chosen based on highest coefficient of correlation and least MSE for training, testing and validation. The correlation coefficients (R) between the actual and the expected values are 0.99943 for training, 0.9863

for testing, 0.99615 for validation and overall correlation was 0.99769. Therefore, the ANN anticipation for training, validation, and testing is highly substantial and meritorious in terms of correlation. As indicated in indicated in Fig. 4 and, the performance value for the final iteration gave a best result.

Comparison of RSM and ANN Performance

To identify the best model that accurately predict the effect of extraction parameters on the yield, the prediction capabilities of the RSM and ANN models the computed values of all statistical indicators were compared. The result showed that both models indicates high of accurate of the result, since the higher R^2 values were predicted in both cases. However, ANN gave a lower RMSE value when compared to the RSM model. Therefore, ANN was a better modeling tool due to its low RMSE and high extract yield. Owing to higher value of R^2 and lower values of other statistical values of ANN compared to RSM, the accuracy of ANN model is better than that of the RSM model.

Product Characterization

The analyzed characteristics of the extracted pectins were indicated with their values and summarized in table 5.

Table 5: Properties of extracted pectin with their values.

Parameters	Current Value (%)	Previous value (%) and References	
		Rahmani *et al.* (2020)	Mohamed, (2016)
Methoxyl content	6.43	6.23	8.875
Anhydrouronic acid	68.25	70.9	60.95
Acetyle content	0.371	0.4	0.455
EC	63.13	60.4	55.01
MW	604.74	599.74	–

To represent the extent to which carboxyl groups in pectin molecules exist as the methyl ester, esterification capacity (EC) is an essential molecular index for pectin classification. Since result indicates, higher degree of esterification above50 percentage, it can be considered as high methoxyl (HM) pectin. Pectin's has high tendency to form gel rapidly at higher temperature and have a great effective action on the lipid profile, when degree of esterification is above 50 percent (Brouns *et al.* 2012) molecular weight (MW; Dominiak *et al.* 2014). However, esterification capacity shows only the ratio between methanol-esterified carboxyl groups and free carboxyl groups, whereas the methoxyl rate represents to the amount of methoxyl groups in a sample (Fakayode & Abobi, 2018). So, the EC could not be judged separately, as it does not show the actual amount of methyl esterification, especially when the galacturonic acid amount is low. High methoxyl pectins (above 50% EC) require a relatively a low pH for gel formation and large concentration of soluble solids, while pectins having methoxyl below 50% EC, establish in flexible gels the using the movement of multivalent cations, which cross-hyperlink the galacturonic acid chains (Garna, Mabon, Wathelet, & Paquot, 2004). In present study, the esterification capacity (EC) of the hydrated pectin was 63.13% similar to previous studies as shown in table 4.

