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Citric acid extraction of pectin from tropical fruit peels of passion fruit, dragon fruit and soursop

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Abstract

Pectin has been intensively used as natural gelling agent and stabilizer to alter rheological properties in food ingredients by most food processing industries in achieving desired textural quality. Three tropical fruit peels, the yellow passion fruit, red dragon fruit, and soursop were selected for optimised extraction of pectin using citric acid extraction by varying pH from 2.0 to 4.5 and extraction time from 30 to 120 min. Peels of yellow passion fruit and dragon fruit gave pectin yield of 14.24% and 12.56% with degree of esterification (DE) of 55.54% and 47.88% at optimised extraction conditions of pH 2.37 and extraction times of 58.47 and 64.67 min, respectively. The soursop peel had relatively low pectin of < 6%. The pH of extraction solvent had significant effect on pectin yield where pH 2.0 was suggested for high pectin yield. The passion fruit and dragon fruit peels which have greater capability for producing pectin are recommended for the pectin industry.

Key words: Pectin, degree of esterification, tropical fruit peels, citric acid, extraction.

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Introduction

Pectin is a carbohydrate polymer which contains at least 65% galacturonic acid units where the acid groups may either be free or in the form of a simple salt, i.e. sodium, potassium, calcium or ammonium or naturally esterified with methanol [1]. Pectins are classified into high (DE > 50) and low (DE < 50) methoxyl pectin in accordance to the percentage of degree of esterification (DE) [2]. The DE is expressed as a percentage of the esterified galacturonic acid units to the total galacturonic acid units in a pectin molecule[1]. The food industry traditionally uses pectin as a thickener, emulsifier, and stabiliser. It is added to jellies and jams as a gelling agent. In food, pectin is used as a fat substitute in spreads, salad dressings and ice-cream. Pectin is normally incorporated with starches in starch-based food in order to improve tolerance of processing conditions, cold storage/freeze-thaw stability and water mobility control, besides achieving modified textural quality [2]. More recently, pectin has been used in edible food packaging and film manufacturing industries because of its antimicrobial property[1, 3]. In pharmaceutical terms, pectin is used to lower blood cholesterol levels and treat diarrhea [4].

With many ways of pectin usage and its health benefits, the world demand for pectin has increased and pectin production is urgently needed in the market [5]. Pectin production has been extensively studied and this included recovery from by-products from food processing industries such as fruit wastes. The recovery of pectin from industries' by-products were pomaces of grape [6], *Citrus junos* [7], peach [8] and apple [9, 10] from winemaking and juice processing industries, and rapeseed cake [11] from oil extraction. Fruit waste in the form of peels, such as the orange [4, 5], pomelo [12, 13], passion fruit [14, 15], grapefruit [16], lime [17], banana [18], mango[19] and dragon fruit [20, 21] are also popular sources of obtaining pectin.

The yellow passion fruit (*Passiflora edulis* var. *flavicarpa*), red dragon fruit (*Hylocereus polyrhizus*) and soursop (*Annona muricata* L.) are popular tropical fruits in Southeast Asia. They are commonly processed into juice and their waste in the form of peels may be utilised for pectin extraction. These three fruits may be an alternative source to western fruits such as apple and citrus fruits for pectin extraction. Pectin extraction from fruit peels also provides an alternative way to reduce cost of biological waste disposal whilst generating income for fruit juice processing industry.

Numerous extraction methods were reported in obtaining pectin from various fruit peels. Most extraction methods have used an acidic extraction solvent. The acidic condition of extraction solvent is adjusted to a pH between 2 to 3 using mineral acids, such as sulphuric acid [22], hydrochloric acid [13, 16, 23], nitric acid [8, 13, 14], or organic acids of citric [6, 20, 24], acetic [14] and tartaric acid [9, 14]. The usage of strong mineral acids which are corrosive may be hazardous to health. The waste liquor generated from the pectin extraction industrial processes may also lead to a burden to the environment and a high treatment cost due to strong acidity [24].

