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OPTIMIZATION OF PRESSURISED INTERMITTENT MICROWAVE ASSISTED EXTRACTION OF PECTIN FROM THAI SOYBEAN HULLS

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ABSTRACT

Soybean (*Glycine max*), a major crop worldwide, is used to produce oil for cooking and for industrial purposes such as production of soy milk, soy sauce and biodiesel fuel. The hulls are mainly used as animal feed but they can be used for pectin extraction for food industries; but, the methods currently used for extraction have limited efficiency. Therefore, pressurised intermittent microwave-assisted extraction (PIMAE) was tested to extract pectin from soybean hulls from either flakes (SHF) or flakes ground into powder (SHP). Pressurised intermittent microwave-assisted extraction reduced both extraction time and solvent consumption. It also uses a by-product of a processed crop and is an environmentally friendly technology. Box-Behnken response surface design was utilized to study and optimize the effects of processing variables, which included pH from 1.5 to 2.5, pulse ratios from 1.0 to 1.51 and extraction time from 5 to 15 min on pectin yield. The degree of esterification of pectin was measured since it affects pectin's commercial value as a gelling and thickening agent. The amount of pectin extracted increased with increasing extraction time and reduced as the pH and pulse ratio increased. The conditions that resulted in the highest pectin yield were pH 2 pulse ratio 1.0 and extraction time 15 min, which gave 6.42% from SHF samples and pH 1.5, pulse ratio 1.0 and extraction time 10 min, which gave 12.09% from SHP samples. The conditions that produced the highest percentage of esterification were pH 2.5, pulse ratio 1.25 and extraction time of 5 min, which gave 80.81% for SHF and 80.59% for SHP samples. The extracted pectin contained high levels of methoxy pectin. Levels of pectin yield and degree of esterification were below the predicted theoretical values from the Box-Behnken response surface design, despite the good fit of the models, which were 0.99 R^2 for SHF and 0.98 R^2 for SHP for yields of pectin and 0.97 R^2 for SHF and 0.98 R^2 for SHP for the degree of esterification of pectin. In these optimal conditions, experimental yield correlated with predicted yields.

Key words: soybean hulls, pressurised microwave assisted extraction, pectin, Box-Benhken, degree of esterification

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INTRODUCTION

Pressurised intermittent microwave assisted extraction (PIMAE) combines microwave energy and traditional solvent extraction to heat polar solvents in contact with solids. This is done in order to partition compounds between the solid raw material and the solvent. This technique can reduce both extraction time and solvent consumption [1, 2]. Pressurised intermittent microwave assisted extraction improves extraction and solvent penetration, which gives higher temperatures and pressure throughout the raw material. It has low energy consumption, high extraction efficiency, short extraction time, selectivity of extraction, low pollution and uses less solvent. It is combined with intermittent operation of the microwave energy.

In the last 10 years, intermittent microwave extraction (IMAE) techniques have been utilized in drying and extraction of heat-sensitive important substances from plants, medicines and biomaterials. It has been successfully used to extract pectin since it avoids overheating and is better at balancing heat of samples during mass transfer processes [3]. The improved efficiency of intermittent microwave extraction compared to the continuous process is also because of efficiency of the pulsed heat supply [4]. Swamy and Muthukumarappan [5] showed that intermittent processing yielded higher pectin retrieval from banana peel compared to continuous processing microwave extraction (MAE), and is also comparable to other modern extraction techniques including supercritical fluid extraction (SFE), subcritical water extraction (SWE) and ultrasound-assisted extraction (UAE), due to its simplicity and low cost. Wang and Weller [6] considered both economic and practical aspects of extraction processes and concluded that MAE was a strong novel technique for the extraction of nutraceuticals. It has been successfully used for the extraction of pectin from the peel of fruit. Pectin consists of a backbone of α-D-(1, 4) galacturonic acid (GalA) residues, which are partially esterified with methyl alcohol or acetic acid in carboxylic acid. Pectin is classified into high methoxyl and low methoxyl pectin, depending on whether its degree of esterification (DE) is greater or less than 50% [7].

Pectin is the most important and widely used polysaccharide in the food industry. It is used as a gelling, colloid stabilizing and a thickening agent in foods including jams, jellies, confectionery, fruit juice, dairy and bakery products, nutraceutical and functional foods [8, 9]. The degree of esterification on pectin molecules can affect their commercial use as gelling and thickening agents. Additionally, pectin has been shown to have properties that can be used in pharmaceuticals, including wound healing, drug delivery, lipase inhibition, apoptosis induction of human cancer cell, anti-colon cancer activities, immunostimulation, anti-ulcer, anti-metastasis, insulin, gastric inhibitory polypeptides reduction and cholesterol decreasing effects [10, 11]. Soybean hulls are a source of dietary fibre and have been shown to reduce blood serum cholesterol [12].

The seed coats (hulls) of soybean are a major by-product of the soybean processing industry. Hulls constitute about 8% of soybean seed weight and contain about 86% complex carbohydrates and are used as a source of dietary fibre [12]. The insoluble carbohydrate fraction of cell walls of soybean hulls consists of 30% pectin, 50% hemicellulose, and 20% cellulose [13] and a small proportion of lignin. Camiscia *et al*.

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[14] compared soybean hulls with sugarcane bagasse, wheat straw and rice hulls and showed they have higher levels of lignocelluloses. Gnanasambandam and Proctor [15] reported that pectin from soybean hulls is a natural, inexpensive food grade form. It has been reported that IMAE can be successfully used to extract important substances from plants including pectin from pomelo peel [16] and banana peel [5], piperine-oleoresin from both black and white pepper [17, 18], β-carotene from carrot peel [19] and polysaccharides from seaweed (*Porphyra dentate*) [20]. Various techniques have been used to extract pectin, including extraction by hydrochloric acid [21] and hot-compressed water at temperatures of 110 to 180ºC [13]. However, since it has not been previously tested, the aim of the study was the optimization of pressurised intermittent microwave-assisted extraction of pectin from soybean hull flakes and soybean hull powder, by evaluating the effects of grinding methods on yield and degree of esterification of pectin.

MATERIALS AND METHODS

Materials

All chemicals used were of analytical grade. The hulls of Thai soybeans (*Glycine max* {L.} Merr. cv. 'Chiang Mai 60') were obtained as a byproduct from a local soybean processing company, Kim Kin Pattana Co., Ltd., Samut Sakhon, Thailand.

Preparation of soybean hulls powder

The soybean hulls were washed twice in tap water in order to ensure they were fully clean, dried in a hot air oven at 60ºC for 6 h and then milled into flakes. Half these dried milled flakes were then ground into powder using an electric stainless-steel grinding machine (Multipurpose Disintregrator Li Xiang-010A) with a particle diameter below 50 mesh (SHP) as shown in Figure 1. The other half was retained as flakes (SHF). All the samples were kept in vacuum polyethylene film bags and stored at 4ºC, in order to slow any possible deterioration, until required for analysis.

