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Article

Pectin Extraction from Residues of the Cocoa Fruit (*Theobroma cacao* L.) by Different Organic Acids: A Comparative Study

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Abstract: Ecuador is the world's fifth largest cocoa producer, generating hundreds of annual tons of residues from this fruit. This research aims to value this residual (cocoa pod husk) by using it as raw material to obtain pectin, which is widely used in the food and pharmaceutical industries. For them, the extraction of three different organic acids with GRAS status (and therefore safe for their use), citric, malic and fumaric acids, were studied. In addition, two other factors, temperature (70-90°C) and extraction time (60-90 min), were explored in a central composite design of experiments. Under the conditions of the experiment, it was obtained that the best yields were reached for citric acid, malic acid and fumaric acid and for ~86 min of extraction time. The temperature did not show a significant influence on the yield. The pectins obtained under optimal conditions were characterised, showing their similarity with commercial pectin. However, the equivalent weight and the esterification degree of the pectin obtained with fumaric acid classify it as having a high equivalent weight and a low degree of esterification. It differs significantly from the other two acids, perhaps due to the limited solubility of fumaric acid.

Keywords: pectin; cocoa pod husk valorisation; citric acid; malic acid; fumaric acid; response surface methodology; central composite design

1. Introduction

Ecuador is the fifth largest producer of cocoa in the world, with more than 327,000 tons per year of cocoa beans [1] and one of the leading exporters of fine-flavour cocoa, reaching 65% of the market in 2017 [2] and 54% in 2020 [3].

The chocolate paste is obtained from the roasted seeds of the cocoa fruit, which only represent about 10% of the weight of the whole fruit [4]. Thus, in Ecuador, more than 295,100 tons of residues from cocoa production are generated per year.

Cocoa residues are made up of the cocoa pod husk (CPH), the mucilage that covers the seeds and the cover that comes off when the latter is roasted [5]. However, the majority component of the residues is formed by the CPH reaching between 90-93% wt. of the total cocoa residue [5].

CPH is formed mainly by cellulose (35.0%), lignin (14.6%), hemicellulose (11.0%), pectin (6.1%) and proteins (5.9%), in addition to mineral salts, ash and water [6,7].

Pectin, on the other hand, is the natural polysaccharide that forms part of cell walls and tissues in higher plants. It is essential in plant physiology, defence, and typical development [8]. In addition, pectin gives plant tissues the mechanical resistance and flexibility they possess [9].

Pectin has numerous uses in the food, cosmetic and pharmaceutical industries [10,11]. For example, it has been used as a gelling and thickening agent [12], and it has also been part of the formulation of food [13], cosmetics [11], and pharmaceutical applications [14–19].

Interestingly, pectin can be obtained from agricultural, and food waste [20–24], which considerably lowers its production costs and reduces the environmental impact these wastes can exert on ecosystems [25–28].

CPH can be an attractive raw material for obtaining cellulose [29,30], antioxidant compounds [31], and pectin [9,21,32]. However, the pectin extraction yields from CPH depend on several factors [33]. For example, the chosen solvent, the solvent/CPH ratio used, the extraction temperature, and the duration of the process, among others, influence the extraction process yields [34].

CPH-pectin can be extracted using acid solvents, and the extraction process can be assisted or not, using microwaves [35,36] and enzymes [37]. Various acids have been used such as hydrochloric [33] and nitric [38] acids, and some organic acids like ascorbic [39], oxalic [36], acetic, and citric [40] acids. Among all, the latter is the most common choice.

Finally, the response surface methodology (RSM) is an experimental design and analysis tool aimed at finding the conditions for which one or several responses of an experiment are optimized [41,42] and has been used successfully in the optimization of numerous agro-industrial processes [43]. The extraction of pectin has not been an exception [36,44–47].

This work aims to find, through the RSM, the best temperature and contact time conditions that maximize the yield of pectin extraction from dehydrated CPH using three organic acids. One already used before is citric acid, and two other organic acids are considered with GRAS status: malic and fumaric acids.

2. Materials and Methods

2.1. Raw materials

The cocoa fruits in this work belong to the CCN-51 variety and come from the Lita Parish, Ibarra Canton, Imbabura Province, Ecuador.

2.2. Chemicals

Food-grade reagents citric acid monohydrate ($C_6H_8O_7{\cdot}H_2O$, CAS 5949-29-1, food additive code: E330), L-malic acid ($C_4H_6O_5$, CAS 97-67-6, E296), and fumaric acid ($C_4H_4O_4$, CAS 110-17- 8, E297, were supplied by Sucroal S.A. (https://sucroal.com.co, Recta Cali – Palmira Km 18. Palmira, Valle del Cauca, Colombia).

2.3. Preparation of dehydrated cocoa pod husk

All the experiments were carried out in Cayambe, Pichincha province, Ecuador. The city is located at the coordinates 0°02′38″N 78°09′22″W and 2,830 m above sea level.

The cocoa residues were washed with abundant tap water to remove any debris or traces of dirt on their surface, and then after drying it was weighed.

Cutting the shell, it darkens rapidly, evidence of oxidation or maybe due to the action of hydrolytic enzymes. The enzymatic inactivation process was carried out, adding water to cover the wet residuals and then it was heated up to 75 °C for 8 minutes. Subsequently, it is drained and cut into small pieces 3 mm wide to facilitate drying at 50 °C for about six hours until the residues turn amber.

Finally, the dehydrated-CPH (d-CPH) residues were crushed in an Oster mill until obtaining a low moisture content powder was stored in vacuum-sealed polyethene bags and stored at 4°C until used in acid hydrolysis experiments (Figure 1, left side).

2.4. Pectin extraction experiments from dehydrated CPH

The acid solutions were prepared by diluting about 20 g of each acid with deionized water until an acid solution of pH 2.5 was obtained, according to the procedure described elsewhere [33].

Subsequently, 6 g of d-CPH have placed in 100 ml Schoot-flasks, and the acid solution was added in a proportion of 16 ml for each gram of d-CPH (a substrate-extractant ratio of 1:16 (w/v)), according to the variants of the central composite design (CCD) of experiments to be carried out (see Table 2).

The temperature of all the experiments was between 70 and 90 °C and was controlled in a recirculating water bath with temperature control (± 1 °C). The process times were between 60 and 90 min, according to the CCD variants of experiments (Table 2).

At the end of each acid extraction time, the flasks were placed in an ice box for about 10 min, to rapidly cool the mixture, after which the solid residues were separated from the liquid acid phase. To the latter, a similar volume of separated supernatant was mixed Diagram of the process for obtaining dehydrated CPH (left side) and pectin from it (right side) with an equal volume of 96% ethyl alcohol at -15 °C, and the mixture was stirred for a few seconds until it was completely homogeneous. Then the samples were placed at 4°C in a conventional refrigerator, for about 30 min at rest, to facilitate the precipitation of the pectin.