Although the use of strong acids provides high extraction yields and time-saving advantages, the extracted pectin is known to undergo degradation [7]. The citric acid, being a weak organic acid that is naturally concentrated in a great number of plant fruits, especially the citrus plants has been used for pectin extraction in various studies [25] and found to be better than other extractors in terms of better degree of esterification, macromolecular and gelling properties [15]. The objective of this study were to investigate the influences of pH adjusted by citric acids and extraction time on pectin yield and degree of esterification from tropical fruit peels of passion fruit, red dragon fruit and soursop.

Material and Methods

Preparation of raw materials: Yellow passion fruit (*Passiflora edulis* var. *flavicarpa*) and red dragon fruit (*Hylocereus polyrhizus*) at the same stage of ripeness and with similar peel colours were selected from the same harvest at the Multi-Rich Pitaya Orchard, Selangor (Malaysia) during the months of January to April 2012. The soursop (*Annona muricata* L.) fruits were obtained from a fruit wholesales in Selayang, Selangor (Malaysia). The fruits were first washed twice with distilled water and then the flesh was separated from the fruit peels. The peels were dried in a convection oven (UM 500, Memmert GmbH, Germany) at 55°C until a constant weight was achieved. The dried peels were then milled into 534.56 – 634.21 µm mesh size powdered fruit peel using an electronic miller (DFT-150, Dickson, China). The dragon fruit peel powder was packaged in a polyethylene bag and stored at -15°C in a freezer (ACF15F, Acson, Malaysia). All chemicals used were of analytical grade as supplied by Systerm Sdn Bhd, Selangor (Malaysia).

Particle size of fruit peel powder: The particle size of the three fruit peel powders, passion fruit, dragon fruit and soursop, were measured using the Mastersizer 2000 (Malvern Instruments, Worcestershire, United Kingdom) equipped with the Scirocco 2000 dry dispersion unit, connected to a computer equipped with Malvern software. Particle size distribution of each fruit peel sample was performed in order to eliminate result inconsistency due to differences in particle size of raw material. An averaged fruit peel powder particle size distribution graph was plotted with particle size (µm) axis versus volume (%) (Fig. 1). The averaged particle size for passion fruit, dragon fruit and soursop peel powder were 544.7 ± 84 µm, 634.2 ± 26 µm and 534.6 ± 18 µm, respectively.

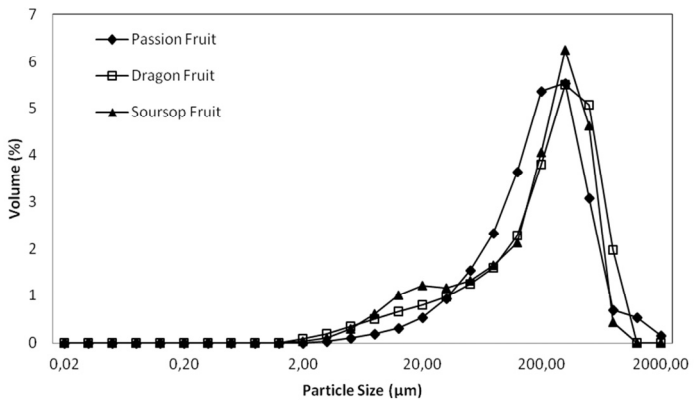

Figure 1. Averaged fruit peel powder particle size distribution plotted as volume (%) versus particle size (ìm).

Citric acid extraction procedure: Pectin was extracted using different pH and extraction times. A total of 10 g fruit peel powder, measured on an analytical balance (B204-S, MK II, Mettler Toledo, Switzerland) scale was blended with 250 ml distilled water and acidified with different volumes of 0.1 N citric acid to meet the designed pH of 2.0, 3.3 and 4.5. The mixture was then stirred using a stirrer until all fruit peel powder was evenly wetted by distilled water in homogenous form. The pectin extraction procedure was continued by treating the acidified samples at 70°C for 30, 75 or 120 min in a shaking water bath (Lab Companion 37L, Jeio Tech, Korea). The mixture was kept at room temperature for 24 hours.