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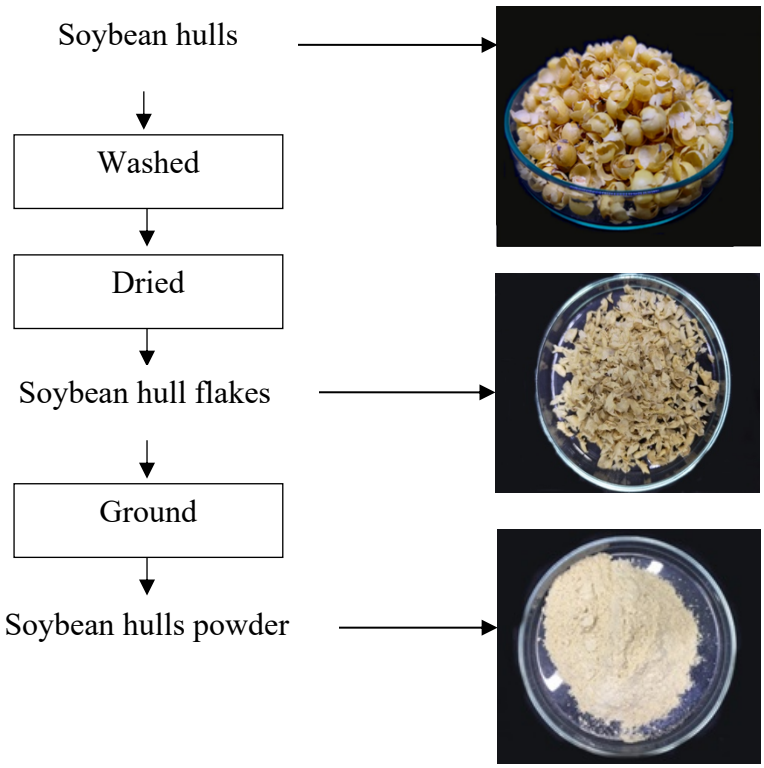

Figure 1: Preparation of soybean hull flakes (SHF) and soybean hulls powder (SHP) from soybean hulls

Pressurised intermittent microwave assisted extraction of pectin

Pressurised intermittent microwave assisted extraction of pectin was performed using a microwave pressure cooker (Prestige, 56846-C, 3.25L) and a 20 litre household microwave oven (Samsung ME711K/XST).

For pectin extraction 10 g (db) of each sample was mixed with deionized water at a ratio of 1:20 (w/v) in a 1 litre glass bowl. Three levels of pH were tested (1.5, 2 or 2.5) by adding the appropriate amount of HCl (0.5 mol L^{-1}). Each sample was then placed in the microwave pressure cooker and three pulse ratios (1, 1.25 or 1.51) and three times (5, 10 or 15 min) were tested.

The pulse ratios were calculated using Soysal *et al*. [22]:

$$\text{pulse ratio} = \frac{(\tau_{on}+\tau_{off})}{\tau_{on}} \quad \text{(Eq.1)}$$

Where τ_{on} is the turn on time (sec) of the microwave field and τ_{off} is the turn off time (sec) of the microwave field.

One cycle time was set to be $\tau_{on} + \tau_{off}$. When the pulse ratio = 1, it means that τ_{on} = 60 sec and τ_{off} = 0 sec. (timing of power on during utilized in the PIMAE). When the pulse ratio = 1.25, it means that τ_{on} = 24 sec and τ_{off} = 6 sec. When the pulse ratio = 1.51, it means that τ_{on} = 20 sec and τ_{off} =10 sec.

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Pectin purification procedure

After extraction, the pH of each sample was adjusted to 7 using NaOH (0.5 mol L^{-1}). The supernatant was concentrated with a rotary evaporator and collected by centrifugation at 6000 rpm for 20 min. Precipitation was carried out by adding isopropyl alcohol (IPA) in a ratio of 1:2 (precipitate:PA, v/v). The mixture was then stirred for 10 min at room temperature (approximately 25°C) followed by storage at 4°C for 48 h and then drying at 60°C in an oven to obtain the crude pectin, thereafter this crude pectin was used for analysis.

Analysis of yield of pectin

The yield of pectin (YP) extraction was calculated from the following equation (Eq.2) [8]

$$\text{Pectin yield (\%)} = \frac{\text{Weight of dry sample (g)}}{\text{Weight of initial sample (g)}} \times 100 \qquad \text{(Eq.2)}$$

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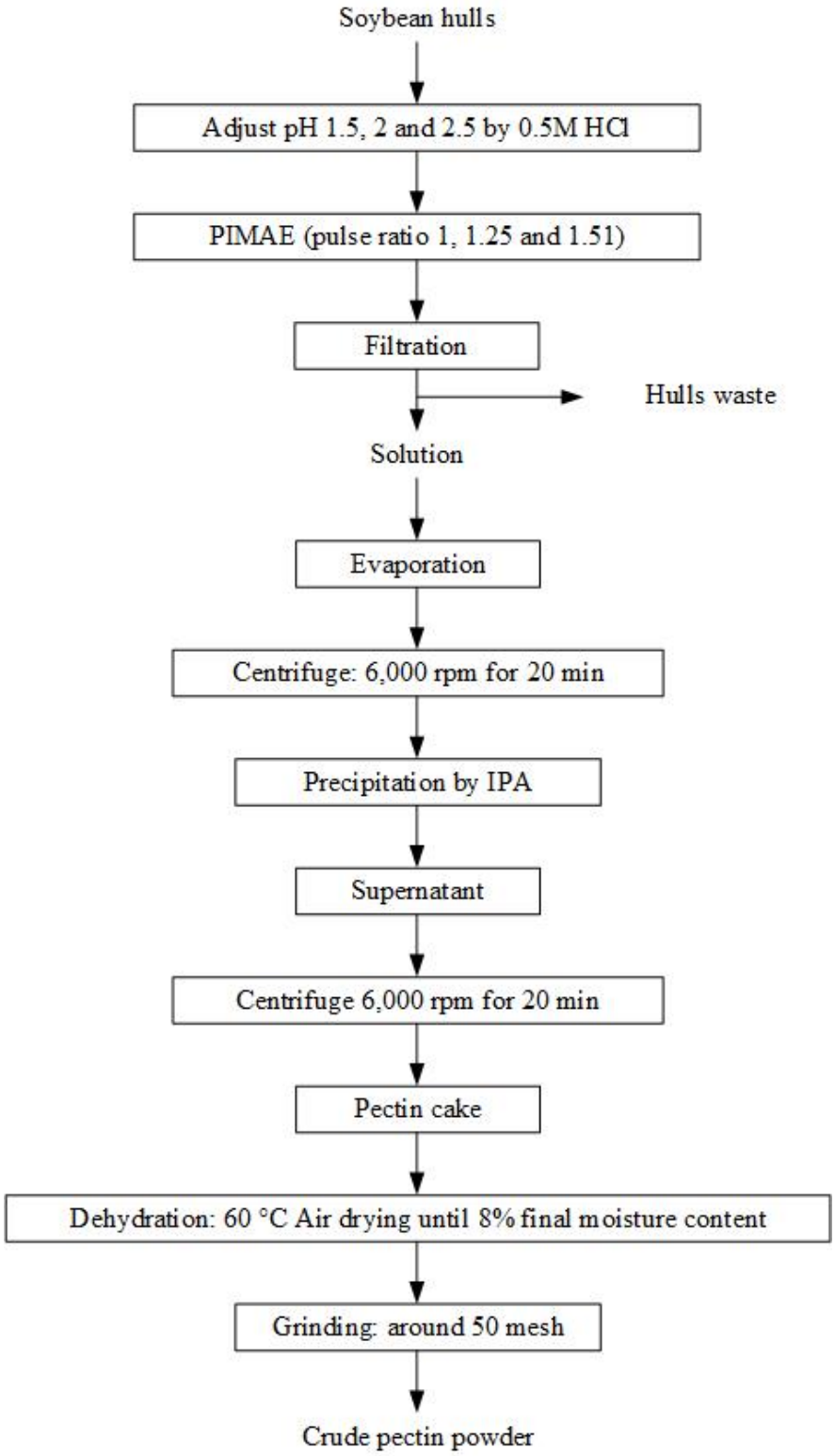