Finally, the contents of each bottle were carefully decanted and filtered through a white Muslim cloth to separate the pectin obtained. The moist pectin was dried in an oven at around 50 °C for 4 h and then weighed on an analytical balance.

The dry material was stored in the vacuum-sealed polyethene bags at 4 °C until it was used in the characterisation studies carried out later (Figure 1, right side).

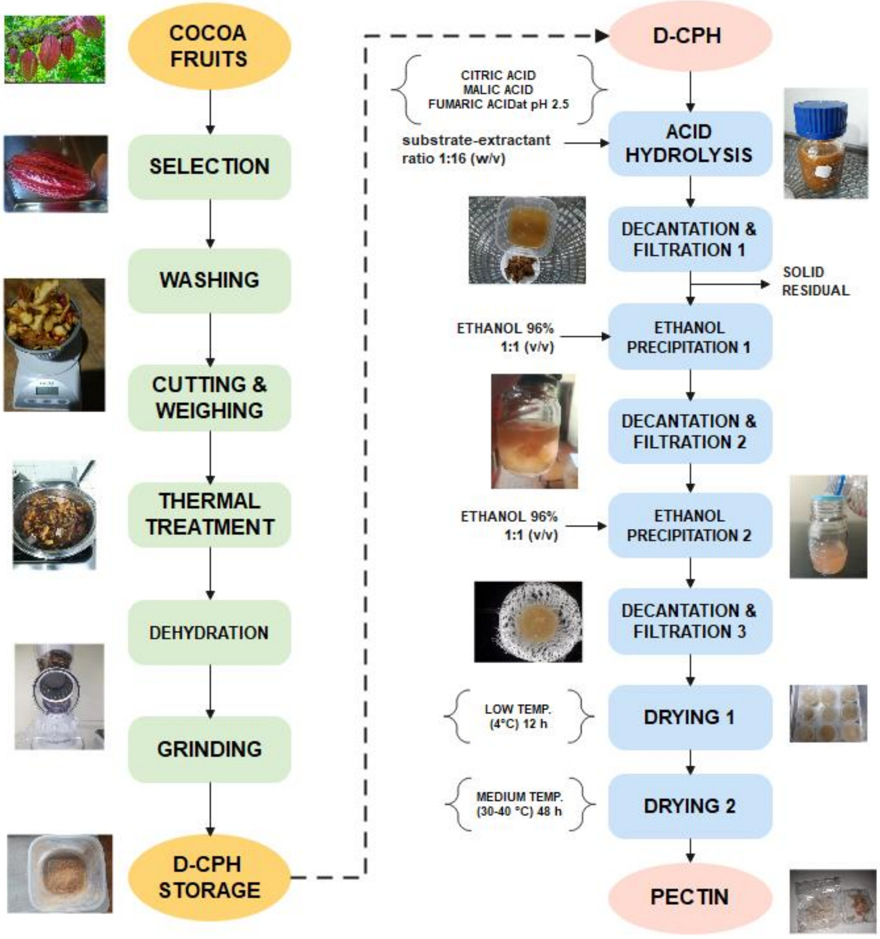


Figure 1. Diagram of the process for obtaining dehydrated CPH (left side) and pectin from it (right side).

The pectin yields obtained (g/kg) by acid hydrolysis were calculated from the modification of the method used by other researchers [33,48]:

$$Yield\left(\frac{g}{kg}\right) = \frac{Dry\ pectin\ (g)}{d{-}CPH\ (kg)} \quad (1)$$

2.5. *Central-composite design of experiments in response surface methodology*

To find the combination of temperature, extraction time, and type of acid, with which maximum yield of pectin performance is obtained, a central composite design (CCD) of experiments of the response surface methodology (RSM) was carried out.

Three factors were used, two continuous quantitative factors (A: extraction time (min) and B: extraction temperature (°C)), and a nominal qualitative factor (C: type of organic acid). Of the latter, three organic acids were used: C[1]: Citric acid, C[2]: Malic acid, and C[3]: Fumaric acid.

Two blocks of experiments were used, which corresponded to two batches of cocoa fruits used, all from the same supplier.

The values of each factor used, and their coded variables are shown in Table 1.

Table 1. Factors used (real and coded) in the CCD experiments of the present study.

	Coded factors				
	-1.414	-1.000	0.000	+1.000	+1.414
A: Time (min)	60	64	75	86	90
B: Temperature (°C)	70	73	80	87	90
C: Organic acid	C[1]: Citric acid		C[2]: Malic acid	C[3]: Fumaric acid	

Design-Expert released 13.013.0 (Stat-Ease, Inc., MN, USA), a statistical software package that was employed to manage and analyze all the experiments.

The experimental results of CCD of RSM could fit with a second-order polynomial equation by multiple regression techniques.

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum \sum_{i<j=3} \beta_{ij} X_i X_j + \beta_{123} X_1 X_2 X_3 + \varepsilon = \hat{Y} + \varepsilon \quad (2)$$

Where Y and $\hat{Y}$ are the response and the predicted by quadratic model response (yield of pectin), β_0 is the model intercept coefficient β_i, β_{ii} and β_{ij} are regression coefficients of the linear, quadratic, and interactive terms, respectively, X_i are the factors under study (time and temperature of pectin extraction, and organic acid type), and ε is the residual error.

2.6. *Characterization of CHP-pectins.*

2.6.1. FTIR analysis of CHP-pectins

The final dry pectin samples, obtained from the acid extractions with the three organic acids in this study, as well as the reference commercial pectin, were analyzed by IR spectrometry, using an Agilent Cary 630 FTIR (Agilent Technologies Inc., 5301 Stevens Creek Blvd, Santa Clara, CA 95051, USA), in a range of wavenumber between 400 and 4000 cm^{-1} in 32 scans with a resolution of 4 cm^{-1}. In addition, an ATR sampling technique was used on a single rebound diamond crystal.

2.6.2. Determination of equivalent weight, methoxyl content (MeO), anhydrouronic acid content (AUA), and degree of esterification (DE) of CHP-pectins

Equivalent weight was determined according to the methodology described elsewhere [49,50]. First, five hundred milligrams of dry pectin sample from dehydrated CPH were moistened with 2 ml of ethanol and dissolved in 100 ml of deionised CO_2-free sterile water. Then, 1 g NaCl and 6 drops phenol-red indicator was added and mixed vigorously until dissolved all the pectin. After that, the mix was carefully titrated with 0.1 N NaOH until the colour turned pink (pH 7.5).