The precipitated pectin was recovered by using a refrigerated centrifuge (Mikro 22R, Hettich, Germany) at 6000 rpm for 10 min. Water bath heat-treated samples were then filtered and a double volume of 95% ethanol (1:2 v/v) was added to allow pectin precipitation. The samples were stored in dark conditions at room temperature of 25°C for 24 hours to allow pectin flotation which was then separated by filtration and subsequently washed twice using 70% ethanol. Acetone was added in a drop-wise manner and until the top liquid phase completely cleared to remove unwanted color of the pectin [26]. The resulting pectin substance was dried in a conventional oven (UM500, Memmert GmbH, Schwabach, Germany) at 65°C until constant weight was reached. The percentage yield of the fruit peel pectin was determined as gram of product obtained per 10 g of fruit peel powder following equation (1):

$$Pectin\ yield\ (\%) = \frac{Product\ obtained\ (g)}{10\ (g)\ Fruit\ peel\ powder} \times 100\ \% \quad (1)$$

Determination of degree of esterification (DE): The degree of esterification is defined as the ratio of esterified galacturonic acid groups to the galacturonic acid groups present [27]. Degree of esterification is an important molecular index for pectin classification that describes the extent to which carboxyl group in pectin molecules exist as the methyl ester. DE is calculated from the neutralization and saponification equivalents of the pectic carboxyl groups [28]. The DE values of the pectin samples were determined by the titration method of Bochek *et al.* [29] with slight modification. Dried pectin (0.2 g) was placed in a conical flask for titration and wetted with ethanol to convert the free carboxyl groups to protonated form. Distilled water (20 ml) was mixed with pectin and the mixture was shaken automatically in a water bath at 45°C until the pectin dissolved completely. The resulting solution was titrated with 0.1 N NaOH with a few drops of phenolphthalein as indicator. The titration volume was recorded as the initial titration volume when pink color appears. Then, another 10 ml of 0.1 N NaOH solution was added to neutralise the polygalacturonic acid sample after determination of the free carboxyl groups and mixture turned into red color. The conical flask was plugged with a stopper and the mixture was stirred at room temperature for 2 hours to de-esterify the pectin. Ten ml of 0.1 N HCl was added to neutralise the unreacted NaOH and the mixture was further titrated with 0.1 N NaOH in the presence of phenolphthalein. The number of the esterified carboxyl groups was calculated from the volume of 0.1 N NaOH solution used for titration [26]. The final titration volume of NaOH was recorded and DE was calculated using equation (2):

$$DE\ (\%) = \left(\frac{Final\ titration\ volume\ (ml)}{Initial\ titration\ volume(ml) + Final\ titration\ volume(ml)}\right) \times 100\ \% \quad (2)$$

Experimental design and statistical analysis: Response surface methodology (Design Expert 8, Stat-Ease Inc, US) was used to determine the optimum conditions for pectin extraction from soursop, passion fruit, and red dragon fruit peels. Two independent variables were designed using central composite design (CCD). Based on preliminary studies, the minimum, maximum and mean values for the two variables studied, the extraction pH and extraction time were set as 2.0, 3.3, and 4.5 and 30, 75 and 120 min, respectively (Table 1). The complete design consisted of 13 runs including four axial experiments (levels $\pm\alpha$), four factorial experiments (levels -1 and +1) and five replicates in centre point. The centre points were performed to minimize the effect of unexplained variability in the observed responses due to systematic errors. The response functions measured were pectin yield and DE of the extracted pectin. A second-order polynomial model equation, Eq. (3) was defined and fitted for each response from the experimental data.

$$Y = C_0 + C_1X_1 + C_2X_2 + C_{11}X_1^2 + C_{22}X_2^2 + C_{12}X_1X_2 \quad (3)$$

Y indicates the estimated response, C_0,C_1, C_2, C_{11}, C_2, and C_{12} are constant coefficients where C_0 is a constant, C_1 and C_2 are coefficients for linear terms, C_{11} and C_{22} are coefficients for quadratic terms, and C_{12} is the coefficient for interaction terms. X_1 and X_2 are the uncoded values of the independent variables of pH and extraction time in minutes, respectively.