Figure 2: Flow diagram of pectin extraction from soybean hulls using pressurised intermittent microwave-assisted extraction (PIMAE)

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Analysis of degree of esterification (DE)

The degree of esterification of pectin samples was determined by the titration method [23] with slight modifications. First, dried pectin powder (500 mg) was placed in a 250 mL flask, to which 2 mL isopropyl alcohol and 100 mL deionized water were added. After dissolving the samples, five drops of phenolphthalein reagent were added and the solution titrated against NaOH (0.5 M). The end-point was recorded as the first titer or V_1. Subsequently, 10 mL of NaOH (0.5 M) were added for hydrolysis, and the solution was stirred for 15 min then, 10 mL HCl (0.5 M) were added and the solution was stirred vigorously until the pink color completely disappeared. After adding 5 drops of phenolphthalein reagent, the excess HCl was titrated against NaOH (0.5 M) until a pale pink color was obtained. The volume of NaOH that was required was recorded as the second titer or V_2.

The DE of pectin was calculated as follows [24]:

$$DE\ (\%) = \frac{V^2\ (mL)}{V^1\ (mL) + V^2\ (mL)} \times 100 \qquad \text{(Eq.3)}$$

Experimental set-up and characterization using pressurised intermittent microwave assisted extraction of pectin

In the present study, Response Surface Methodology coupled with Box-Behnken Design (BBD) was employed to investigate the individual and interactive effects of the process variables on the extraction process. pH (X_1), pulse ratio (X_2) and extraction time (X_3) were selected as independent variables and were coded in range -1 to 1. Table 1 shows the response variables selected when modeling pectin extraction. The pectin extraction yield was calculated from equation (Eq.2). All computations and graphics in this study were performed using the statistical software Design Expert 7.0 (Stat-Easy Inc., Minneapolis, USA). Seventeen experiments were carried out, which consisted of 12 randomized experiments and 5 replications of center points. Analysis of variance was employed to analyze the data and indicate the significance at the $p<0.05$ level.
Experimental data were also fitted to a second-order polynomial model (Eq.4) in order to obtain a regression coefficient.

$$\text{Yield or DE}\ (\%) = \beta_0 + \sum_{i=1}^{a} \beta_i X_i + \sum_{i=1}^{a} \beta_{ij} X_i X_j + \sum_{i=1}^{a} \beta_{ii} X_i^{\,2} \qquad \text{(Eq.4)}$$

Where β_0 was a constant coefficient, β_i, β_{ii} and β_{ij} were the coefficients of the linear, quadratic and interactive terms, respectively and X_i and X_j were coded variables.

RESULTS AND DISCUSSION

Experimental design and analysis

There was a total of 17 runs for optimizing the three individual parameters used in the current Box-Behnken design. The values of the responses at different experimental combinations for variables show that the pectin yield of SHF ranged between 0.47 to 6.42% and SHP 7.78 to 12.09%. Table 2 shows the predicted values for SHF and SHP were obtained by solving models of (Eq.5) and (Eq.6). The predicted results were close to the experimental results with a residual error of not more than ± 0.3. The highest yields

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were from pH 2, pulse ratio 1 and extraction time 15 min for SHF, and pH 1.5, pulse ratio 1 and extraction time 10 min for SHP.

The highest yields of pectin from at the replicated central point from SHF were 4.20 to 4.71% and SHP samples 10.26 to 10.88%. Also, the pectin yields increased with decreasing particle size, indicating that the decrease in particle size improved the extraction of the pectin. The independent variables had a significant (p<0.05) effect on pectin yield with the particle size decreasing as the pectin yield increased, which was due to the grinding breaking the cell walls, thus allowing compounds to easily pass out of the cells. Similar results were previously reported for the extraction of pectin from sugar beet pulp, where different particle sizes were achieved by traditional heating methods [25]. In addition, pectin extraction, using the instantaneous controlled pressure drop process from the expanded vegetal matrix affected extraction as has previously been shown for orange peel [26, 31], which supports the current results.

The degree of esterification of samples varied between 70.45 and 80.81% for SHF and between 70.44 and 80.59% for SHP. The highest DE was from the combination of pH 2.5, pulse ratio 1.25 and extraction time of 5 min. The DE of pectin from SHF and SHP sample at the replicated central point were 70.45 to 72.61 and 70.44 to 72.39 %, respectively. The predicted DE from SHF and SHP pectin for these factors were obtained by solving models of (Eq.7) and (Eq.8), with the predicted results similar to the experimental results.