Another important characteristic in classifying the functional behavior of pectin is equivalent weight (EW) since, gelling tendency of individual pectin's are linked very closely with EW. The higher equivalent
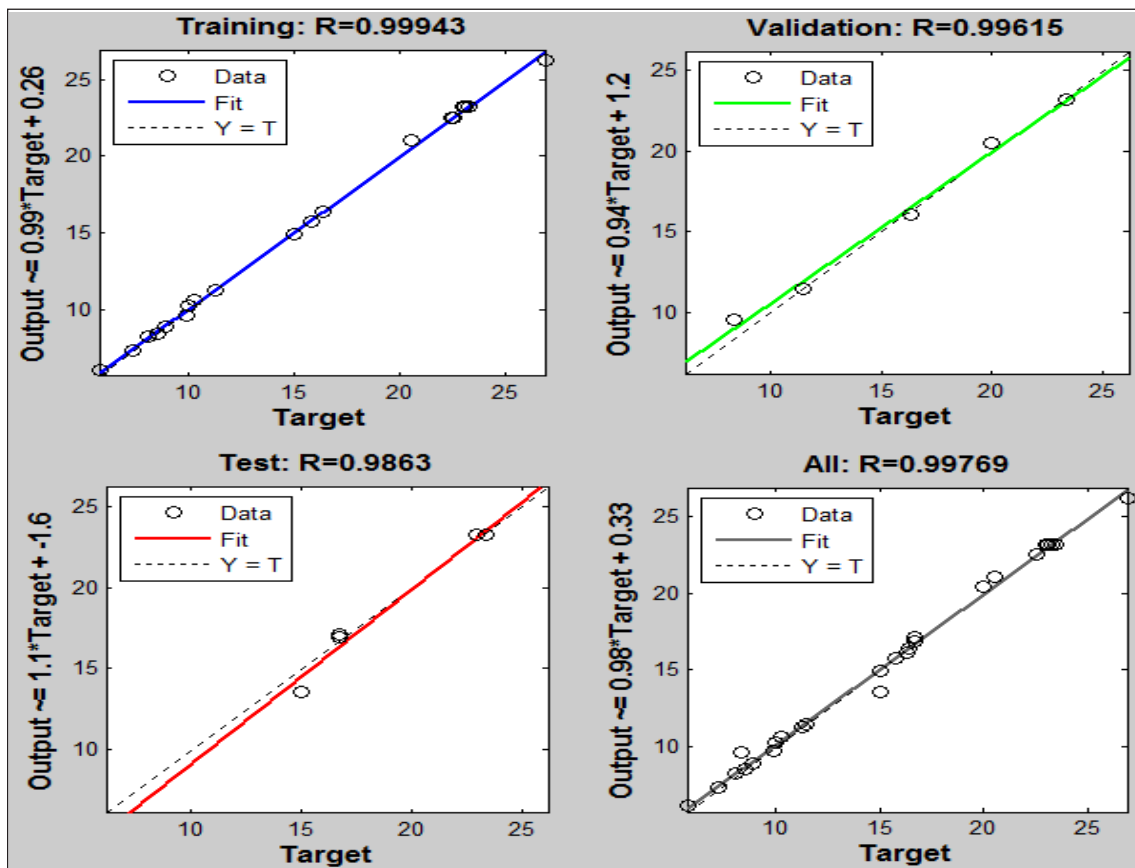
Fig. 4: Correlation coefficients for mean pectin yield (ANN)

weight, has greater gel formation, while the lower equivalent weight, indicates larger partial degradation of the pectin which is non-profitable (Hardy, 1924) strawberries, rhubarb stem and apples, Schryver and Haynies [1916] employed a hot dilute solution of ammonium oxalate to extract pectinogen from the washed and dried material. Subsequently, Farnell [1923], working in conjunction with Schryver, showed that oxalic acid could be substituted for the salt, and indeed, is preferable in so far that it remains in solution when the extracted pectinogen is precipitated by alcohol, whereas ammonium oxalate is strongly adsorbed by the alcohol-gel. Farnell also demonstrated that solutions of ammonium sulphate and of carbonic acid are capable of extracting appreciable amounts of pectinogen from dried turnip, but that water alone is without effect. He suggested that pectinogen is loosely combined with calcium in plant tissues, and is liberated therefrom by any reagent which precipitates the metal. Carre [1922] has, however, shown that dilute hydrochloric acid is an effective extractor of pectinogen, a result which is not concordant with this generalisation. In examining the factors which influence the extraction of pectinogen from the fibre of sugar cane, Farnell (private communication and thus the raise or diminish of the equivalent weight could be subjected to the content of free acid (Mohamed, 2016). The average equivalent weight of orange peel pectin in this study found to be 604.74 kDa closest to data reported by Altaf *et al.* (2015) at different time, temperature and pH combinations. Extraction was done at temperatures 60°C, 70°C and 80°C for

time 30 mins, 45 mins and 60 mins, and pH 2, 2.5 and 3.0 respectively. Using hydrochloric acid, the yield of pectin was higher (ranging from 2.8%-16%, that the equivalent weigh of the pectin extracted form papaya fruits using HCl and Citric acids were 912.17 and 455.1 respectively and stated that, pectin produced at lower pH value, has higher equivalent weight, since lower pH can lead polymerization of pectin into an extended chain, and in turn decrease the amount of free acid..