Table 1. Levels of independent variables established according to the CCD.

Independent variables		Independent variables level				
Uncoded	Coded	-α	-1	0	1	α
pH	X_1	2.0	2.4	3.3	4.1	4.5
Extraction time (min)	X_2	30	43	75	107	120

Results and Discussion

Effects of pH and extraction time on pectin yield and degree of esterification: Table 2 shows that the passion fruit peel gave the highest pectin yield of 14.60% and highest DE of 67.31%. The DE of passion fruit peel was in the range from 41.67% to 67.31% which indicated higher proportion of high methoxyl pectin. Pectin obtained from dragon fruit peel has lower yield than passion fruit peel and has tendency towards low methoxyl pectin because the DE obtained was at a lower value between 36.54% to 57.81%. Fig. 2 shows the effects of pH on pectin yield and degree of esterification. Within the levels studied, lower pHs of 3.3 and 2.0 contributed significantly larger percentage of pectin yields extracted from both passion and dragon fruit peels, compared to pH 4.5 (Fig. 2a). At lower pH, the highly hydrated carboxylate groups are repressed in the larger hydrogen ion concentration and therefore, converted into slightly hydrated carboxylic acid groups [2]. The loss of charge was able to reduce the repulsion of the polysaccharide molecules leading to precipitated pectin at lower pH. The decreased pH was also able to promote the liberation of pectin molecules from the peel during the acid-washing stage because the interaction of pectins means that the hemicelluloses fractions are cleaved [30]. Yapo *et al.* [22] reported that an increase in acid strength plays an important role in increasing the content of galacturonic acid as pectin is a polymer of galacturonic acid [31].

Pectin yield obtained from soursop peel was marginally higher at higher pH 4.5 compared to pH 3.3 and pH 2.0. The soursop gave the lowest pectin yield and this might be explained by pectinesterase (PE) available in soursop peel. Pectinesterase is a heat-resistant enzyme involved in the maceration and soft-rotting of plant tissue during fruit ripening [32]. During pectin extraction, pectinesterase presents in soursop fruit [33] catalyses the deesterification of pectin into pectate and methanol. Thus, catalysed deesterification of pectin by pectinesterase and its heat resistant behaviour may cause the low soursop pectin yield even though the extraction was conducted at relatively high temperature of 70°C.

Due to the low amount of pectin yield obtained from soursop peel, it was not possible to determine its degree of esterification (DE) and therefore, the influence of pH on DE was presented for passion and dragon fruit peels only (Fig. 2b). The passion fruit peels gave higher DE consistently at all levels. Pinheiro *et al.*[26] observed that a lower citric acid concentration of 0.086% w/v with extraction time of 60 min gave a maximum pectin DE of 78.59% extracted from passion fruit peel. In this work lower pH did not seem to give higher DE. This difference of DE trend was most probably due to the different method employed for pectin extraction. Pinheiro *et al.* [26] conducted pectin extraction using reflux condensation system at 97°C, while for this study, extraction was conducted out in a shaking water bath at 70°C. Methacanon *et al.* [13] reported that pectin extracted from spongy white peel of pomelo at pH 3.0 gave higher DE than that at pH 2.0. Nevertheless, pectins obtained in this work have DE > 50 where it can be categorized as high methoxyl pectin. Seixas *et al.* [14] also obtained pectin with DE ranging from 50.00% to 64.56% from passion fruit peels, irrespective of types of acids used. Analysis of variance indicated that the pH value has significant effect on pectin yield ($p < 0.05$) and it has no significant effect on DE ($p > 0.05$).

Fig. 3 shows the effect of extraction time on pectin yield and DE. Similarly, DE of soursop peel is not tested and presented in Fig. 3b due to low amount of pectin yield obtained. Results demonstrated that the extraction time of 75 min was more favourable to higher pectin yields from both passion and dragon fruit peels (Fig. 3a). Extraction time required to obtain maximum pectin yield from various fruit peels varied such that the spongy white peel of pomelo required 90 min [13], and the dragon fruit peel needed 67 min [20], to get the maximum yield.