Data fitting and statistical analysis

From the Box-Behnken design model, the conditions for achieving maximum yield were expressed in Equations (Eq.5) to (Eq.8):

$$\text{Pectin yield of SHF (\%)} = +16.315-4.32669X_1-10.90096X_2+0.099867X_3-1.78431X_1X_2 +0.3270X_1X_3+0.23333+0.5590X_1^2+3.45636X_1^2-0.032410X_3^2 \quad \text{(Eq.5)}$$

$$\text{Pectin yield of SHP (\%)} = +1.2560.34271X_1+11.79154X_2+0.93433X_3+0.76471X_1X_2+0.049X_1X_3-0.21373X_2X_3- 0.6830X_1^2-5.70165X_1^2-0.024830X_3^2 \quad \text{(Eq.6)}$$

$$\text{DE of SHF (\%)} = +80.032-5.63818X_1 -0.87814X_2-0.85191X_3 -5.05882X_1\ X_3 - 0.2860X_1X_3 -0.65294X_2\ X_3+4.5530X_1^2 +8.31603X_2^2+0.08223X_3^2 \quad \text{(Eq.7)}$$

$$\text{DE of SHP (\%)} =+131.585-23.06028X^1-70.64179X^2 +0.2284X^3+11.96078X^1X^2- 0.6090X^1X^3-0.61569X^2X^3+4.9330X_1^2+22.92580X_1^2+0.062330X_3^2 \quad \text{(Eq.8)}$$

Where, X_i is a coded independent factor (X_1= pH, X_2= pulse ratio and X_3= extraction time).

Accuracy and variance analysis of the regression model

The results of ANOVA are presented in Table 3 and show the effects of linear and quadratic terms, where the quadratic polynomial regression fitted well to the

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experimental data. To evaluate the accuracy and validity of the model, the ANOVA for yield was carried out (Table 3). The results of pectin yield from SHF had an f-value of 73.16, indicating that the deviation in the responses could be clarified by the regression equation and that the model was highly significant (p<0.0001). Probability values for the main effect for pulse ratio (X_2) and extraction time (X_3) were lower, which in turn indicated that the fitness of the model was highly significant (p<0.0001), whilst the interaction between pH, pulse ratio and extraction time was significant (p<0.05), confirming that the model was significant.

The results of pectin yield from SHP showed that they approximated to the results of SHF, with an f-value of 50. Probability values of the main effect for pH (X_1), pulse ratio (X_2) and extraction time (X_3) were below p<0.0001 and interactions between the main effects were p<0.05. The results also demonstrated that the proposed regression model for SHF and SHP pectin yield was satisfactory with high values of R^2 (0.99 and 0.98) and adj-R^2 (0.98 and 0.96. It is clear from the above that the form of the model chosen to represent the actual relationship between the response and independent variables gave good correlations.

Low values of coefficient of variance clearly exhibited a very high degree of precision and good reliability in our experiments.

The results of ANOVA showed the f-values for the DE from SHF pectin as 28.48 and from SHP pectin as 33.64 (Table 4), which implies the model was significant at p<0.05. There was only a 0.01% chance that a model f-value as large could occur due to noise. Probability values of the main effect for pH (X_1), pulse ratio (X_2), extraction time (X_3) were significantly different (p<0.05) for the DE of SHF pectin. Also, the interactions between pH and pulse ratio (X_1X_2), pH and extraction time (X_1X_3), pulse ratio and extraction time (X_2X_3) did not show a significant effect (p<0.05) on the DE of SHF pectin. However, the main effect of SHF pectin on DE had the highest interactive effect, which was significant at p<0.0001.

The results of degree of esterification from SHP pectin showed probability values of the main effect for pH (X_1) and extraction time (X_3) of p<0.0001, and the interaction between pH and pulse ratio (X_1X_2), pH and extraction time (X_1X_3) had significant probability values (p<0.05). There was no significant p value greater than 0.05 interaction between pulse ratio and extraction time (X_2X_3) on the DE of SHP. Also, the pulse ratio and extraction time had highest level of significant interactive effects on DE of SHP pectin. It can, therefore, be concluded that the proposed regression model for SHF and SHP pectin on DE was satisfactory with high R^2 (0.97 and 0.98), adj-R^2 (0.94 and 0.95) values, respectively (Table 4). The lack of fit for the f-value (Table 4) of 0.13 for SHF and 0.75 for SHP, indicate that they were not statistically significant (p>0.05) relative to the pure error, which were 93.45 and 57.56% and the results could have occurred due to noise.

Determination of optimum conditions

From the results, the optimum conditions for achieving maximum extraction yield of pectin, which were 6.42% for SHF and 12.07% for SHP, were as follows: pH of 2.0, pulse ratio of 1 and extraction time of 15 min.

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The mean values for these experiments, performed in triplicate, were 6.32±0.24% for SHF and 12.16±0.36% for SHP indicating that the experimental yield was approximate to the predicted yield and thus validated the optimized conditions (Table 5). Eskilsson and Björklund [27] also reported that plant particle size and size distribution usually have a significant influence on the efficiency of MAE and that the particle size of the extracted material are usually in the range of 100µm to 2 mm. Fine powder can enhance the extraction because the limiting step of the extraction is often the diffusion of chemicals out of the plant matrix and the larger surface area of a fine powder provides better contact between the plant matrix and the solvent. For example, for MAE of cocaine and finely ground cocoa powder were more easily extracted than larger particles [6, 28].

Effect of independent variables on the yield of soybean hull flakes and soybean hulls powder pectin

The effects of pH on the yield of SHF and SHP pectin extracted (Fig. 3 a-b and Fig. 4 a-b) show that the highest yield was obtained at pH 1.5, and yield decreased slightly with a pH increase of 2 to 2.5. Hamidon and Zaidel [29] had previously showed that low pH might prompt the disruption of hydrogen bonds and ester linkages between pectin and cell wall, which can then increase the rate of diffusion and pectin. Also, low pH can reduce the molecular weight of pectin that can result in partial extraction from plant tissues [30]. The effect of pulse ratio (Fig. 3 b, c, and Fig. 4 b, c) showed that with increasing pulse ratio, pectin yield steadily increased. It has previously been reported that the higher pulse ratio, the more the pectin can easily dissociate into the solvent. This is because relatively long on periods (higher on: off ratio) induces sudden temperature rises and internal pressure increases inside the plant cells and causes swelling, which forces cell walls to rupture [6, 30].

The yield of pectin at the extraction time of 15 min (Fig. 3 b, c, and Fig. 4 b, c) showed a steadily increasing yield as the extraction time increases. It was previously shown that increasing the extraction time allows thermal accumulation within extraction solution that will increase the absorption of microwave energy, promote the dissolution and steadily increase yield [31].

Effect of independent variables on the degree of esterification of soybean hull flakes and soybean hulls powder pectin

The degree of esterification of pectin was between 70.18% and 80.80%; therefore, both SHF and SHP pectin can be considered to be high methoxyl pectin (HMP). High methoxyl pectin has the ability to form gels with sugar and acids; the so-called low water activity gels or sugar-acid-pectin gels. Therefore, HMP can be used as a gelling agent, thickening agent and stabilizer in food. The classical application is giving the jelly-like consistency to jams or marmalades, which would otherwise have a consistency that is too liquid. Pectin can also be used to stabilize acidic protein drinks, such as drinking yogurt, and as a fat substitute in baked goods [32-34]. The DE of both SHF and SHP pectin increased with increased pH and decreased pulse ratio and extraction time (Fig. 4), which are consistent with their results described above.