Equivalent weight was calculated as:

$$Equiv.weight\ (g/mol) = \frac{mass\ sample\ (g)}{V(ml)\ alkali\ \times Conc.(N)\ alkali} \tag{3}$$

Methoxyl (MeO) content was determined according to methodology described elsewhere [49,50]. Briefly, 25 ml of 0.25 N was added to the neutralized solution above described to determine the equivalent weight. New alkali solutions were well-mixed and allowed to stand for half hour in a stoppered flask. Then, 25 ml of 0.25 N HCl was added, and titrated again, as it were made before.

Methoxyl (MeO) content (% wt.) was calculated as:

$$MeO\ (\%) = \frac{V(ml)\ alkali\ \times Conc.(N)\ alkali \times 31}{mass\ sample\ (g) \times 1000} \times 100 \tag{4}$$

where "31" is the molecular weight of the MeO group.

Anhydrouronic acid (AUA) content (% wt.) in pectin samples was obtained according to the following formula:

$$AUA\ (\%) = \frac{176 \times 0.1 \cdot (V_z + V_y)}{m \times 1000} \times 100 \tag{5}$$

Using the titration volumes of alkali obtained above to determine both the equivalent weight (V_z, ml) and the methoxyl content (V_y, ml), and the mass of samples (m, g).

Degree of esterification (DE) in pectin samples as calculated according to the expression reported by others as:

$$DE(\%) = \frac{176 \times MeO(\%)}{31 \times AUA(\%)} \times 100 \tag{6}$$

Degree of esterification (DE, %) can also be calculated by determining the peak areas in FTIR spectra of pectin's, corresponding to their free carboxyl groups (~1630 cm^{-1}) and the esterified groups (~1740 cm^{-1}) according to the equations [51,52]:

$$R = \frac{A_{1740}}{A_{1740} + A_{1630}} \times 100 \tag{7}$$

$$DE(\%) = 124.7 \cdot R + 2.2013 \tag{8}$$

3. Results

3.1. CCD experiments and the model analysis

CCD experiments to find the best extraction conditions (extraction time and temperature) for each of the three organic acids with GRAS status (citric, malic or fumaric acids) were carried out in two blocks according to the fruits processed to obtain the dehydrated CPH material (d-CPH) that was used as starting raw material to obtain pectin from d-CPH (Table 2).

Table 2. CCD experiments for the maximisation of the yield of pectin.

Std	Block	Run	A: Time (min)	B: Temperature (°C)	C: Organic-acid (-)	Yield (g/kg)
32	1	1	86	87	Fumaric acid	4.0
31	1	2	64	87	Fumaric acid	3.6
17	1	3	64	87	Malic acid	2.4
33	1	4	75	80	Fumaric acid	1.0
18	1	5	86	87	Malic acid	5.4
4	1	6	86	87	Citric acid	4.6
21	1	7	75	80	Malic acid	4.4
29	1	8	64	**73**	Fumaric acid	1.6
5	1	9	75	80	Citric acid	5.0
1	1	10	64	**73**	Citric acid	4.2
15	1	11	64	**73**	Malic acid	2.2
6	1	12	75	80	Citric acid	5.0
19	1	13	75	80	Malic acid	4.8
16	1	14	86	**73**	Malic acid	4.6
3	1	15	64	87	Citric acid	1.8
35	1	16	75	80	Fumaric acid	1.0
30	1	17	86	**73**	Fumaric acid	1.4
2	1	18	86	**73**	Citric acid	4.2
20	1	19	75	80	Malic acid	4.4
34	1	20	75	80	Fumaric acid	4.6
7	1	21	75	80	Citric acid	5.8
23	2	22	90	80	Malic acid	5.6
40	2	23	75	80	Fumaric acid	1.0
28	2	24	75	80	Malic acid	4.4
9	2	25	90	80	Citric acid	9.4
10	2	26	75	70	Citric acid	6.8
22	2	27	60	80	Malic acid	1.6
12	2	28	75	80	Citric acid	5.0
38	2	29	75	70	Fumaric acid	2.4
24	2	30	75	70	Malic acid	3.6
14	2	31	75	80	Citric acid	4.8
41	2	32	75	80	Fumaric acid	1.2
26	2	33	75	80	Malic acid	4.6
11	2	34	75	90	Citric acid	5.2
37	2	35	90	80	Fumaric acid	4.0
8	2	36	60	80	Citric acid	5.4
39	2	37	75	90	Fumaric acid	3.0
13	2	38	75	80	Citric acid	5.0
25	2	39	75	90	Malic acid	5.0
27	2	40	75	80	Malic acid	4.4
42	2	41	75	80	Fumaric acid	1.0
36	2	42	60	80	Fumaric acid	0.8

With the data shown above (Table 2), the following model was obtained, in terms of the codified factors, of the pectin yield:

$$\hat{Y}(g/kg) = 3.81 + 1.01 \cdot A + 1.34 \cdot C[1] + 0.2857 \cdot C[2] \quad (R^2 = 0.6). \tag{9}$$

While, in terms of actual factors, the equations that best represented the experimental data were:

Citric acid: $\hat{Y}_{CA}(g/kg) = -1.98440 + 0.095221 \cdot Time(min)$ (10)

Malic acid: $\hat{Y}_{MA}(g/kg) = -3.04154 + 0.095221 \cdot Time(min)$ (11)

Fumaric acid: $\hat{Y}_{FA}(g/kg) = -4.95583 + 0.095221 \cdot Time(min)$ (12)

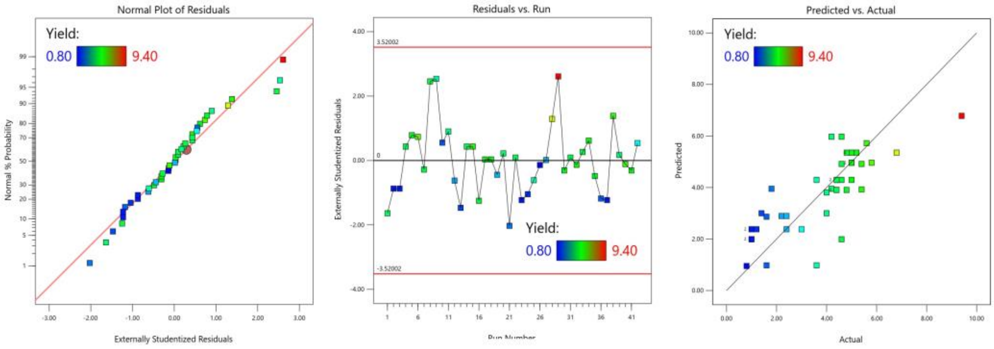
The analysis of variance (ANOVA) for the yield model shows the significance of each term in the model (Table 3).