DE of extracted pectin decreased non-significantly ($p>0.05$) with increasing extraction time (Fig. 3b). A longer treatment time may have caused pectin degradation with prolonged heating. Similar results were reported on pectin extracted from passion fruit peels [26, 34] and mandarin orange [35]. They explained that DE was partially degraded with a longer extraction time. The degradation is mainly caused by the depolymerisation mechanism of galacturonan chains of pectin which is known as beta-elimination [36]. The use of citric acid, a weak acid for pectin extraction is supported by other studies [37, 38] that a high percentage of DE was obtained from treatment using low concentration of citric acid. These explain that DE extraction has affinity towards using citric acid.

Table 2. Pectin yield and degree of esterification at various pH and extraction time.

Run	pH	Time (min)	Passion fruit peel			Dragon fruit peel			Soursop peel		
			Pectin yield	DE	Pectin type	Pectin yeld	DE	Pectin type	Pectin yield	DE	Pectin type
1	2.0(-α)	75(0)	14.60	63.45	HM	12.62	52.33	HM	0.80	-	-
2	2.4(-1)	43(-1)	13.25	46.35	LM	13.04	44.75	LM	2.06	46.15	LM
3	2.4(-1)	107(1)	13.63	44.35	LM	12.26	51.52	HM	0.43	-	-
4	3.3(0)	30(-α)	8.38	58.92	HM	5.16	49.30	LM	0.87	-	-
5[a]	3.3(0)	75(0)	9.87	56.62	HM	11.06	42.86	LM	2.02	42.68	LM
6[a]	3.3(0)	75(0)	10.37	46.94	LM	11.22	45.45	LM	0.83	-	-
7[a]	3.3(0)	75(0)	9.32	52.94	HM	10.34	46.24	LM	1.25	-	-
8[a]	3.3(0)	75(0)	10.48	58.51	HM	7.80	36.54	LM	0.98	-	-
9[a]	3.3(0)	75(0)	12.31	48.68	LM	8.08	49.06	LM	0.54	-	-
10	3.3(0)	120(α)	2.25	41.67	LM	6.02	38.16	LM	5.87	44	LM
11	4.1(1)	43(-1)	5.11	52.63	HM	6.74	48.39	LM	0.41	-	-
12	4.1(1)	107(1)	4.34	60.53	HM	8.71	56.14	HM	0.28	-	-
13	4.5(α)	75(0)	5.82	67.31	HM	2.79	57.81	HM	4.74	47.65	LM

[a] centre point

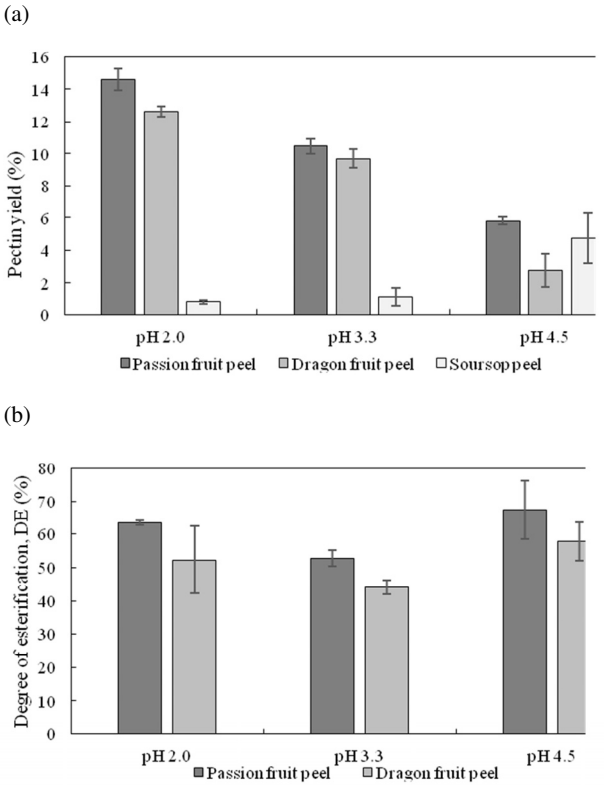

Figure 2. Effect of pH on (a) pectin yield and (b) degree of esterification from fruit peels at centre point of 75 min extraction time.