The maximum interactions at pH 2.5 of pectin were at the higher levels of DE, with higher pulse ratio and reduced extraction time. The reduction of DE of pectin in lower

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pH, pulse ratio and short extraction time was probably because of the de-esterification of galacturonic acid chains. These results are consistent with previous work by Pasandide *et al*. [35] and Hosseini *et al*. [36].

The main effect of pulse ratio and the interaction between pulse ratio, extraction time and pH did not show a significant ($p > 0.05$) effect on DE of the pectin, the higher the pulse ratio, the higher was the extraction efficiency.

At pH 2 and 2.5, pulse ratio and extraction time favored the formation of pectin with high DE (Table 2), which is in accordance with previous studies [37, 38]. In general, pectin extracted in low pulse ratio, long extraction time and low pH had low DE. This is because these harsh conditions promote the de-esterification of polygalacturonic chains [33, 39, 40]. From the current research, using PIMAE, the highest yield of pectin extracted from soybean hulls, 12.16% (Table 5), was low compared with using hydrochloric acid where results were 16.31% and DE was 18.02% at 90°C for 60 min [21] and hot-compressed water at 150ºC for 60 min it was 19.4% [13].

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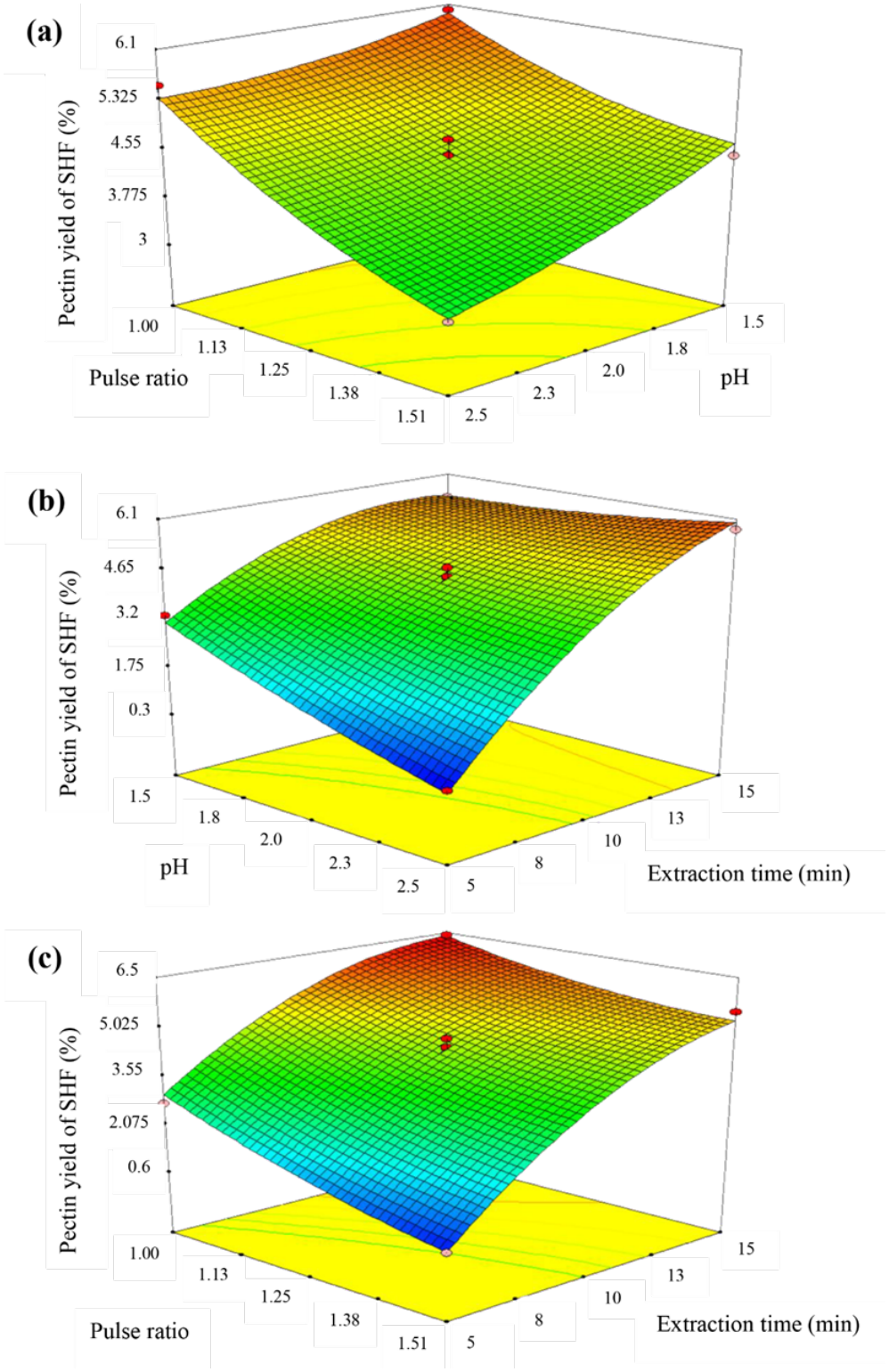

Figure 3: 3-D response surface plots showing the effect of process variables on the yield of soybean hull flakes (SHF): (a) effect of pH and pulse ratio: (b) effect of pH and extraction time: (c) effect of pulse ratio and extraction time