Table 3. ANOVA for CCD of experiments on the yield of pectin extraction.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Block	1.60	1	1.60			
Model	88.00	3	29.33	20.70	< 0.0001	*significant*
A-Time	24.48	1	24.48	17.28	0.0002	
C-OA [1]	63.52	2	31.76	22.41	< 0.0001	
Residual	52.43	37	1.42			
Lack of Fit	43.18	25	1.73	2.24	0.0728	*not significant*
Pure Error	9.25	12	0.7711			
Cor total	142.03	41				

[1] OA: Organic acid.

Additionally, normality (Figure 2 a) and their residuals distribution (Figure 2 b), and correspondence between the values obtained by the model with the actual values (Figure 2 c), were checked.



(a) (b) (c)

Figure 2. Analysis of the model of the responses for the yield of pectin extraction (**a**) Normality plot of the residuals for the yield model shown in Equation (7); (**b**) Student's t external distribution of the residuals; and (**c**) correspondence between the actual values of the yield responses with the values obtained with the model shown in Equation (7).

All the analyses showed the usefulness of the pectin yield model and suggested that the yield of the pectin model could be used to navigate on their surfaces to find the optimal values.

3.2 Optimisation of pectin yield model

By optimizing the yield of the pectin model with the maximum levels of importance (5 (+++++)), in which factors A: time and C: Organic acid, both were in range, and for a non-significant ($p > 0.05$) temperature factor (B: Temp.), it was equal to 80 °C (Table 4).

Table 4. Search criteria for the optimal condition for the yield of pectin extraction.

Name	Goal	Lower Limit	Upper Limit	Importance
A: Time	is in range	64.39	85.61	3
B: Temperature	is equal to 80.0	72.93	90.00	3
C: Organic acid	is in range	Citric acid	Fumaric acid	3
Yield	maximize	0.80	9.40	5

After performing the optimal procedure, three maximum possible values for pectin yield were obtained for each organic acid used in this study (Table 5).

Table 5. Optimum values of pectin yield obtained with the constrains shown in Table 4.

Number	Time	Temperature	Organic acid	$\hat{Y}(g/kg)$
1	85.607	80.00	Citric acid	6.167
2	85.607	80.00	Malic acid	5.110
3	85.607	80.00	Fumaric acid	3.196

These optimal values can be represented graphically, depending on each of the factors under study (Figure 3).

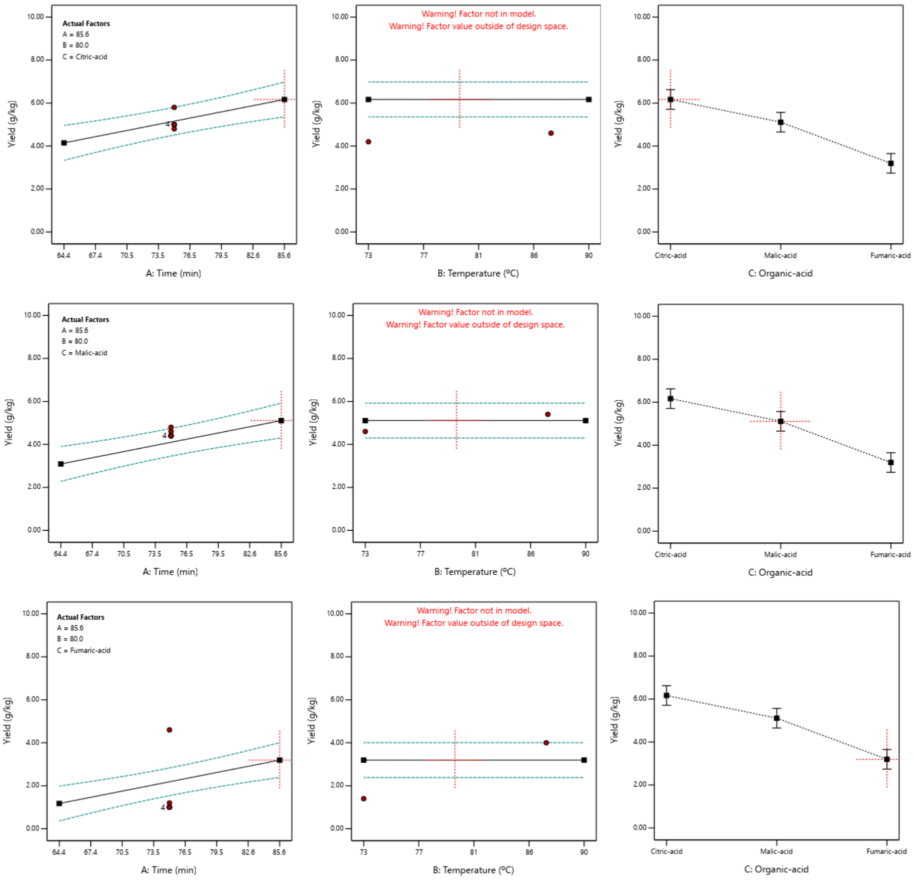


Figure 3. Values of the pectin yield model in relation to factors A: time, B: Temp., and C: Organic acid, for the three organic acids used in this study. Upper side: Citric acid; Middle Side: Malic acid, and Bottom Side: Fumaric acid.

Moreover, as suggested by the yield models for each of the three organic acids used in the present study, only the extraction time and the acid type significantly influence ($p < 0.05$) the extraction yield.

3.3 Confirmation experiments for the pectin yield model

Six complementary, confirmatory experiments were carried out in unison and under the conditions suggested in Table 5 (for an extraction time and temperatures of around 86 min and 80 °C, respectively) to verify the validity of the obtained model. As a result, the pectin yields for each of the organic acids are within the ranges suggested by the models' equations (10) to (12) (Table 6).

Table 6. Confirmatory experiments of the validity of the pectin yield model for each of the organic acids used in this study.

Organic acid	Predicted Mean	Std Dev	n	SE Pred	95% PI low	Data Mean	95% PI high
Citric acid	6.17	1.19	6	0.63	4.89	5.73	7.44
Malic acid	5.11	1.19	6	0.63	3.83	4.70	6.39
Fumaric acid	3.20	1.19	6	0.63	1.92	3.53	4.47

The complementary validation experiments corroborate that there are significant differences in the acid extraction yield between the three organic acids used in this study, although the real values obtained are somewhat lower than those of the models for citric and malic acid, and somewhat higher than the ones. predicted for fumaric acid (Table 6).

3.4 Characterisation of the pectin from d-CPH by using three organic acids

The pectin samples obtained from the confirmatory experiments were used to carry out the characterisation studies.

FTIR spectra of the pectin samples obtained from d-CPH and extracted with three GRAS-type organic acids demonstrate their chemical similarity. They are also similar to those of the FTIR spectrum of the commercial pectin used as a reference and shown elsewhere [52](Figure 4).