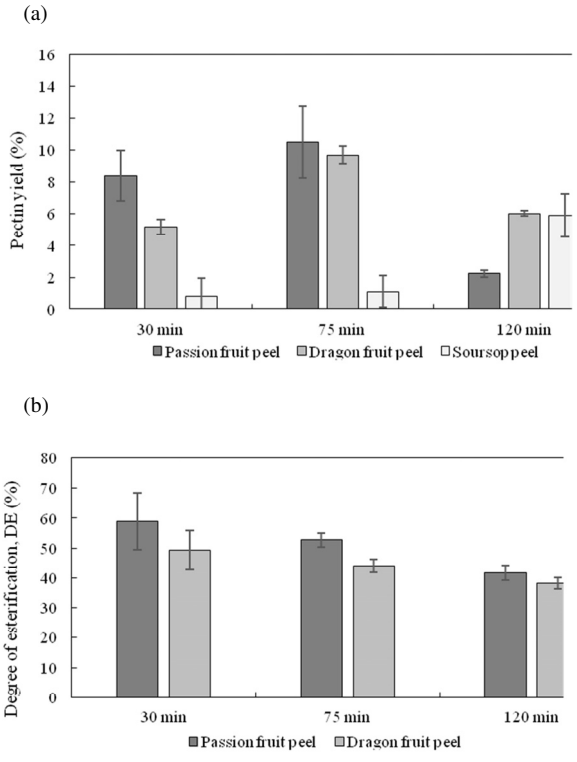

Figure 3. Effect of extraction time on (a) pectin yield and (b) degree of esterification from fruit peels at centre point pH 3.3.

Model selection and verification of pectin yield: Table 3 shows a summary of regression coefficients (C) and significant levels (p) of the independent variables of pH (X_1) and extraction time (X_2) on pectin yield and DE. The passion fruit pectin yield was more significantly affected by pH at $p < 0.001$ than extraction time at p

Table 3. Regression coefficients and probability values for each response.

Term	Pectin yield (Y_1)				Pectin DE (Y_2)			
	Passion fruit		Dragon fruit		Passion fruit		Dragon fruit	
	C	*p*	*C*	*p*	*C*	*p*	*C*	*p*
Constant	14.616	0.0000	17.843	0.0000	112.705	0.0000	116.771	0.0000
X_1	-5.128	0.0004*****	-4.260	0.0087***	-40.948	0.2018*	-45.980	0.3310*
X_2	0.330	0.1016**	0.104	0.7267	0.077	0.3819	-0.041	0.9381
$X_1.X_1$	0.256	0.7654	-0.143	0.9026	5.892	0.1268**	7.322	0.0271***
$X_2.X_2$	-0.002	0.0102***	-0.001	0.2259*	-0.003	0.3052*	0.0001	0.9813
$X_1.X_2$	-0.010	0.7478	0.024	0.5733	0.088	0.5028	0.009	0.9305

X_1: pH; X_2:Extraction time. *p<0.5,**p<0.15,***p<0.05,****p<0.005,*****p<0.001, denoting different significant effects.