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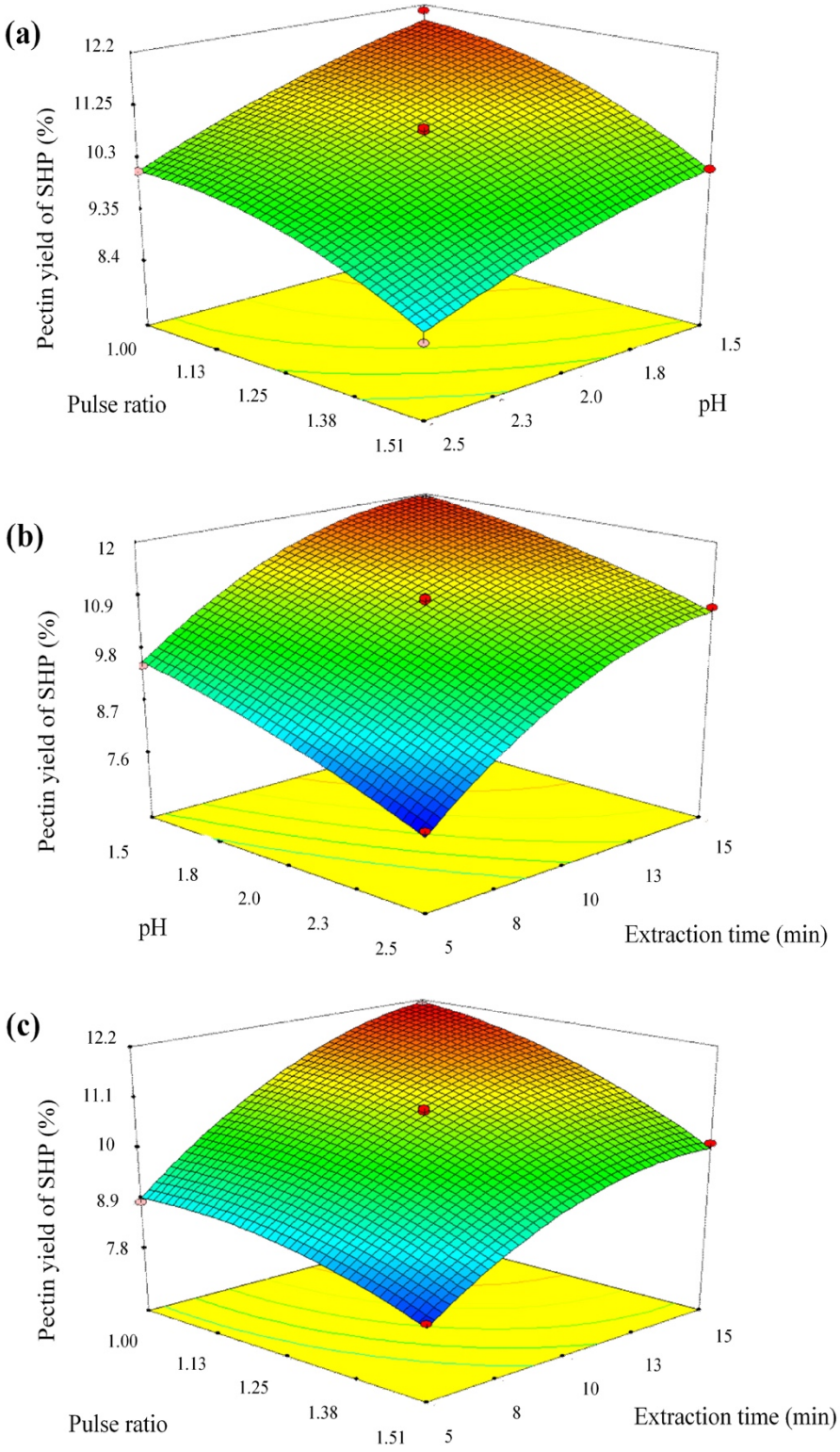

Figure 4: 3-D response surface plots showing the effect of process variables on the yield of soybean hulls powder (SHP): (a) effect of pH and pulse ratio: (b) effect of pH and extraction time: (c) effect of pulse ratio and extraction time

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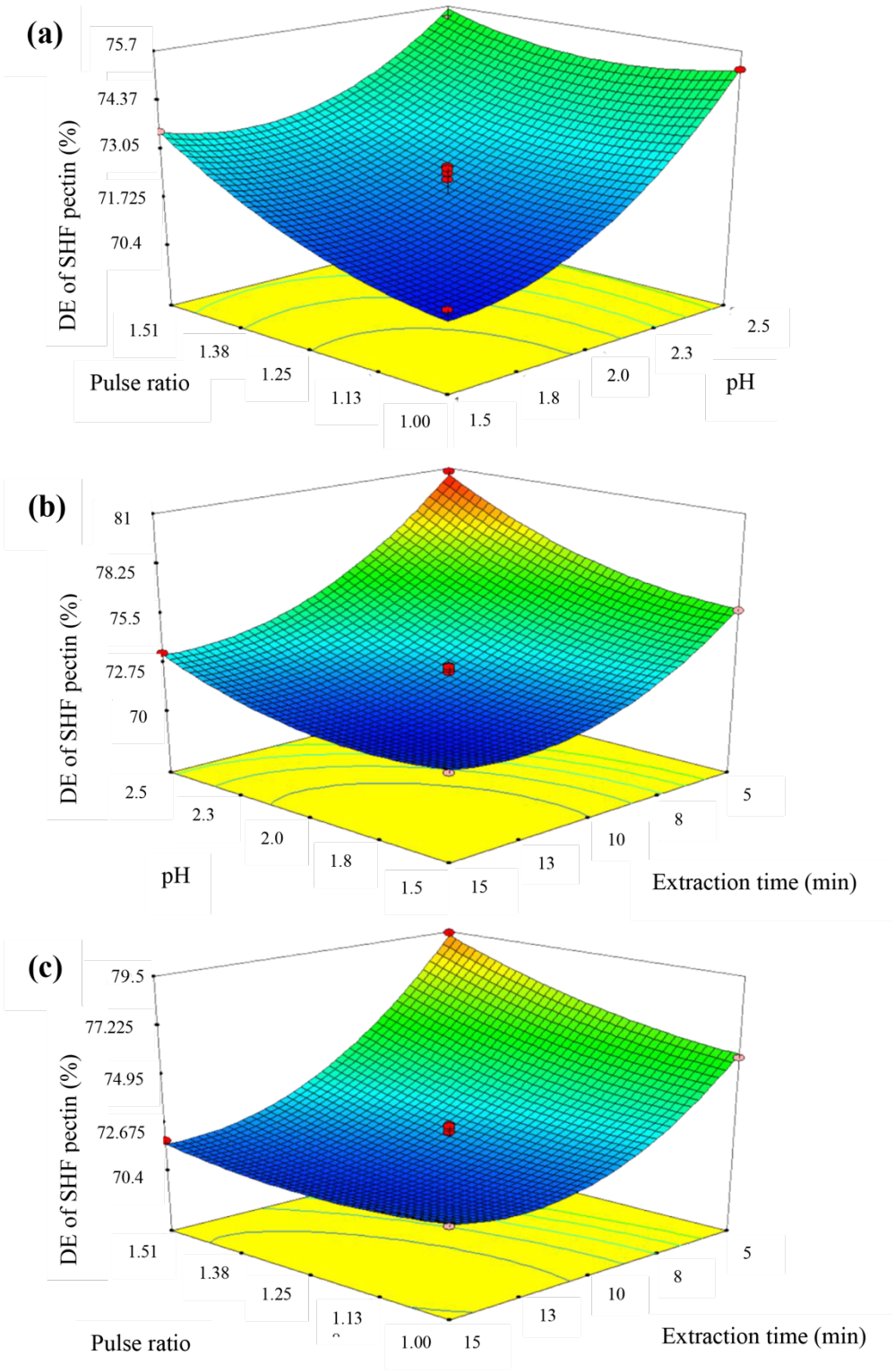