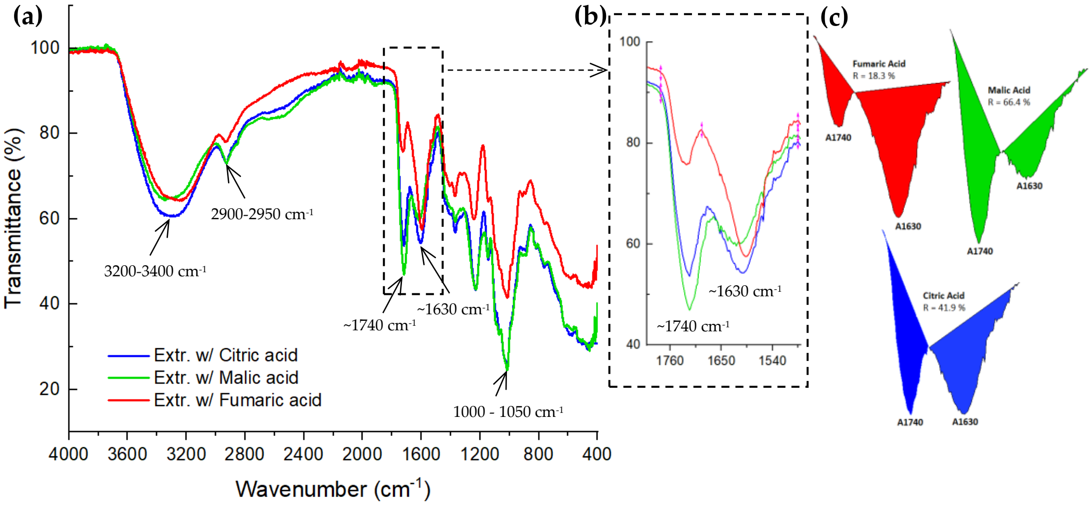


Figure 4. FTIR spectra of the pectin obtained from d-CPH and (a) extracted with the different organic acids employed in this study. (b) The peaks and their areas are analysed in the zone close to ~1740 and ~1630 cm^{-1}, which correspond to the esterified and free carboxyl groups, respectively. (c) Areas at ~1740 cm^{-1} and ~1630 cm^{-1} for each FTIR spectrum and the calculation of the R-value (eq. 7).

Additionally, the equivalent weight, the MeO and AUA contents (%wt.), and the degree of esterification (DE, %) of the pectin samples obtained from d-CPH by extraction with the three organic acids used in this work were determined (Figure 5).

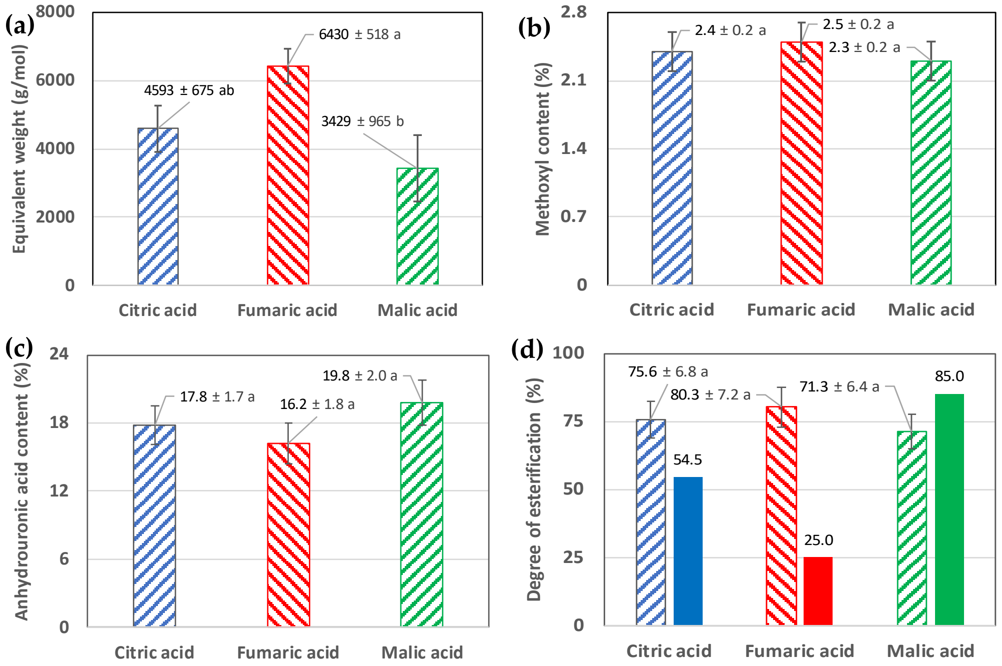


Figure 5. Characterisation of the pectin obtained from d-CPH and extracted with the different organic acids employed in this study (citric, malic, and fumaric acids). (a) Equivalent weight (g/mol); (b) Methoxyl (MeO) content (%); (c) Anhydrouronic acid (AUA) content (%); (d) Degree of esterification (DE, %). The bars filled with a pattern represent the DE (%) values determined according to the titration method (Eq. 6), while the full-colored bars are the results calculated from the FTIR spectra and the calculation of the areas (A_{1740} and A_{1630}, Figure 4 b) and Eqs. (7-8). All values: mean ± standard deviation (n = 3). In each graph, equal letters denote non-statistically significant differences ($p < 0.05$).

4. Discussion

The process of preparation of the CPH and its dehydration could be extended the storage time of this raw material. In this way, the raw material could be available for more time and not depend on its availability only during the harvest periods of the cocoa fruit (Figure 1, left side).

Decantation-filtration characterized the extraction process with different GRAS-status type organic acids as the separation method. It was used in the initial solid-liquid separation processes after the extraction time off and in the two processes of ethanolic precipitation for the purification of pectin (Figure 1, right side). However, the decantation-filtration process seems less efficient than those other methods, such as press-filtration or centrifugation.

Due to the above, the yields obtained in the present study are lower than those obtained by similar studies. Thus, for example, pectin yielded of 5.55–7.70 % [9] and 6.10-9.20 % [53] dry wt., and 18.12 % [40], 11.52 % [54], and 9.00 % [55] wet-fresh wt. has been reported elsewhere for extraction with citric acid, while in the present work, a modest 0.62 % wt. was reached.