The methoxyl content (MC) is an instrument tool used to control the setting time, the gel strength and to find the functional properties of pectin (Twinomuhwezi, Godswill, & Kahunde, 2020). Kanmani, (2014) established that, depending on the origin of raw material used, method of extraction, in addition to the method used for determination of methoxyl content, the MC of pectin usually varies from 0.2–12%. The result indicated that 6.23% of methoxyl content was achieved. Since the methoxyl content was below 12%, this pectin has lower ester characteristic, which implies that it is desirable in terms of quality, and in addition due to it has above 50% DE.

As indication of Food Chemical Codex (FCC), Food and Agriculture Organization (FAO), and European Union (EU), pectin must contains at least 65% of galacturonic acid (Willats, Knox, & Mikkelsen, 2006), since anhydrouronic acid amount is used to represent the gelling capabilities of given pectin. The higher value means, the produced pectin has a lower amount of protein. In present study, the result indicated that 68.25% of anhydrouronic acid was achieved. A minimum value of anhydrouronic acid (65%) for commercial pectins has been specified by FAO (Twinomuhwezi *et al.* 2020). This criterion has limited the potential sources of food and pharmaceutical pectins.

Acetyl value (AV); the gelling capacity of pectin diminished with the raise in the degree of acetylation (Fakayode & Abobi, 2018). The inhibition of gelormation will be created when acetyl formation is found in pectin. Other researcher shown that the gelling capacity of pectin diminished with raise in the degree of acetylation and samples holding 3.5%-4.0% acetyl brings weak gels while gelling capacity restored at levels around 2.4% acetyl (Mohamed, 2016). Based on acetyl value and compared to previous study, the result indicates that orange peel pectin has good gelling capacity (0.371%) indicated in table 5.

CONCLUSION

The ultrasound-assisted extraction of pectin was optimized by the combination of both response surface methodology (RSM) and artificial neural network multi-layer back propagation (ANNMBP). The objectives of study were extraction of pectin from orange peel using ultrasound assisted extraction and response surface method and artificial neural network technique. The accuracy of the two models were studied to compare the performances of the two models to making decision for achievement of optimum process parameters during extraction of the pectin.

The following finding are absorbed from the effects of extraction parameters. The ultrasound assisted extraction and pH of citric acid solution applied for the extraction of pectin were found to be strongly influenced the yield. However, pH solution was highly significant compared to ultrasound power. As well as interaction between ultrasound and pH solution were found to be strongly influenced the extraction yield of pectin. The optimal conditions for extraction were irradiation time of 22.5 min, pH of 1.5, and ultrasound power of 155W and liquid-solid ratio 22.5:1 mL/ g. Under these conditions, yield of pectin was 26.87% experimentally, while 26.74 and 26.93% of yield were predicted by RSM and ANN model respectively.

The extracted pectin of orange peel was categorized as high methoxyl pectin since it has 63.13% degree of esterification that is above 50% affirmed by FTIR detection.

The prediction of yield was investigated by using RSM and ANN model and found to be in good agreement with experimental data, however, the prediction of ANN model is found to be better than RSM. The results indicates that, ANN model is much more accurate in estimating the values of pectin yield and mean square error when compared with the RSM.

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Acronyms

A	:	Irradiation time
ANOVA	:	Analysis of Variance
B	:	pH: power of hydrogen
C	:	Ultrasound power
C.V	:	Coefficient of variation
D	:	Liquid to solid ratio
KOH	:	Potassium hydroxide
R^2	:	Coefficient of determination
RSM	:	Response surface method
Std. Dev.	:	Standard deviation
FTIR	:	Fourier Transform Infrared spectroscopy
ANN	:	Artificial neural network
W	:	Watt

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