Figure 5: 3-D response surface plots showing the effect of process variables on the degree of esterification of the soybean hull flakes (SHF) pectin: (a) effect of pH and pulse ratio: (b) effect of pH and extraction time: (c) effect of pulse ratio and extraction time

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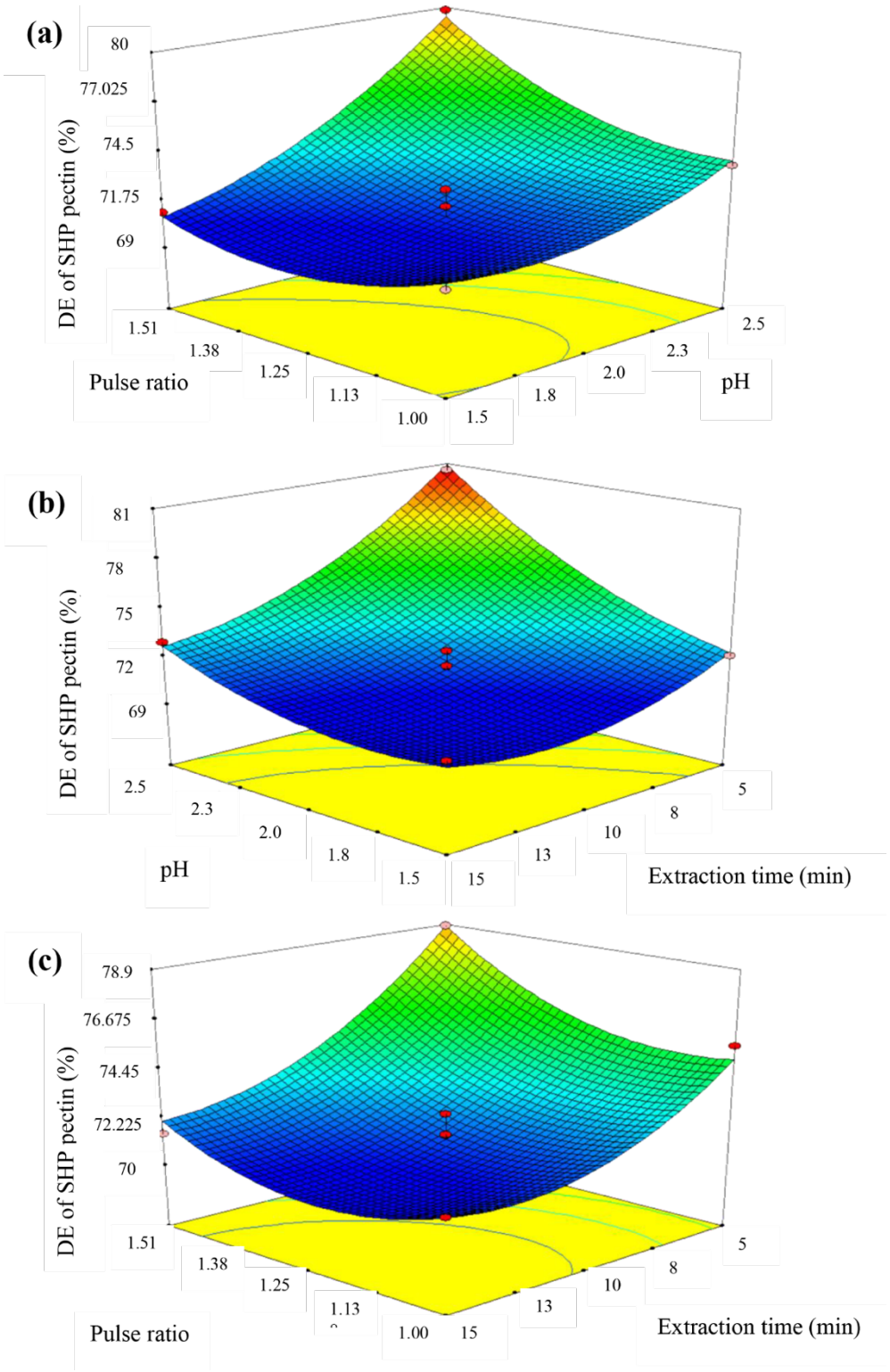

Figure 6: 3-D response surface plots showing the effect of process variables on the degree of esterification of the soybean hulls powder (SHP) pectin: (a) effect of pH and pulse ratio: (b) effect of pH and extraction time: (c) effect of pulse ratio and extraction time

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CONCLUSION

In this study, pressurised intermittent microwave-assisted extraction (PIMAE) was optimized for the extraction of pectin from soybean hulls. For three factors at three levels, Box–Behnken response surface experimental design was successfully employed to optimize the individual and interactive effect of the process variables of pH, pulse ratio and extraction time on the maximum extraction yield and degree of esterification of pectin from the soybean hulls. Comparison of soybean hull flakes and flakes that had been ground into a powder indicated that grinding enhanced the pectin extract yield because of improved access of the solvent to the pectin molecules. On the basis of the extraction level, soybean hulls pectin can be classified as high methoxyl type with a degree of esterification higher than 50%. The PIMAE conditions that resulted in the highest yield were pH 1.5, pulse ratio 1 and extraction time 10 min, which gave pectin yields of up to 12.09%. The yield of pectin extracted was shown to be dependent on the extraction technique, pH, temperature and extraction time.

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Table 1: Process variables and their ranges

Factor	Code	Level		
		-1	0	1
pH	X_1	1.5	2	2.5
pulse ratio	X_2	1	1.25	1.51
Extraction time (min)	X_3	5	10	15

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Table 2: Box-Behnken design matrix together with experimental, predicted value and residual error on pectin yield and degree of esterification