CCD experiment suggest that the extraction yields under the conditions of the experiment comply with the following relationship $Y_{CA} > Y_{MA} > Y_{FA}$, as shown by the relationships established between the yield models, such as:

$$\hat{Y}_{MA}(g/kg) = \hat{Y}_{CA}(g/kg) - 1.06 = \hat{Y}_{FA}(g/kg) + 1.91 \qquad (13)$$

When carrying out the model validation experiments (n = 6) what the models predicted was confirmed and it was observed that $\bar{Y}_{CA} > \bar{Y}_{MA} > \bar{Y}_{FA}$, although the differences between the yields were slightly different from those shown with the models (Eq. 14).

$$\bar{Y}_{MA}(g/kg) = \bar{Y}_{CA}(g/kg) - (1.03 \pm 0.16) = \bar{Y}_{FA}(g/kg) + (1.17 \pm 0.31) \qquad (14)$$

The explanation for the behaviour observed in the acid extraction yields needs to be clarified. However, it is possibly related to the presence of three carboxyl groups in citric acid compared to the two that have both malic acid and fumaric acid, on the one hand, and to the low solubility of fumaric acid (4.9 g/L at 20°C) concerning malic acid (558.0 g/L at 20°C) and citric acid (592.0 g/L at 20°C).

The first confers certain advantages to citric acid compared to the other two acids, so its acid hydrolysis action could be more effective. Moreover, the second does not allow the fumaric acid to remain soluble throughout the extraction process and can thus exert its role of hydrolysis on the d-CPH solid material.

The FT-IR spectra of the pectin obtained from d-CPH with the organic acids with GRAS status are very similar to each other (Figure 4), and close to those reported for commercial pectin [9,44,52]. In FTIR spectra observed the -OH peaks at 3400–3200 cm^{-1}. These peaks represent many polyhydroxy compounds present in pectin (Figure 4 a). The -CH, -CH_2, and -CH_3 stretches of galacturonic acid methyl esters absorb at 2900 - 2950 cm^{-1} (Figure 4 a). Peaks ~1750 cm^{-1} correspond to the C=O stretch observed in the ester and derived from the acetyl (-$COCH_3$) group (Figure 4 a). Finally, peaks ~1630 cm^{-1} are related to the -OH tensile vibration band, and the bands at 1000–1050 cm^{-1} belong to C-O bending or stretching (Figure 4 a).

Their esterification degrees were calculated based on the areas of the peaks at ~1740 cm−1 and ~1630 cm−1 of the pectin's FT-IR spectra (Figure 4 b). Pectin extracted with citric (DE = 54.5 %) and malic (DE = 85 %) acids had high esterification degrees (DE > 50 %). Whilst pectin extracted with fumaric acid (DE = 25 %) could be classified as a low esterification degree. These results differed from the titration method results, where all the pectins had high-esterification degrees (DE ≈ 75 %), and all of them were statistically similar ($n = 3$, $p < 0.05$) (Figure 5 d).

Regarding the values of equivalent weight, methoxyl content, and anhydrouronic acid content (Figure 5) of the pectin obtained by acid extraction with organic acids such as citric acid, malic acid and fumaric acid from d-CPH, these values are different from those reported by other authors. For example, the values obtained for the yield, methoxyl content, degree of esterification, and the equivalent weight of the pectin obtained from CPH using hydrochloric acid for 60 min at 80 °C were 5.50-7.70 %, 3.51-4.86 %, 10.76-19.96 %, and 663.83 - 1549.22 g/mol, respectively [9]. It is possible that the combined use of a strong acid, such as HCl, for less time than the one used in the present work results in a lower degree of esterification and higher equivalent weight and methoxyl contents.

However, further studies must be carried out to find the causes of such differences, especially concerning the degree of esterification, which is closely related to pectin's applications as a gelling and thickening agent.

5. Conclusions

In the present work, pectin was obtained from d-CPH, using acid extraction with three organic acids with GRAS status. The pectin obtained had lower yields than previous reports, which is attributed to the pectin isolation and purification procedures. However, the yields were significantly different, with citric acid being the highest and fumaric acid the lowest. The pectin obtained were very similar in appearance, and their FT-IR spectra demonstrate their similarity to each other, and the commercial pectin spectra published by other authors.

However, the pectin obtained with fumaric acid showed differences, concerning those obtained with citric acid and malic acid, regarding the equivalent weight and the degree of esterification, which may be related to the lower solubility of this organic acid.