< 0.15. The pectin yield and DE for both passion and dragon fruit peels are well described in quadratic regression models as given in equations (4) to (7) with R^2 of 0.89, 0.69, 0.54 and 0.60:

$$Y_{1p} = 14.62 - 5.13X_1 + 0.33X_2 - 0.01X_1X_2 + 0.26X_1^2 - 0.002X_2^2 \quad (4)$$

$$Y_{1d} = 17.84 - 4.26X_1 + 0.10X_2 + 0.02X_1X_2 - 0.14X_1^2 - 0.001X_2^2 \quad (5)$$

$$Y_{2p} = 112.71 - 40.95X_1 + 0.077X_2 + 0.09X_1X_2 + 5.89X_1^2 - 0.003X_2^2 \quad (6)$$

$$Y_{2d} = 116.77 - 45.98X_1 - 0.041X_2 + 0.01X_1X_2 + 7.32X_1^2 + 0.0001X_2^2 \quad (7)$$

where, Y_{1p} and Y_{1d} are pectin yield for passion and dragon fruit peels respectively and Y_{2p} and Y_{2d} are the DE. The correlations between the observed and predicted responses are presented in Fig. 4.

Fig. 5a-d shows the response surface plots of pectin and DE extracted from passion and dragon fruit peels as affected by pH and extraction time. A lower pH and longer extraction gave higher pectin yields for both the passion fruit (Fig. 5a) and dragon fruit (Fig. 5b). For DE, a higher pH was favourable (Fig. 5c-d). The optimised extraction conditions were of pH 2.37 and extraction times of 58.47 and 64.67 min for the passion fruit and dragon fruit peels giving pectin yield of 14.24% and 12.56% with DE of 55.54% and 47.88%, respectively. These optimised values were used to verify the experimental pectin yield and DE values of 13.03% and 58.69% for the passion fruit and 11.64% and 52.51% for the dragon fruit, respectively. The calculated percentage error values for the passion fruit and dragon fruit pectin yield were 9.29% and 7.90%

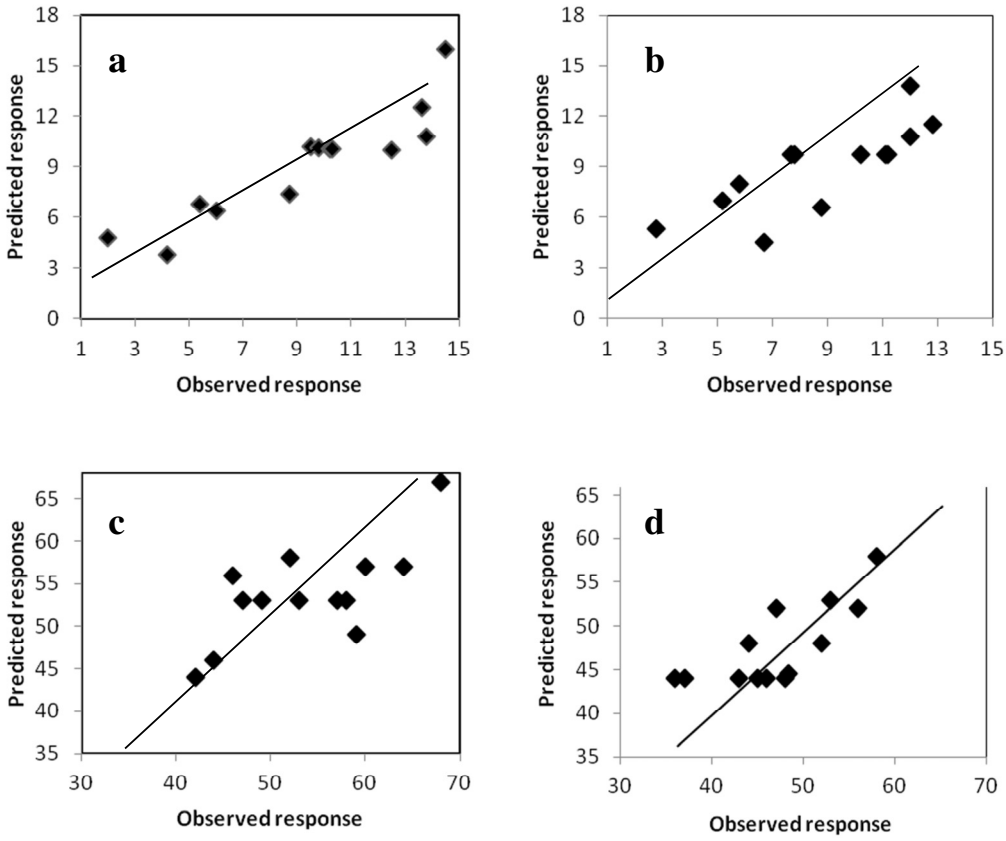

Figure 4. Correlation of predicted responses versus observed responses, (a) passion fruit pectin yield, (b) dragon fruit pectin yield, (c) passion fruit pectin DE, and (d) dragon fruit pectin DE.