Std	Run	Independent variables			Pectin yield (%)						Degree of esterification (%)					
					Experimental		Predicted		Residual error		Experimental		Predicted		Residual error	
		X_1	X_2	X_3	SHF	SHP	SHF	SHP	SHF	SHP	SHF	SHP	SHF	SHP	SHF	SHP
4	1	2.5	1	10	5.55	10.07	5.35	10.08	0.20	-0.01	75.21	73.78	75.21	74.03	0.00	-0.25
8	2	2.5	1.25	15	5.80	10.68	6.03	10.61	-0.23	0.07	73.34	72.92	73.24	72.65	0.10	0.27
1	3	1.5	1.51	10	4.45	10.12	4.65	10.11	-0.20	0.01	73.55	71.07	73.55	70.82	0.00	0.25
15	4	2	1.25	10	4.45	10.88	4.43	10.75	0.02	0.13	72.43	72.39	72.04	71.20	0.39	1.19
17	5	2	1.25	10	4.71	10.87	4.43	10.75	0.28	0.12	70.45	71.43	72.04	71.20	-1.59	0.23
10	6	2	1	5	2.74	8.83	3.01	8.93	-0.27	-0.10	75.79	75.51	75.96	74.91	-0.17	0.60
5	7	1.5	1.25	5	3.31	9.45	3.11	9.55	0.20	-0.10	75.73	72.07	75.80	72.30	-0.07	-0.23
14	8	2	1.25	10	4.42	10.81	4.43	10.75	-0.01	0.06	72.24	71.41	72.04	71.20	0.20	0.21
13	9	2	1.25	10	4.26	10.85	4.43	10.75	-0.17	0.10	72.61	70.46	72.04	71.20	0.57	-0.74
7	10	1.5	1.25	15	5.37	11.86	5.45	11.99	-0.08	-0.13	71.12	70.49	71.28	70.16	-0.16	0.33
2	11	2.5	1.51	10	3.08	8.49	3.13	8.68	-0.05	-0.19	75.34	79.83	75.63	79.46	-0.29	0.37
12	12	2	1	15	6.42	12.07	6.40	12.15	0.02	-0.08	71.52	71.28	71.64	71.26	-0.12	0.02
16	13	2	1.25	10	4.20	10.26	4.43	10.75	-0.23	-0.49	72.55	70.44	72.04	71.20	0.51	-0.76
9	14	2	1.51	5	0.63	7.96	0.65	7.88	-0.02	0.08	79.45	78.84	79.33	78.86	0.12	-0.02
11	15	2	1.51	15	5.50	10.11	5.23	10.01	0.27	0.10	71.85	71.47	71.68	72.07	0.17	-0.60
6	16	2.5	1.25	5	0.47	7.78	0.43	7.68	0.04	0.10	80.81	80.59	80.62	80.87	0.19	-0.28
3	17	1.5	1	10	6.01	12.09	5.96	11.90	0.05	0.19	70.84	71.12	70.55	71.49	0.29	-0.37

Where, Coded X1: pH, X2:pulse ratio, X3: extraction time, SHF: soybean hull flakes and SHP: soybean hulls powder

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Table 3: Analysis of variance for SHF and SHP yield of soybean hulls pectin

Source	Sum of squares	DF	Mean square	F-Value	P-Value
Yield of SHF pectin					
Model	46.43	9	5.16	73.16	< 0.0001
X_1-pH	2.25	1	2.25	31.87	0.0008
X_2-Pulse ratio	6.23	1	6.23	88.37	< 0.0001
X_3-Extraction time	31.76	1	31.76	450.48	< 0.0001
X_1X_2	0.21	1	0.21	2.94	0.1303
X_1X_3	2.67	1	2.67	37.92	0.0005
X_2X_3	0.35	1	0.35	5.02	0.06
X_1^2	0.08	1	0.08	1.17	0.316
X_2^2	0.21	1	0.21	3.02	0.126
X_3^2	2.76	1	2.76	39.21	0.0004
Residual	0.49	7	0.07		
Lack of Fit	0.34	3	0.11	2.82	0.1708
Pure Error	0.16	4	0.04		
Cor Total	46.92	16			
C.V. %	6.32				
R^2	0.99				
Adj R^2	0.98				
Pred R^2	0.88				
Yield of SHP pectin					
Model	27.60	9	3.07	50.00	< 0.0001
X_1-pH	5.28	1	5.28	86.11	< 0.0001
X_2-Pulse ratio	5.09	1	5.09	82.96	< 0.0001
X_3-Extraction time	14.31	1	14.31	233.34	< 0.0001
X_1X_2	0.04	1	0.04	0.62	0.4569
X_1X_3	0.06	1	0.06	0.98	0.3555
X_2X_3	0.30	1	0.30	4.84	0.0637
X_1^2	0.12	1	0.12	2.00	0.2
X_2^2	0.58	1	0.58	9.44	0.018
X_3^2	1.62	1	1.62	26.45	0.0013
Residual	0.43	7	0.06		
Lack of Fit	0.15	3	0.05	0.68	0.6067
Pure Error	0.28	4	0.07		
Cor Total	28.03	16			
C.V. %	2.43				
R^2	0.98				
Adj R^2	0.97				
Pred R^2	0.90				

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Table 4: Analysis of variance for SHF pectin and SHP pectin on DE of soybean hulls Pectin

Source	Sum of squares	DF	Mean square	F-Value	P-Value
DE of SHF pectin					
Model	133.20	9	14.80	28.48	0.0001
X_1-pH	22.65	1	22.65	43.58	0.0003
X_2-Pulse ratio	5.83	1	5.83	11.22	0.0123
X_3-Extraction time	71.70	1	71.70	137.99	< 0.0001
X_1X_2	1.66	1	1.66	3.20	0.1166
X_1X_3	2.04	1	2.04	3.94	0.0877
X_2X_3	2.77	1	2.77	5.34	0.0542
X_1^2	5.46	1	5.46	10.50	0.0142
X_2^2	1.23	1	1.23	2.37	0.1676
X_3^2	17.79	1	17.79	34.25	0.0006
Residual	3.64	7	0.52		
Lack of Fit	0.33	3	0.11	0.13	0.9345
Pure Error	3.30	4	0.83		
Cor Total	136.83	16			
C.V. %	0.98				
R^2	0.97				
Adj R^2	0.94				
Pred R^2	0.92				
DE of SHP pectin					
Model	178.26	9	19.81	33.64	< 0.0001
X_1-pH	62.55	1	62.55	106.24	< 0.0001
X_2-Pulse ratio	11.33	1	11.33	19.24	0.0032
X_3-Extraction time	54.34	1	54.34	92.29	< 0.0001
X_1X_2	9.30	1	9.30	15.80	0.0054
X_1X_3	9.27	1	9.27	15.75	0.0054
X_2X_3	2.46	1	2.46	4.19	0.0800
X_1^2	6.40	1	6.40	10.88	0.0132
X_2^2	9.36	1	9.36	15.89	0.0053
X_3^2	10.22	1	10.22	17.36	0.0042
Residual	4.12	7	0.59		
Lack of Fit	1.49	3	0.50	0.75	0.5756
Pure Error	2.63	4	0.66		
Cor Total	182.38	16			
C.V. %	1.05				
R^2	0.98				
Adj R^2	0.95				

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Pred R^2	0.85

Table 5: Validation results at optimum parameters as pH of 2.0, pulse ratio of 1, extraction time of 15 min

Pectin yield (%)	Optimized data (predicted)	Experimental data[a]
Soybean hull flakes (SHF)	6.43	6.32±0.24
Soybean hulls powder (SHP)	12.09	12.16±0.36

[a] Mean ± standard deviation of triplicate determinations from experiments

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