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References

1. FAOSTAT *Food and Agriculture Organization of the United Nations - Statistics Division, Http://Www.Fao.Org/Faostat/En/#data/QC (Accessed 08.03.2022)*; 2022;
2. Díaz-Montenegro, J.; Varela, E.; Gil, J.M. Livelihood Strategies of Cacao Producers in Ecuador: Effects of National Policies to Support Cacao Farmers and Specialty Cacao Landraces. *J Rural Stud* **2018**, *63*, doi:10.1016/j.jrurstud.2018.08.004.
3. Ketema, M.W.; Tennhardt, L.; Cramon-Taubadel, . Stephan v. Profitability of Small-Scale Cocoa Production in Ecuador. *Conference Proceedings: Tropentag* **2021**.
4. Mansur, D.; Tago, T.; Masuda, T.; Abimanyu, H. Conversion of Cacao Pod Husks by Pyrolysis and Catalytic Reaction to Produce Useful Chemicals. *Biomass Bioenergy* **2014**, *66*, doi:10.1016/j.biombioe.2014.03.065.
5. Porto de Souza Vandenberghe, L.; Kley Valladares-Diestra, K.; Amaro Bittencourt, G.; Fátima Murawski de Mello, A.; Sarmiento Vásquez, Z.; Zwiercheczewski de Oliveira, P.; Vinícius de Melo Pereira, G.; Ricardo Soccol, C. Added-Value Biomolecules' Production from Cocoa Pod Husks: A Review. *Bioresour Technol* **2022**, *344*, doi:10.1016/j.biortech.2021.126252.
6. Campos-Vega, R.; Nieto-Figueroa, K.H.; Oomah, B.D. Cocoa (Theobroma Cacao L.) Pod Husk: Renewable Source of Bioactive Compounds. *Trends Food Sci Technol* 2018, *81*.
7. Ouattara, L.; Kouadio, E.; Soro, D.; Soro, Y.; Benjamin Yao, K.; Adouby, K.; Drogui, A.; Tyagi, D.; Aina, P. Cocoa Pod Husk: A Review. *Bioresources* **2021**, *16*.
8. Mohnen, D. Pectin Structure and Biosynthesis. *Curr Opin Plant Biol* 2008, *11*.
9. Musita, N.- Characteristics of Pectin Extracted from Cocoa Pod Husks. *Pelita Perkebunan (a Coffee and Cocoa Research Journal)* **2021**, *37*, doi:10.22302/iccri.jur.pelitaperkebunan.v37i1.428.
10. de Cindio, B.; Gabriele, D.; Lupi, F.R. Pectin: Properties Determination and Uses. In *Encyclopedia of Food and Health*; 2015.
11. Lupi, F.R.; Gabriele, D.; Seta, L.; Baldino, N.; de Cindio, B.; Marino, R. Rheological Investigation of Pectin-Based Emulsion Gels for Pharmaceutical and Cosmetic Uses. *Rheol Acta* **2015**, *54*, doi:10.1007/s00397-014-0809-8.
12. Gawkowska, D.; Cybulska, J.; Zdunek, A. Structure-Related Gelling of Pectins and Linking with Other Natural Compounds: A Review. *Polymers (Basel)* 2018, *10*.
13. Lara-Espinoza, C.; Carvajal-Millán, E.; Balandrán-Quintana, R.; López-Franco, Y.; Rascón-Chu, A. Pectin and Pectin-Based Composite Materials: Beyond Food Texture. *Molecules* 2018, *23*.
14. Mamani Crispín, P.L.; Ruiz Caro, R.; Veiga, Ma.D. Pectin: Pharmaceutical and Therapeutic Uses. *Anales de la Real Academia Nacional de Farmacia* **2012**, *78*.
15. Satapathy, S.; Rout, J.R.; Kerry, R.G.; Thatoi, H.; Sahoo, S.L. Biochemical Prospects of Various Microbial Pectinase and Pectin: An Approachable Concept in Pharmaceutical Bioprocessing. *Front Nutr* 2020, *7*.
16. Tkalec, G.; Knez, Z.; Novak, Z. Encapsulation of Pharmaceuticals into Pectin Aerogels for Controlled Drug Release. *Advanced technologies* **2015**, *4*, doi:10.5937/savteh1502049t.
17. Tyagi, V.; Sharma, P.K.; Malviya, R. Pectins And Their Role in Food and Pharmaceutical Industry. *Journal of Chronotherapy and Drug Delivery* **2015**, *6*.
18. Srivastava, P.; Malviya, R. Sources of Pectin, Extraction and Its Applications in Pharmaceutical Industry - an Overview. *Indian J Nat Prod Resour* 2011, *2*.
19. Sutapa, B.M.; Dhruti, A.P.G.; Gopa, R.B. Therapeutic and Pharmaceutical Benefits of Native and Modified Plant Pectin. *Journal of Medicinal Plants Research* **2018**, *12*, doi:10.5897/jmpr2017.6542.
20. Mellinas, C.; Ramos, M.; Jiménez, A.; Garrigós, M.C. Recent Trends in the Use of Pectin from Agro-Waste Residues as a Natural-Based Biopolymer for Food Packaging Applications. *Materials* 2020, *13*.
21. Kley Valladares-Diestra, K.; Porto de Souza Vandenberghe, L.; Ricardo Soccol, C. A Biorefinery Approach for Pectin Extraction and Second-Generation Bioethanol Production from Cocoa Pod Husk. *Bioresour Technol* **2022**, *346*, doi:10.1016/j.biortech.2021.126635.

22. Zoghi, A.; Vedadi, S.; Esfahani, Z.H.; Gavlighi, H.A.; Khosravi-Darani, K. A Review on Pectin Extraction Methods Using Lignocellulosic Wastes. *Biomass Convers Biorefin* 2021.
23. Desniorita; Nazir, N.; Novelina; Sayuti, K. Sustainable Design of Biorefinery Processes on Cocoa Pod: Optimization of Pectin Extraction Process with Variations of PH, Temperature, and Time. *Int J Adv Sci Eng Inf Technol* **2019**, *9*, doi:10.18517/ijaseit.9.6.10670.
24. Sabater, C.; Villamiel, M.; Montilla, A. Integral Use of Pectin-Rich by-Products in a Biorefinery Context: A Holistic Approach. *Food Hydrocoll* 2022, *128*.
25. Otles, S.; Despoudi, S.; Bucatariu, C.; Kartal, C. Food Waste Management, Valorization, and Sustainability in the Food Industry. In *Food Waste Recovery: Processing Technologies and Industrial Techniques*; 2015 ISBN 9780128004197.
26. Garcia-Garcia, G.; Woolley, E.; Rahimifard, S.; Colwill, J.; White, R.; Needham, L. A Methodology for Sustainable Management of Food Waste. *Waste Biomass Valorization* **2017**, doi:10.1007/s12649-016-9720-0.
27. Lin, Z.; Ooi, J.K.; Woon, K.S. An Integrated Life Cycle Multi-Objective Optimization Model for Health-Environment-Economic Nexus in Food Waste Management Sector. *Science of the Total Environment* **2022**, *816*, doi:10.1016/j.scitotenv.2021.151541.
28. Girotto, F.; Alibardi, L.; Cossu, R. Food Waste Generation and Industrial Uses: A Review. *Waste Management* **2015**, doi:10.1016/j.wasman.2015.06.008.
29. Azmin, S.N.H.M.; Hayat, N.A.B.M.; Nor, M.S.M. Development and Characterization of Food Packaging Bioplastic Film from Cocoa Pod Husk Cellulose Incorporated with Sugarcane Bagasse Fibre. *Journal of Bioresources and Bioproducts* **2020**, *5*, doi:10.1016/j.jobab.2020.10.003.
30. Akinjokun, A.I.; Petrik, L.F.; Ogunfowokan, A.O.; Ajao, J.; Ojumu, T.V. Isolation and Characterization of Nanocrystalline Cellulose from Cocoa Pod Husk (CPH) Biomass Wastes. *Heliyon* **2021**, *7*, doi:10.1016/j.heliyon.2021.e06680.
31. Campos-Vega, R.; Nieto-Figueroa, K.H.; Oomah, B.D. Cocoa (Theobroma Cacao L.) Pod Husk: Renewable Source of Bioactive Compounds. *Trends Food Sci Technol* 2018, *81*.
32. Adi-Dako, O.; Ofori-Kwakye, K.; Frimpong Manso, S.; Boakye-Gyasi, M. el; Sasu, C.; Pobee, M. Physicochemical and Antimicrobial Properties of Cocoa Pod Husk Pectin Intended as a Versatile Pharmaceutical Excipient and Nutraceutical. *J Pharm (Cairo)* **2016**, *2016*, doi:10.1155/2016/7608693.
33. Chan, S.Y.; Choo, W.S. Effect of Extraction Conditions on the Yield and Chemical Properties of Pectin from Cocoa Husks. *Food Chem* **2013**, *141*, 3752–3758, doi:10.1016/j.foodchem.2013.06.097.
34. Hamidon, N.H.; Zaidel, D.N.A. Effect of Extraction Conditions on Pectin Yield Extracted from Sweet Potato Peels Residues Using Hydrochloric Acid. *Chem Eng Trans* **2017**, *56*, doi:10.3303/CET1756164.
35. Sarah, M.; Hisham, H.; Rizki, M.; Erwinda, R. Effect of Power and Time in Pectin Production from Cocoa Pod Husk Using Microwave-Assisted Extraction Technique. *International Journal of Renewable Energy Development* **2020**, *9*, doi:10.14710/ijred.9.1.125-130.
36. Pangestu, R.; Amanah, S.; Juanssilfero, A.B.; Yopi; Perwitasari, U. Response Surface Methodology for Microwave-Assisted Extraction of Pectin from Cocoa Pod Husk (Theobroma Cacao) Mediated by Oxalic Acid. *Journal of Food Measurement and Characterization* **2020**, *14*, doi:10.1007/s11694-020-00459-4.
37. Hennessey-Ramos, L.; Murillo-Arango, W.; Vasco-Correa, J.; Astudillo, I.C.P. Enzymatic Extraction and Characterization of Pectin from Cocoa Pod Husks (Theobroma Cacao L.) Using Celluclast® 1.5 L. *Molecules* **2021**, *26*, doi:10.3390/molecules26051473.
38. Vriesmann, L.C.; Teófilo, R.F.; Petkowicz, C.L.D.O. Optimization of Nitric Acid-Mediated Extraction of Pectin from Cacao Pod Husks (Theobroma Cacao L.) Using Response Surface Methodology. *Carbohydr Polym* **2011**, *84*, 1230–1236, doi:10.1016/j.carbpol.2011.01.009.
39. Priyangini, F.; Walde, S.G.; Chidambaram, R. Extraction Optimization of Pectin from Cocoa Pod Husks (Theobroma Cacao L.) with Ascorbic Acid Using Response Surface Methodology. *Carbohydr Polym* **2018**, *202*, doi:10.1016/j.carbpol.2018.08.103.
40. Marsiglia, D.E.; Ojeda, K.A.; Ramírez, M.C.; Sánchez, E. Pectin Extraction from Cocoa Pod Husk (Theobroma Cacao L.) by Hydrolysis with Citric and Acetic Acid. *Int J Chemtech Res* **2016**, *9*.
41. Myers, R.; Montgomery, D.; Anderson-Cook, C. *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*; 4th ed.; John Wiley & Sons, Inc: Hoboken, New Jersey, 2016; ISBN 978-1-118-91601-8.
42. Khuri, A.I. A General Overview of Response Surface Methodology. *Biom Biostat Int J* **2017**, doi:10.15406/bbij.2017.05.00133.
43. Pais-Chanfrau, J.M.; Núñez-Pérez, J.; del Carmen Espin-Valladares, R.; Vinicio Lara-Fiallos, M.; Enrique Trujillo-Toledo, L. Uses of the Response Surface Methodology for the Optimization of Agro-Industrial Processes. In *Response Surface Methodology in Engineering Science*; Palanikumar Kayaroganam, Ed.; Intech: London, UK, 2021; pp. 1–21 ISBN 978-1-83968-918-5.
44. Mada, T.; Duraisamy, R.; Guesh, F. Optimization and Characterization of Pectin Extracted from Banana and Papaya Mixed Peels Using Response Surface Methodology. *Food Sci Nutr* **2022**, *10*, doi:10.1002/fsn3.2754.
45. Duwee, Y.S.; Kiew, P.L.; Yeoh, W.M. Multi-Objective Optimization of Pectin Extraction from Orange Peel via Response Surface Methodology: Yield and Degree of Esterification. *Journal of Food Measurement and Characterization* **2022**, *16*, doi:10.1007/s11694-022-01305-5.