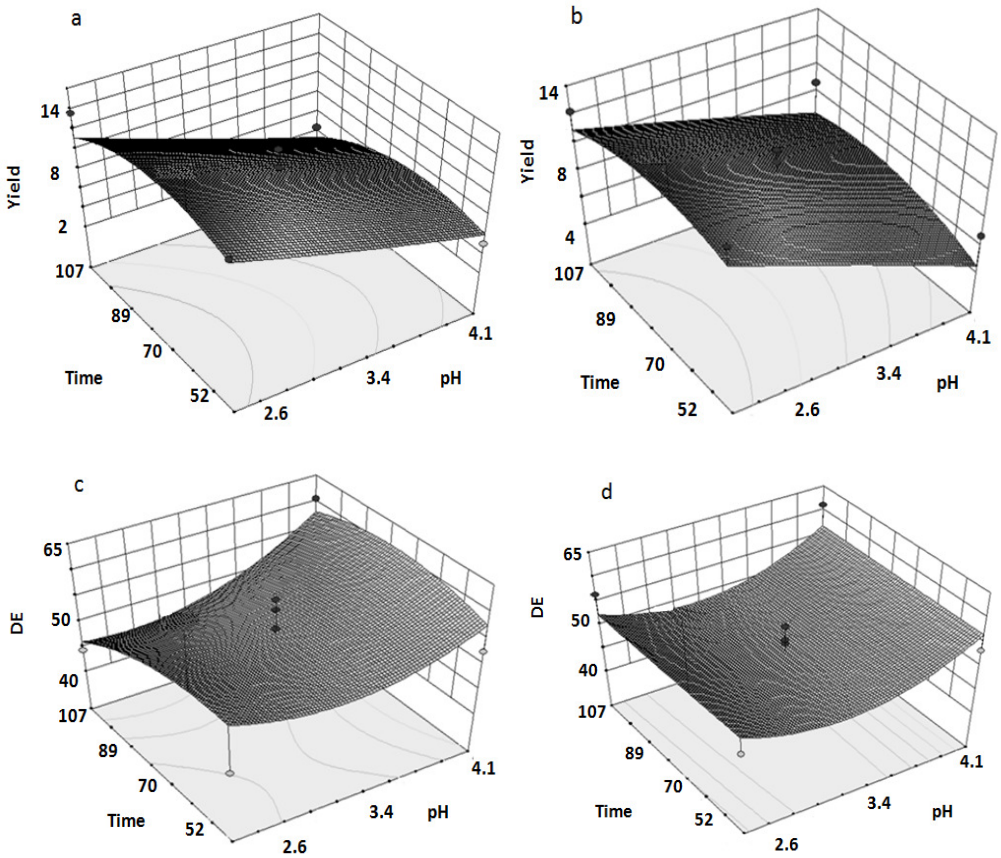

Figure 5. Effect of pH and extraction time on (a) passion fruit pectin yield, (b) dragon fruit pectin yield; (c) passion fruit pectin DE, (d) dragon fruit pectin DE.

while the DE values were 5.37% and 8.82%. These percentage errors which are lower than 10% indicated a good fit [39]. It also indicates that there are no significant differences between the predicted and experimental values and it was a good fit to the model [39].

Conclusions

The optimised extraction of pectin from passion fruit and dragon fruit peels produced yield of more than 12% with DE values about 50%. The soursop peel is not suitable for extraction as it gave very low pectin yield of less than 6%. The value of pH was the more important factor which affected pectin yield compared to extraction time.

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