46. Sundarraj, A.A.; Thottiam Vasudevan, R.; Sriramulu, G. Optimized Extraction and Characterization of Pectin from Jackfruit (Artocarpus Integer) Wastes Using Response Surface Methodology. *Int J Biol Macromol* **2018**, *106*, doi:10.1016/j.ijbiomac.2017.08.065.
47. Pradhan, D.; Abdullah, S.; Pradhan, R.C. Optimization of Pectinase Assisted Extraction of Chironji (Buchanania Lanzan) Fruit Juice Using Response Surface Methodology and Artificial Neural Network. *International Journal of Fruit Science* **2020**, *20*, doi:10.1080/15538362.2020.1734895.
48. Seggiani, M.; Puccini, M.; Pierini, M.; Giovando, S.; Forneris, C. Effect of Different Extraction and Precipitation Methods on Yield and Quality of Pectin. *Int J Food Sci Technol* **2009**, *44*, doi:10.1111/j.1365-2621.2008.01849.x.
49. Khamsucharit, P.; Laohaphatanalert, K.; Gavinlertvatana, P.; Sriroth, K.; Sangseethong, K. Characterization of Pectin Extracted from Banana Peels of Different Varieties. *Food Sci Biotechnol* **2018**, doi:10.1007/s10068-017-0302-0.
50. Ranganna, S. Handbook of Analysis and Quality Control for Fruit and Vegetable Products. In *Handbook of analysis and quality control for fruit and vegetable products*; 1986.
51. Pappas, C.S.; Malovikova, A.; Hromadkova, Z.; Tarantilis, P.A.; Ebringerova, A.; Polissiou, M.G. Determination of the Degree of Esterification of Pectinates with Decyl and Benzyl Ester Groups by Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) and Curve-Fitting Deconvolution Method. *Carbohydr Polym* **2004**, *56*, doi:10.1016/j.carbpol.2004.03.014.
52. Güzel, M.; Akpınar, Ö. Valorisation of Fruit By-Products: Production Characterization of Pectins from Fruit Peels. *Food and Bioproducts Processing* **2019**, *115*, doi:10.1016/j.fbp.2019.03.009.
53. Vásquez, Z.S.; de Carvalho Neto, D.P.; Pereira, G.V.M.; Vandenberghe, L.P.S.; de Oliveira, P.Z.; Tiburcio, P.B.; Rogez, H.L.G.; Góes Neto, A.; Soccol, C.R. Biotechnological Approaches for Cocoa Waste Management: A Review. *Waste Management* 2019, *90*.
54. Amponsah, S.K.; Yeboah, S.; Kukuia, K.K.E.; N'Guessan, B.B.; Adi-Dako, O. A Pharmacokinetic Evaluation of a Pectin-Based Oral Multiparticulate Matrix Carrier of Carbamazepine. *Adv Pharmacol Pharm Sci* **2021**, *2021*, doi:10.1155/2021/5527452.
55. Vriesmann, L.C.; Teófilo, R.F.; Lúcia de Oliveira Petkowicz, C. Extraction and Characterization of Pectin from Cacao Pod Husks (Theobroma Cacao L.) with Citric Acid. *LWT - Food Science and Technology* **2012**, *49*, 108–116, doi:10.1016/j.lwt.2012.04.018.